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[Continued on next page]

(54) Title: METHODS OF USE OF FLUOROQUINOLONE COMPOUNDS AGAINST BACTERIA

(57) Abstract: This invention relates, in part, to newly identified methods of using quinolone antibiotics, particularly a gemifloxacin compound against certain bacteria, especially pathogenic bacteria.

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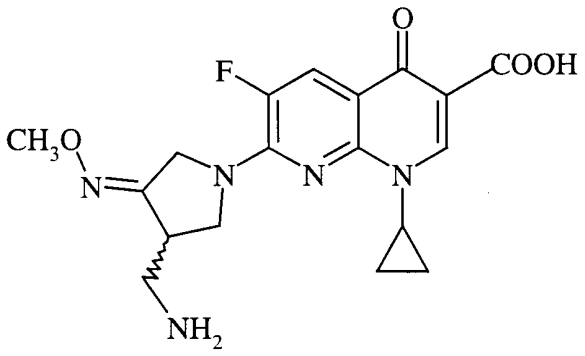
METHODS OF USE OF FLUOROQUINOLONE COMPOUNDS AGAINST BACTERIA

This invention relates, in part, to newly identified methods of using quinolone antibiotics, particularly a gemifloxacin compound against bacteria, particularly pathogenic bacteria.

BACKGROUND OF THE INVENTION

Quinolones have been shown to be effective to varying degrees against a range of bacterial pathogens. However, as diseases caused by these pathogens are on the rise, there exists a need for antimicrobial compounds that are more potent than the present group of quinolones.

Gemifloxacin mesylate (SB-265805) is a novel fluoroquinolone useful as a potent antibacterial agent. Gemifloxacin compounds are described in detail in patent application PCT/KR98/00051 published as WO 98/42705. Patent application EP 688772 discloses novel quinoline(naphthyridine)carboxylic acid derivatives, including anhydrous (R,S)-7-(3-aminomethyl-4-methoxyiminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid of formula I.



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PCT/KR98/00051 discloses (R,S)-7-(3-aminomethyl-4-*syn*-methoxyimino-pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid methanesulfonate and hydrates thereof including the hemihydrate and sesquihydrate.

The method according to claims 54 wherein the gemifloxacin compound is gemifloxacin mesylate sesquihydrate.

Provided herein is a significant discovery made using a gemifloxacin compound against certain bacterial species, particularly medically important pathogens in need of more effective antibiotics. This invenion thereby fills an unmet medical need.

An aspect of the invention is directed to the use of a gemifloxacin compound agianst

Acinetobacter species, demonstrating the activity of the gemifloxacin compounds herein used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Prevotella denticola/loeschii group, Veillonella* spp., *Prevotella heparinolytica, Prevotella intermedia, Prevotella melaninogenica, Porphyromonas* spp. (*Porphyromonas cangingivalis*; *Porphyromonas cansulci*; *Porphyromonas circumdentaria*; and *Porphyromonas levii*), *Prevotella bivia, Prevotella buccae-oris* group, *Porphyromonas canoris, Porphyromonas gingivalis, Porphyromonas macaccae, Peptostreptococcus micros, Peptostreptococcus prevotii, Porphyromonas asaccharolyticus, Fusobacterium varium, Peptostreptococcus asaccharolyticus, Peptostreptococcus magnus, Fusobacterium* spp group 1 (*Fusobacterium gonidiaformans, Fusobacterium naviforme, Fusobacterium necrophorum, Fusobacterium nucleatum, and Fusobacterium nucleatum* ss *animalis*), *Fusobacterium* spp. group 2 (*Fusobacterium mortiferum, Fusobacterium necrogenes,* and *Fusobacterium ulcerans*), *Fusobacterium russii, Clostridium difficile, Clostridium inocuum, Clostridium ramosum, Bacteroides ureolyticus, Bilophila wadsworthia, Clostridium clostridioforme, Anaerobiospirillum succiniciproducens, Bacteroides gracilis, Bacteroides tectum, Actinomyces odontolyticus, Actinomyces israelii*, and *Anaerobiospirillum thomasii*, especially Peptostreptococcus and Porphyromonas species, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

A further aspect of the invention is directed to the use of a gemifloxacin compound against *Salmonella* spp. and *Shigella* spp. , demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

Another aspect of the invention is directed to the use of a gemifloxacin compound against pneumococcal bacteria, including penicillin-susceptible, intermediate and resistant (including ciprofloxacin-resistant) pneumococci., demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

Still another aspect of the invention is directed to the use of a gemifloxacin compound against maxillary sinus pathogens, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against Gram negative, non-fermenting , demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Chlamydia pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Moraxella*

catarrhalis, Mycoplasma pneumoniae and *Legionella* spp., demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against respiratory tract bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against bacterial meningitis, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against Gram negative bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against Gram positive or Gram negative aerobic bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Streptococci* bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Mycoplasma* bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Legionella* spp., demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Streptococcus pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Haemophilus influenzae* and pneumococci bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against Gram positive bacteria, such as streptococci and staphylococci, and Enterobacteriaceae bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against Gram positive pneumococci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against gonococci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against pneumococci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against aerobic bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against pneumococci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

Another aspect of the invention is directed to the use of a gemifloxacin compound against Gram positive cocci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

Yet another aspect of the invention is directed to the use of a gemifloxacin compound against *Streptococcus pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *enterococci*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against streptococci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Acinetobacter* spp., demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Chlamydia pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against streptococci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Bordetella* spp., demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

An aspect of the invention is directed to the use of a gemifloxacin compound against *Mycoplasma*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

Another aspect of the invention is directed to the use of a gemifloxacin compound against *Streptococcus pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

Still another aspect of the invention is directed to the use of a gemifloxacin compound against *Streptococcus pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein.

As set forth herein in more detail, gemifloxacin compounds are valuable compounds for the treatment of clinical conditions or indications caused by a range of bacteria of the invention, including those bacteria resistant to usual oral therapy.

SUMMARY OF THE INVENTION

An object of the invention is a method for modulating metabolism of *Acintobacter* pathogenic bacteria comprising the step of contacting *Acintobacter* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Acintobacter* pathogenic bacteria is selected from the group consisting of: *A. baumannii, A. anitratus, A. lwoffii, A. calcoaceticus* or *Acinetobacter* spp.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Acintobacter* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Acintobacter* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *A. baumannii, A. lwoffii, or A. calcoaceticus.*

An object of the invention is a method for modulating metabolism of anaerobic pathogenic bacteria comprising the step of contacting anaerobic pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said anaerobic pathogenic bacteria is selected from the group consisting of: *Prevotella denticola/loeschii group*, *Veillonella* spp., *Prevotella heparinolytica*, *Prevotella intermedia*, *Prevotella melaninogenica, Porphyromonas* spp. (*Porphyromonas cangingivalis*; *Porphyromonas cansulci*; *Porphyromonas circumdentaria*; and *Porphyromonas levii*), *Prevotella bivia*, *Prevotella buccae-oris* group, *Porphyromonas canoris*, *Porphyromonas gingivalis*, *Porphyromonas macaccae, Peptostreptococcus micros*, *Peptostreptococcus prevotii*, *Porphyromonas asaccharolyticus, Fusobacterium varium*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus magnus, Fusobacterium* spp group 1 (*Fusobacterium gonidiaformans*, *Fusobacterium naviforme, Fusobacterium necrophorum, Fusobacterium nucleatum, and Fusobacterium nucleatum* ss *animalis*), *Fusobacterium* spp. group 2 (*Fusobacterium mortiferum, Fusobacterium necrogenes,* and *Fusobacterium ulcerans*), *Fusobacterium russii, Clostridium difficile*, *Clostridium inocuum*, *Clostridium ramosum, Bacteroides ureolyticus*, *Bilophila wadsworthia*, *Clostridium clostridioforme, Anaerobiospirillum succiniciproducens*, *Bacteroides gracilis*, *Bacteroides tectum, Actinomyces odontolyticus*, *Actinomyces israelii*, or *Anaerobiospirillum thomasii.*

Also provided by the invention is a method of treating or preventing a bacterial infection by anaerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with anaerobic pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Prevotella denticola/loeschii group*, *Veillonella* spp., *Prevotella heparinolytica*, *Prevotella intermedia*, *Prevotella melaninogenica, Porphyromonas* spp. (*Porphyromonas cangingivalis*; *Porphyromonas cansulci*; *Porphyromonas circumdentaria*; and *Porphyromonas levii*), *Prevotella bivia*, *Prevotella buccae-oris* group, *Porphyromonas canoris*, *Porphyromonas gingivalis*, *Porphyromonas macaccae, Peptostreptococcus micros*, *Peptostreptococcus prevotii*, *Porphyromonas asaccharolyticus, Fusobacterium varium*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus magnus, Fusobacterium* spp group 1

(*Fusobacterium gonidiaformans*, *Fusobacterium naviforme*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum*, *and Fusobacterium nucleatum* ss *animalis*), *Fusobacterium* spp. group 2 (*Fusobacterium mortiferum*, *Fusobacterium necrogenes*, and *Fusobacterium ulcerans*), *Fusobacterium russii*, *Clostridium difficile*, *Clostridium inocuum*, *Clostridium ramosum*, *Bacteroides ureolyticus*, *Bilophila wadsworthia*, *Clostridium clostridioforme*, *Anaerobiospirillum succiniciproducens*, *Bacteroides gracilis*, *Bacteroides tectum*, *Actinomyces odontolyticus*, *Actinomyces israelii*, or *Anaerobiospirillum thomasii*.

An object of the invention is a method for modulating metabolism of pathogenic Enterococcal bacteria comprising the step of contacting pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Serratia* spp., *Providencia stuartii*, *Salmonella* spp., *Stenotrophomonas maltophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus* MSSA, *Staphylococcus aureus* MRSA, *Staphylococcus epidermidis* MSSE, *Staphylococcus epidermidis* MRSE, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Streptococcus lancefield* Gp B, *Streptococcus millerii*, *Streptococcus pneumoniae*, Quinolone-resistant *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Clostridium perfringens*, *Clostridium difficile*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevoti*, and *Bacteroides fragilis*.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Enterobacter* spp., *Staphylococcus aureus* MSSA, *Staphylococcus epidermidis* MSSE, *Staphylococcus epidermidis* MRSE, *Streptococcus pneumoniae*, Quinolone-resistant *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevoti*, and *Bacteroides fragilis*..

An object of the invention is a method for modulating metabolism of *Salmonella* spp. and *Shigella* spp. pathogenic bacteria comprising the step of contacting *Salmonella* spp. and *Shigella* spp. pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Salmonella* spp. and *Shigella* spp. pathogenic bacteria is selected from the group consisting of: *Salmonella* spp. and *Shigella* spp.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Salmonella* spp. and *Shigella* spp. pathogenic bacteria comprising the step of administering an

antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Salmonella* spp. and *Shigella* spp. pathogenic bacteria.

An object of the invention is a method for modulating metabolism of pneumococcal pathogenic bacteria comprising the step of contacting pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: penicillin-susceptible, intermediate and resistant (including ciprofloxacin-resistant) pneumococci.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: penicillin-susceptible, intermediate and resistant (including ciprofloxacin-resistant) pneumococci.

An object of the invention is a method for modulating metabolism of maxillary sinus pathogenic bacteria comprising the step of contacting maxillary sinus pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said maxillary sinus pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae, Haemophilus influenzae, Haemophilus parainfluenzae, Moraxella catarrhalis, Staphylococcus aureus, Peptostreptococcus spp., Bacteroides urealyticus, Enterobacteriaceae,* non-fermentative Gram negative *bacilli, Neisseria meningitidis, Bacteroides spp., beta-hemolytic Streptococcus* and Gram negative rods.

Also provided by the invention is a method of treating or preventing a bacterial infection by maxillary sinus pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with maxillary sinus pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected

from the group consisting of: *Streptococcus pneumoniae, Haemophilus influenzae, Haemophilus parainfluenzae, Moraxella catarrhalis, Staphylococcus aureus, Peptostreptococcus spp., Bacteroides urealyticus*, Enterobacteriaceae, non-fermentative Gram negative bacilli, *Neisseria meningitidis, Bacteroides spp.*, beta-hemolytic Streptococcus and Gram negative rods.

An object of the invention is a method for modulating metabolism of Gram negative, non-fermenting pathogenic bacteria comprising the step of contacting Gram negative, non-fermenting pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said Gram negative, non-fermenting pathogenic bacteria is selected from the group consisting of: *Pseudomonas. Aeruginosa, Acinetobacter* spp., *Stenotrophomonas maltophilia* and *Burkholderia cepacia* bacteria.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram negative, non-fermenting pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with Gram negative, non-fermenting pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Pseudomonas. Aeruginosa, Acinetobacter* spp., *Stenotrophomonas maltophilia* and *Burkholderia cepacia* bacteria.

An object of the invention is a method for modulating metabolism of *Chlamydia pneumoniae* or other respiratory tract pathogenic bacteria comprising the step of contacting *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria is selected from the group consisting of: *Chlamydia pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae* and *Legionella* spp..

Also provided by the invention is a method of treating or preventing a bacterial infection by *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected

from the group consisting of: *Chlamydia pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae* and *Legionella* spp.

An object of the invention is a method for modulating metabolism of respiratory tract pathogenic bacteria comprising the step of contacting respiratory tract pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said respiratory tract pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae* and *Haemophilus influenzae.*

Also provided by the invention is a method of treating or preventing a bacterial infection by respiratory tract pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with respiratory tract pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Streptococcus pneumoniae* and *Haemophilus influenzae.*

An object of the invention is a method for modulating metabolism of bacterial meningitis pathogenic bacteria comprising the step of contacting bacterial meningitis pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said bacterial meningitis pathogenic bacteria is *Streptococcus pneumoniae.*

Also provided by the invention is a method of treating or preventing a bacterial infection by bacterial meningitis pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with bacterial meningitis pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is *Streptococcus pneumoniae.*

An object of the invention is a method for modulating metabolism of Gram negative pathogenic bacteria comprising the step of contacting Gram negative pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said Gram negative pathogenic

bacteria is selected from the group consisting of: *Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, Pseudomonas aeruginosa, Morganella morganii, Proteus spp., Stenotrophomonas maltophilia, Klebsiella oxytoca, Citrobacter freundii, Acinetobacter sp.*, and *Serratia spp.*

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram negative pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with Gram negative pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, Pseudomonas aeruginosa, Morganella morganii, Proteus spp., Stenotrophomonas maltophilia, Klebsiella oxytoca, Citrobacter freundii, Acinetobacter sp.*, and *Serratia spp.*

An object of the invention is a method for modulating metabolism of Gram positive or Gram negative aerobic pathogenic bacteria comprising the step of contacting Gram positive or Gram negative aerobic pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said Gram positive or Gram negative aerobic pathogenic bacteria is selected from the group consisting of: *Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Proteus vulgaris.*

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive or Gram negative aerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with Gram positive or Gram negative aerobic pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Proteus vulgaris.*

An object of the invention is a method for modulating metabolism of *Streptococci*

pathogenic bacteria comprising the step of contacting *Streptococci* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Streptococci* pathogenic bacteria is *Streptococcus* spp.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococci* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Streptococci* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is *Streptococcus* spp.

An object of the invention is a method for modulating metabolism of *Mycoplasma* pathogenic bacteria comprising the step of contacting *Mycoplasma* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Mycoplasma* pathogenic bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma penetrans* and *Ureaplasma urealyticum*.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Mycoplasma* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Mycoplasma* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma penetrans* and *Ureaplasma urealyticum*.

An object of the invention is a method for modulating metabolism of *Legionella* spp. pathogenic bacteria comprising the step of contacting *Legionella* spp. pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Legionella* spp. pathogenic bacteria is selected from the group consisting of: *Legionella pneumophilia*, *Legionella bozemanii*,

Legionella wadsworthii, Legionella jordanis, Legionella dumoffii, Legionella longbeacheae, Legionella micdadei, and Erithromycin-resistant strains of *Legionella spp.*.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Legionella* spp. pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Legionella* spp. pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Legionella pneumophilia, Legionella bozemanii, Legionella wadsworthii, Legionella jordanis, Legionella dumoffii, Legionella longbeacheae, Legionella micdadei*, Erithromycin-susceptible strains of *Legionella spp* and Erithromycin-resistant strains of *Legionella spp.*

An object of the invention is a method for modulating metabolism of *Streptococcus pneumoniae* pathogenic bacteria comprising the step of contacting *Streptococcus pneumoniae* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is *Streptococcus pneumoniae.*

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Streptococcus pneumoniae* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is *Streptococcus pneumoniae* .

An object of the invention is a method for modulating metabolism of *Haemophilus influenzae* or pneumococci pathogenic bacteria comprising the step of contacting *Haemophilus influenzae* or pneumococci pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: *Haemophilus influenzae* and pneumococci.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Haemophilus influenzae* and pneumococci pathogenic bacteria comprising the step of

administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Haemophilus influenzae* or pneumococci pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Haemophilus influenzae* and pneumococci bacteria.

An object of the invention is a method for modulating metabolism of Gram positive bacterial pathogens, such as streptococci and staphylococci, and Enterobacteriaceae pathogenic bacteria comprising the step of contacting the pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: Gram positive bacterial pathogens, such as streptococci and staphylococci, and Enterobacteriaceae bacteria.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive bacterial pathogens, such as streptococci and staphylococci, or Enterobacteriaceae pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with Gram positive bacterial pathogens, such as streptococci and staphylococci, or Enterobacteriaceae pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: Gram positive bacterial pathogens, such as streptococci and staphylococci, or Enterobacteriaceae bacteria.

An object of the invention is a method for modulating metabolism of Gram positive pneumococcal pathogenic bacteria comprising the step of contacting Gram positive pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said Gram positive pneumococcal pathogenic bacteria is *Streptococcus pneumoniae*.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with Gram positive pneumococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is

Streptococcus pneumoniae.

An object of the invention is a method for modulating metabolism of gonococcal pathogenic bacteria comprising the step of contacting gonococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said gonococcal pathogenic bacteria is selected from the group consisting of: *Neisseria gonorrhoeae,* including Ciprofloxacin-resistant strains of *Neisseria gonorrhoeae.*

Also provided by the invention is a method of treating or preventing a bacterial infection by gonococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with gonococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Neisseria gonorrhoeae,* including Ciprofloxacin-resistant strains of *Neisseria gonorrhoeae.*

An object of the invention is a method for modulating metabolism of pneumococcal pathogenic bacteria comprising the step of contacting pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: pneumococcal pathogenic bacteria.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: pneumococcal pathogenic bacteria.

An object of the invention is a method for modulating metabolism of aerobic pathogenic bacteria comprising the step of contacting aerobic pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said aerobic pathogenic bacteria is

selected from the group consisting of: *Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Proteus vulgaris*.

Also provided by the invention is a method of treating or preventing a bacterial infection by aerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with aerobic pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Proteus vulgaris*.

An object of the invention is a method for modulating metabolism of pneumococcal pathogenic bacteria comprising the step of contacting pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pneumococcal pathogenic bacteria is *S. pneumoniae*.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is *S. pneumoniae*.

An object of the invention is a method for modulating metabolism of Gram positive coccal pathogenic bacteria comprising the step of contacting Gram positive coccal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said Gram positive coccal pathogenic bacteria is selected from the group consisting of: *Enterococcus faecalis, Enterococcus faecium, Enterococcus* spp., *Staphylococcus aureus, Staphylococcus epidermidis*, Coagulase-negative staphylococci, *Streptococcus pneumoniae, Streptococcus* β-hemolytic, *Streptococcus* viridans group,

Bacillus spp., and *Corynebacterium* spp.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive coccal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with Gram positive coccal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, Coagulase-negative staphylococci, *Streptococcus pneumoniae*, *Streptococcus* β-hemolytic, *Streptococcus* viridans group, *Bacillus* spp., and *Corynebacterium* spp.

An object of the invention is a method for modulating metabolism of *Streptococcus pneumoniae* pathogenic bacteria comprising the step of contacting *Streptococcus pneumoniae* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae* .

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Streptococcus pneumoniae* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Streptococcus pneumoniae* .

An object of the invention is a method for modulating metabolism of *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria comprising the step of contacting *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Also provided by the invention is a method of treating or preventing a bacterial infection

by *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

An object of the invention is a method for modulating metabolism of *enterococcal* pathogenic bacteria comprising the step of contacting *enterococcal* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *enterococcal* pathogenic bacteria is selected from the group consisting of: *Enterococcus faecalis* and *Enterococcus faecium*

Also provided by the invention is a method of treating or preventing a bacterial infection by *enterococcal* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *enterococcal* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Enterococcus faecalis* and *Enterococcus faeci*um.

An object of the invention is a method for modulating metabolism of streptococcal pathogenic bacteria comprising the step of contacting streptococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said streptococcal pathogenic bacteria is selected from the group consisting of: *Streptococcus pyogenes*, *Streptococcus agalactiae* and viridans streptococci.

Also provided by the invention is a method of treating or preventing a bacterial infection by streptococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with streptococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected

from the group consisting of: *Streptococcus pyogenes*, *Streptococcus agalactiae* and viridans streptococci.

An object of the invention is a method for modulating metabolism of *Acinetobacter* spp. pathogenic bacteria comprising the step of contacting *Acinetobacter* spp. pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Acinetobacter* spp. pathogenic bacteria is selected from the group consisting of: *Acinetobacter baumannii, Acinetobacter calcoaceticus, Acinetobacter lwoffii,* and *Acinetobacter anitratus.*

Also provided by the invention is a method of treating or preventing a bacterial infection by *Acinetobacter* spp. pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Acinetobacter* spp. pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Acinetobacter baumannii, Acinetobacter calcoaceticus, Acinetobacter lwoffii,* and *Acinetobacter anitratus.*

An object of the invention is a method for modulating metabolism of *Chlamydia pneumoniae* pathogenic bacteria comprising the step of contacting *Chlamydia pneumoniae* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: *Chlamydia pneumoniae.*

Also provided by the invention is a method of treating or preventing a bacterial infection by *Chlamydia pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Chlamydia pneumoniae* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Chlamydia pneumoniae.*

An object of the invention is a method for modulating metabolism of streptococcal pathogenic bacteria comprising the step of contacting streptococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said streptococcal pathogenic bacteria is selected from the group consisting of: *Streptococcus pyogenes, Streptococcus agalactiae* and viridans streptococci.

Also provided by the invention is a method of treating or preventing a bacterial infection by streptococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with streptococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Streptococcus pyogenes, Streptococcus agalactiae* and viridans streptococci.

An object of the invention is a method for modulating metabolism of *Bordetella* spp. pathogenic bacteria comprising the step of contacting *Bordetella* spp. pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Bordetella* spp. pathogenic bacteria is selected from the group consisting of: *B.pertussis*, *B. parapertussis*, and *B. bronchiseptica*.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Bordetella* spp. pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Bordetella* spp. pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *B.pertussis*, *B. parapertussis*, and *B. bronchiseptica*.

An object of the invention is a method for modulating metabolism of *Mycoplasma* pathogenic bacteria comprising the step of contacting *Mycoplasma* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said *Mycoplasma* pathogenic bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma penetrans* and *Ureaplasma urealyticum*.

Also provided by the invention is a method of treating or preventing a bacterial infection

by *Mycoplasma* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Mycoplasma* pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium, Mycoplasma penetrans* and *Ureaplasma urealyticum.*

An object of the invention is a method for modulating metabolism of *Streptococcus pneumoniae* pathogenic bacteria comprising the step of contacting *Streptococcus pneumoniae* pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae* .

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with *Streptococcus pneumoniae* pathogenic bacteria.

A preferred method is provided wherein said modulating metabolism is inhibiting growth of said bacteria or killing said bacteria.

A further preferred method is provided wherein said contacting said bacteria comprises the further step of introducing said composition into a mammal, particularly a human.

Further preferred methods are provided by the invention wherein said bacteria is *Streptococcus pneumoniae* .

Still further preferred methods comprise a gemifloxacin compound selected from the group consisting of gemifloxacin mesylate, gemifloxacin mesylate hydrate, gemifloxacin mesylate hemihydrate and gemifloxacin mesylate sesquihydrate.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows MIC_{50} and MIC_{90} values (μg/ml) for 5 species of *Acinetobacter.*

Figure 2 shows percentage of isolates with MICs above the breakpoint.

Figure 3 shows ranges of MICs (μg/ml).

Figures 4a-4g show bactericidal activity of gemifloxacin against *A. baumannii* ATCC 19606.

Figure 4 shows the bactericidal activity of trovafloxacin against *A. baumannii* ATCC 19606.

Figure 4c shows the bactericidal activity of moxifloxacin against *A. baumannii* ATCC 19606.

Figure 4d shows the bactericidal activity of levofloxacin against *A. baumannii* ATCC 19606.

Figure 4e shows the bactericidal activity of ciprofloxacin against *A. baumannii* ATCC 19606.

Figure 4f show the bactericidal activity of grepafloxacin against *A. baumannii* ATCC 19606.

Figure 4g shows the bactericidal activity of sparfloxacin against *A. baumannii* ATCC 19606.

Figure 5 shows a scattergram of MIC *vs* zone of inhibition for a 1 μg gemifloxacin disk content for all genera except *Pseudomonas species* using the BSAC standardized method of disk testing.

Figure 6 shows a scattergram of MIC *vs* zone of inhibition for a 5 μg gemifloxacin disk content to *Pseudomonas species* using the BSAC standardized method of disk testing

Figure 7 shows a graphical depiction of gemifloxacin time-kill activity against a penicillin-resistant pneumococcus.

Figure 8 shows the MIC distribution of four quinolones against *P. aeruginosa.*

Figure 9 shows the MIC distribution of four quinolones against *Acinetobacter* spp.

Figure 10 shows the efficacy of Gemifloxacin (SB 265805) and comparator agents against RTI in rats caused by *S. pneumoniae* 622286

Figure 11 shows the efficacy of Gemifloxacin and comparator agents against RTI in rats caused by *S. pneumoniae* 305313.

Figure 12 shows the efficacy of Gemifloxacin compared with that of Grepafloxacin (GRP), Levofloxacin and Trovafloxacin (TRV) in a RTI in Rats Caused by S. pneumoniae 305313.

Figure 13 shows the efficacy of Gemifloxacin and comparator agents against RTI in rats

caused by *H. influenzae* HI43.

Figure 14 shows the efficacy of Gemifloxacin and comparator agents against *S. pneumoniae* 1629.

Figure 15 shows the efficacy of Gemifloxacin and comparator agents against *S. pneumoniae* 1629.

Figure 16 shows the mean post antibiotic effects (PAEs) of ciprofloxacin, trovafloxacin, and gemifloxacin in evaluable strains of *S. pneumoniae*

Figure 17 shows the PAEs of ciprofloxacin, trovafloxacin, and gemifloxacin in susceptible strains of *S. pneumoniae*

Figure 18 graphically illustrates percent inhibition by ciprofloxacin.

Figure 19 graphically illustrates percent inhibition by ciprofloxacin.

Figure 20 shows the effect of gemifloxacin administration on the intestinal aerobic microflora of 10 volunteers.

Figure 21 shows the effect of gemifloxacin administration on the intestinal anaerobic microflora of 10 volunteers.

Figure 22 shows the selection of mutant S. *pneumoniae* strains with gemifloxacin.

Figure 23 shows the survival of *Escherichia coli* KL16 treated with gemifloxacin for 3 h at 37 °C.

Figure 24 shows the survival of *Staphylococcus aureus* E3T treated with gemifloxacin for 3 h at 37 °C.

Figure 25 shows the survival of *Streptococcus pneumoniae* C3LN4 treated with gemifloxacin for 3 h at 37 °C.

Figure 26 shows the dose response of *A. baumannii* ATCC 19606 to Gemifloxcain.

Figures 27 shows the bactericidal activity of gemifloxacin against A. baumannii ATCC 19606.

Figure 28 shows the bactericidal activity of trovafloxacin against A. baumannii ATCC 19606.

Figure 29 shows the bactericidal activity of moxifloxacin against *A. baumannii* ATCC 19606

Figure 30 shows the bactericidal activity of levofloxacin against *A. baumannii* ATCC 19606.

Figure 31 shows the bactericidal activity of ciprofloxacin against *A. baumannii* ATCC 19606.

Figure 32 shows the bactericidal activity of grepafloxacin against *A. baumannii* ATCC 19606.

Figure 33 shows the bactericidal activity of sparfloxacin against *A. baumannii* ATCC 19606.

Figure 34 shows the bactericidal activity of sparfloxacin against *A. baumanii* ATCC 19606.

DESCRIPTION OF THE INVENTION

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *A. baumannii, A. anitratus, A. lwoffii, A. calcoaceticus* or *Acinetobacter* spp.

As used herein "gemifloxacin compound(s)" means a compound having antibacterial activity described in patent application PCT/KR98/00051 published as WO 98/42705, or patent application EP 688772, and these applications are incorporated herein by reference.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Acinetobacter* pathogens. An objective of these analyses was to determine the MIC_{50} and MIC_{90} values of gemifloxacin compared with comparator quinolones, including ciprofloxacin, a carbapenem, a cephalosporin and a macrolide by MIC against five species of Acintobacter (i.e., *A. baumannii, A. calcoaceticus, A. lwoffii, A. anitratus* and *Acinetobacter* spp.).

Gemifloxacin is a fluoroquinolone with a spectrum including activity against both Gram positive and Gram negative aerobic bacteria, among other bacteria. In a study discribed herein, an agar dilution method has been used to determine the MICs of 100 clinical isolates of *Acinetobacter* species (47 *A. baumannii*, 18 *A. anitratus*, 18 *A. lwoffii*, 13 *A. calcoaceticus*, 4 *Acinetobacter* spp.) against 12 antimicrobial agents: gemifloxacin (GEM), sparfloxacin (SPA), grepafloxacin (GRE), ciprofloxacin (CIP), moxifloxacin (MOX), trovafloxacin (TRO), levofloxacin (LEV), ofloxacin (OFL), gatifloxacin (GAT), imipenem (IMI), cefuroxime (CEF) and azithromycin (AZI). The $MIC_{50/90}$ values (in μg/ml) are found in Table 1.

Data in Table 1 shows that gemifloxacin has good activity against the four species of *Acinetobacter* and is over eight-fold more potent than ciprofloxacin. The data in Table 1 also demonstrates that gemifloxacin is 2–8-fold more active than grepafloxacin, moxifloxacin, levofloxacin, ofloxacin and gatifloxacin, and within one dilution of the comparator quinolones, sparfloxacin and trovafloxacin. Cross-resistance was seen only within the quinolones and did not extend to the non-quinolone antimicrobials.

Organisms from the genus *Acinetobacter* are increasingly being found in the nosocomial environment where they are responsible for causing secondary infection in the immunocompromised, in particular nosocomial pneumonia. These organisms are innately resistant to much of the antimicrobial armoury available to the clinician, thus, giving them a selective advantage in areas of high antimicrobial usage such as intensive care units. Resistance to ciprofloxacin has been found in the *Acinetobacter* strains within the hospital environment and the search for newer, more potent quinolones continues. Gemifloxacin (SB-265805) is one such quinolone which has shown a good spectrum of activity against both Gram positive and Gram negative organisms. In this study, the *in vitro* activity of gemifloxacin has been compared with eight comparator quinolones including ciprofloxacin, a carbapenem, a cephalosporin and a macrolide by MIC against five species of *Acinetobacter* (*A. baumannii, A. calcoaceticus, A. lwoffii, A. anitratus* and *Acinetobacter* spp.).

In obtaining data for Table 1, all the bacterial strains were bloodstream isolates from the University of Iowa Century Collection, USA. MICs were performed on isosensitest agar following the BSAC guidelines for susceptibility testing (See The British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27(Suppl D)). Breakpoint values were taken from the BSAC guidelines and are as follows: cefuroxime 2 μg/ml; ciprofloxacin 4 μg/ml; imipenem 8 μg/ml; ofloxacin 4 μg/ml; levofloxacin 4 μg/ml; azithromycin 0.5 μg/ml. Where not published, the breakpoints were arbitrarily taken to be 4 μg/ml but may later have been found to be higher.

Results for the MIC determination are in Table 1 and are summarized in Figures 1, 2 and 3. Resistance to the quinolones was independent of resistance to the carbapenem,

Table 1. Activity of gemifloxacin against a variety of *Acinetobacter* species

Antimicrobial agent	MIC (μg/ml) and *Acinetobacter* species (number tested)					
	Acinetobacter baumannii (47)			*Acinetobacter anitratus* (18)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Gemifloxacin	0.015–>128	0.06	16	0.03–64	0.06	32
Ciprofloxacin	0.06–>128	0.5	>128	0.12–128	0.5	128
Levofloxacin	0.06–64	0.25	16	0.12–16	0.25	8
Gatifloxacin	0.03–64	0.25	8	0.03–32	0.12	16
Sparfloxacin	0.008–32	0.03	8	0.015–16	0.03	16
Grepafloxacin	0.015–64	0.06	32	0.015–64	0.06	32
Moxifloxacin	0.015–64	0.12	16	0.06–32	0.12	32
Trovafloxacin	0.015–32	0.03	16	0.015–16	0.03	8

Ofloxacin	0.012–128	0.5	32	0.25–16	0.5	16
Imipenem	0.008–1	0.12	0.25	0.008–2	0.12	0.25
Cefuroxime	8–128	64	128	32–256	64	256
Azithromycin	0.12–128	4	32	1–64	2	64
	Acinetobacter calcoaceticus (13)			*Acinetobacter lwoffii* (18)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Gemifloxacin	0.015–64	0.06	0.12	0.015–32	0.06	1
Ciprofloxacin	0.06–>128	0.5	1	0.015–>128	0.25	8
Levofloxacin	0.06–64	0.25	0.5	0.06–16	0.25	4
Gatifloxacin	0.03–128	0.12	0.25	0.03–8	0.12	4
Sparfloxacin	0.008–32	0.03	0.06	0.008–32	0.06	4
Grepafloxacin	0.015–32	0.06	0.12	0.015–64	0.06	2
Moxifloxacin	0.03–64	0.12	0.12	0.015–16	0.06	2
Trovafloxacin	0.008–32	0.03	0.06	0.008–8	0.03	0.5
Ofloxacin	0.12–>128	0.5	1	0.12–32	0.5	8
Imipenem	0.008–1	0.12	0.25	0.008–2	0.12	0.5
Cefuroxime	16–128	64	128	0.25–128	64	128
Azithromycin	0.5–64	2	4	0.12–32	1	32

	Acinetobacter spp. (4)					
	Range	MIC_{50}	MIC_{90}			
Gemifloxacin	0.008–0.03					
Ciprofloxacin	0.03–0.06					
Levofloxacin	0.008–0.12					
Gatifloxacin	0.03–0.06					
Sparfloxacin	0.008–0.008					
Grepafloxacin	0.015–0.03					
Moxifloxacin	0.008–0.06					
Trovafloxacin	0.008–0.015					

Ofloxacin	0.03–0.25					
Imipenem	0.008–0.12					
Cefuroxime	2–8.0					
Azithromycin	0.12–0.5					

cephalosporin or macrolide (Figure 2). Those organisms showing elevated MICs of ciprofloxacin were mirrored by an increase in MIC of all the other quinolones.

Previous studies have shown gemifloxacin to have good activity against Gram positive respiratory organisms such as *Streptococcus pneumoniae* and *Staphylococcus aureus* with MIC_{50} and MIC_{90} values 32–64-fold more active than ciprofloxacin. In this study it was shown that this high activity is retained against a genus of Gram negative organisms, *Acinetobacter*, with an MIC_{50} and MIC_{90} of gemifloxacin that is 8- and 16-fold less, respectively, than that of ciprofloxacin, and is comparable to trovafloxacin and sparfloxacin. At a species to species level, gemifloxacin is most potent against *A. calcoaceticus* and *A. lwoffii* with MIC_{90}s below the arbitrary breakpoint and 8-fold more potent than ciprofloxacin. The levels of quinolone resistance are similar for the compounds tested (Figure 2), being between 19 and 25%, thus an elevated MIC to one quinolone is mirrored with an increase for all. This does not necessarily mean, however, that treatment failure will occur, as gemifloxacin still demonstrates activity against all but the most ciprofloxacin-resistant isolates.

Gemifloxacin has activity against *Acinetobacter*, a genus of Gram negative organisms. It has considerably better activity than ciprofloxacin and comparable activity to trovafloxacin and sparfloxacin. Gemifloxacin is most potent against *A. calcoaceticus* and *A. lwoffii*. Although the levels of quinolone resistance are similar for the antimicrobial agents tested (19–25%), gemifloxacin demonstrates activity against all but the most ciprofloxacin-resistant isolates.

A further objective of these analyses was to compare the in vitro killing activity of gemifloxacin on *A baumannii* ATCC 19606 against comparitor quinolones TRO, MOX, LEV, CIP, GREand SPA at their respective optimum bactericidal concentration (OBCs) and at four times their MIC.

In experimints, the bactericidal activity of gemifloxacin has been compared to that of trovafloxacin, moxifloxacin, levofloxacin, ciprofloxacin, grepafloxacin and sparfloxacin in a time-kill study at the concentrations of four times the MIC (0.5, 0.25, 1, 2, 2, 0.5, 0.5 μg/ml respectively) and at their optimum bactericidal concentration (OBC) (4, 4, 4, 4, 4, 8, 8 μg/ml, respectively) against the standard *Acinetobacter baumannii* ATCC 19606. At their OBCs there is no significant difference in bactericidal activity between gemifloxacin and the other quinolones, with regrowth

prevented for 24 hours and a reduction in viable cells of over 5 $\log_{10}$. However, at four times the MIC, a concentration that is achievable *in vivo*, gemifloxacin shows an advantage over the other quinolones, reducing the viable count by almost 2 $\log_{10}$ after only 30 minutes compared with a 1 $\log_{10}$ reduction seen with the other drugs, and reducing the viable count by over 4 $\log_{10}$ after 24 hours. This enhanced killing is markedly better than that seen with the Gram negative quinolones, ciprofloxacin and levofloxacin, and also trovafloxacin and sparfloxacin. This data shows that gemifloxacin has excellent bactericidal activity against *A. baumannii* at a concentration eight-fold less than its OBC.

Acinetobacter baumannii is an important opportunistic pathogen that is frequently found in the nosocomial environment where it is responsible for bacteremias, secondary meningitis, urinary tract infections and pneumonias in the immunocompromised. With these patients, a bactericidal drug is usually preferable to a bacteriostatic one. Quinolones are bactericidal but exhibit a bi-phasic dose response, whereby the lethality of a drug increases with its concentration until its optimum bactericidal concentration (OBC) is reached, after which the bactericidal activity decreases with concentration. This study compares the *in vitro* killing activity of gemifloxacin (SB-265805) on *A. baumannii* ATCC 19606 against the comparator quinolones trovafloxacin, moxifloxacin, levofloxacin, ciprofloxacin, grepafloxacin and sparfloxacin at their respective OBCs and at four times their MIC.

The standard *A. baumannii* ATCC 19606 laboratory strain is used throughout the study. Prior to time-kill experiments, the MICs of seven quinolones for *A. baumannii* ATCC 19606 is determined using the agar dilution method following the BSAC guidelines for susceptibility testing. (See The British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27(Suppl D)). To determine the OBC, nutrient broth is inoculated with *A. baumannii* ATCC 19606 and incubated overnight. Doubling dilutions of antimicrobial agent ranging from 0.03–256 μg/ml in nutrient broth are inoculated with the overnight culture and incubated for a further 3 hours. The resulting cultures are serial diluted and plated onto nutrient agar, incubated overnight and the colonies are counted (See Lewin CS, Howard BMA, Ratcliffe NT, Smith JT. 4-Quinolones and the SOS response. *J Med Microbiol* 1989; 29: 139–144).

Log-phase cultures are challenged with a fixed concentration of antimicrobial that corresponded with its OBC or four times MIC. An overnight culture is used to inoculate nutrient broth and incubated for 3 hours. An initial count of microrganisms present (t = 0) is determined by serial dilution and plating onto nutrient agar. The antimicrobial agent was then added and samples are taken every 30 minutes for 3 hours, serially diluted and plated for viable count. A final sample is

taken after 24 hours, diluted and plated for viable counting.

Gemifloxacin demonstrates good bactericidal activity when compared with ciprofloxacin: at four times the MIC, the killing kinetics are almost equal to that of its OBC even though it is one-eighth the concentration. At both the OBC and at four times the MIC, gemifloxacin killed over 99% of microrganisms in 24 hours. Gemifloxacin at four times the MIC killed >2 $\log_{10}$ compared to ciprofloxacin, sparfloxacin and trovafloxacin. This data suggests that gemifloxacin has the potential to treat *Acinetobacter* infections even at low concentrations or multiples of MIC.

Table 2. MIC and OBC values for the antimicrobial agents used against *A. baumannii* ATCC 19606

Antimicrobial agent	OBC (μg/ml)	MIC (μg/ml)	4 x MIC (μg/ml)
Gemifloxacin	4	0.125	0.5
Trovafloxacin	4	0.06	0.25
Moxifloxacin	4	0.25	1
Levofloxacin	4	0.5	2
Ciprofloxacin	4	0.5	2
Grepafloxacin	8	0.125	0.5
Sparfloxacin	8	0.125	0.5

All analyses were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. All parts or amounts set out in the following discussion are by weight, unless otherwise specified.

The invention provides a method for modulating metabolism of *Acinetobacter* pathogenic bacteria. Skilled artisans can readily choose *Acinetobacter* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Provided by the invention is a method of treating or preventing a bacterial infection by *Acinetobacter* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Acinetobacter* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Acinetobacter* pathogenic bacteria is selected from the group consisting of: *A. baumannii, A. anitratus, A. lwoffii,*

A. calcoaceticus or Acinetobacter spp. Other *Acinetobacter* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention also provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Prevotella denticola/loeschii group*, *Veillonella* spp., *Prevotella heparinolytica*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Porphyromonas* spp. (*Porphyromonas cangingivalis*; *Porphyromonas cansulci*; *Porphyromonas circumdentaria*; and *Porphyromonas levii*), *Prevotella bivia*, *Prevotella buccae-oris* group, *Porphyromonas canoris*, *Porphyromonas gingivalis*, *Porphyromonas macaccae*, *Peptostreptococcus micros*, *Peptostreptococcus prevotii*, *Porphyromonas asaccharolyticus*, *Fusobacterium varium*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus magnus*, *Fusobacterium* spp group 1 (*Fusobacterium gonidiaformans*, *Fusobacterium naviforme*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum, and Fusobacterium nucleatum* ss *animalis*), *Fusobacterium* spp. group 2 (*Fusobacterium mortiferum, Fusobacterium necrogenes,* and *Fusobacterium ulcerans*), *Fusobacterium russii, Clostridium difficile*, *Clostridium inocuum*, *Clostridium ramosum, Bacteroides ureolyticus*, *Bilophila wadsworthia*, *Clostridium clostridioforme*, *Anaerobiospirillum succiniciproducens*, *Bacteroides gracilis*, *Bacteroides tectum, Actinomyces odontolyticus*, *Actinomyces israelii*, or *Anaerobiospirillum thomasii*..

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various anaerobic pathogens. An objective of these analyses was to determine the activities of gemifloxacin and comparator agents by an agar dilution method against 419 clinical strains of less commonly identified species of anaerobes. Gemifloxacin is generally more active than trovafloxacin against Gram positive strains by one to two dilutions. Peptostreptococci, such as *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus magnus*, *Peptostreptococcus micros* and *Peptostreptococcus prevotii* and *Porphyromonas* spp., such as, *Porphyromonas asaccharolytica*, *Porphyromonas canoris*, *Porphyromonas gingivalis*, *Porphyromonas macaccae* are all susceptible to ≤0.25 μg/ml of gemifloxacin. *Actinomyces israelii*, *Actinomyces odontolyticus*, *Clostridium innocuum*, *Clostridium clostridioforme*, *Anaerobiospirillum* spp., *Bacteroides tectum, Bacteroides ureolyticus*, *Bacteroides gracilis* (now *Campylobacter gracilis*), *Prevotella intermedia, Prevotella heparinolytica*, and *Prevotella oris–buccae* group have MIC_{90}s of ≤2 μg/ml. *Fusobacterium naviforme and Fusobacterium necrophorum* are also susceptible to ≤2 μg/ml, while *Fusobacterium varium* strains exhibit a bimodal pattern; the other *Fusobacterium* species, such as *Fusobacterium ulcerans,*

Fusobacterium russii, as well as *Veillonella* spp., *Prevotella melaninogenica* group, *Prevotella bivia*, *Clostridium difficile*, and *Bilophila wadsworthia* are relatively resistant to gemifloxacin [MIC_{90}s ≥4 ug/ml].

While pre–market *in vitro* testing of new antimicrobial compounds is often extensive, these studies tend to focus on typical anaerobic bacterial pathogens such as the *Bacteroides fragilis* group and *Clostridium perfringens*. Little or no data is available about the activities of these new compounds against many of the less frequently encountered anaerobic pathogens. As anaerobic susceptibility testing is not routinely performed in most clinical laboratories, the clinician must rely on published studies to help guide both empiric therapy as well as specific therapy in situations that involve less commonly isolated or identified anaerobes or mixed infections at other sites.

The activity of gemifloxacin was determined against the large variety of less usual anaerobic species that are encountered in human clinical infections and compared its activity with that of other commonly used oral agents.

The strains are previously isolated from human clinical specimens from a variety of sources, and are identified by standard criteria. Almost all of these isolates are different from those strains used in a prior study when the same genus and species was used. *Bacteroides fragilis* ATCC 25285, and *Bacteroides thetaiotaomicron* ATCC 29741 are tested simultaneously as control strains. The numbers and species of isolates tested are given in Table 3.

Standard laboratory powders are supplied as follows: gemifloxacin and amoxicillin clavulanate, SmithKline Beecham Pharmaceuticals, Philadelphia, PA; trovafloxacin and azithromycin, Pfizer Inc., New York, NY; clarithromycin, Abbott Laboratories, Abbott Park, IL.; clindamycin, Pharmacia Upjohn Co., Kalamazoo, Mich.; metronidazole, Searle Research & Development, Skokie, IL.; erythromycin, Eli Lilly & Co., Indianapolis, IN; and penicillin G, Sigma Chemical Co., St. Louis, MO, USA. Frozen cultures are transferred at least twice on *Brucella* agar supplemented with hemin, vitamin K_1, and 5% sheep blood to ensure purity and good growth. Susceptibility testing was performed according to NCCLS standards. *Brucella* agar supplemented with hemin, vitamin K_1, and 5% laked sheep blood was the basal medium used. For *Bilophila wadsworthia*, the agar is also supplemented with pyruvate. Antimicrobial agents are reconstituted according to the manufacturers' instructions. Serial twofold dilutions of antimicrobial agents are prepared on the day of the test and added to the media in varying concentrations (μg/ml).

The agar plates are inoculated with a Steers replicator (Craft Machine Inc., Chester, PA,

USA). The inoculum used was 10^5 CFU/spot. Control plates without antimicrobial agents are inoculated before and after each set of drug-containing plates. The MIC was defined as the lowest concentration of an agent that yielded no growth, or a marked change in the appearance of growth as compared to the growth control plate.

The comparative activity of gemifloxacin and other antimicrobial agents is shown in Table 3. Overall, results show that gemifloxacin is active against the Gram positive anaerobes tested as well as the other unusual isolates studied.

Gemifloxacin exhibits activity against Gram positive anaerobes, especially the four *Peptostreptococcus* species and the *Porphyromonas* species. Gemifloxacin is also active against more unusual isolates.

The study, which included 45 strains of peptostreptococci from 4 species, shows that all of these were susceptible to ≤0.25 μg/ml of gemifloxacin. By contrast, a study of 10 strains of peptostreptococci found an MIC_{90} of 2 μg/ml for gemifloxacin. The reason for this discrepancy cannot be accounted for by methodologic variations since both studies used *Brucella* agar and an agar dilution method. Marco *et al* studied 18 strains of peptostreptococci and also found a MIC_{90} of 2 μg/ml (range, ≤0.25–8 μg/ml) for gemifloxacin (See Marco F, Barrett MS, Jones RN. Antimicrobial activity of LB 20304, a fluoronapthyridone, tested against anaerobic bacteria. *J Antimicrob Chemother* 1997; 40: 605–607).

Differences in the susceptibility of various *Clostridium* species to gemifloxacin are apparent in this study, with *Clostrdium clostridioforme* and *Clostridium innocuum* being relatively susceptible while *Clostridium difficile* is often resistant to gemifloxacin. In the current study, the ten *Clostridium ramosum* isolates studied have a MIC_{90} of 1 μg/ml while in another study the MIC_{90} for the 14 isolates studied was 8 μg/ml. With the exception of two strains, all isolates in the two studies are different and most came from blood cultures. The apparent disparity comes from the higher MICs of 3/14 strains in the prior study and highlights the problem of testing small numbers of isolates of a single species.

Table 3. *In vitro* activity of gemifloxacin, trovafloxacin and other oral antimicrobial agents against unusual anaerobic pathogens

	MICs (μg/ml)[a] and isolates tested								
Antimicrobial agent	*Actinomyces odontolyticus* [10]			*Actinomyces israelii* [6]			*Anaerobiospirillum thomasii* [13]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	1–2	2	2	0.5–2	1	–	0.06–0.25	0.125	0.125
Trovafloxacin	2–4	4	4	0.5–2	1	–	0.06–0.5	0.125	0.25
Penicillin G	0.125–0.25	0.125	0.125	≤0.015–0.25	0.03	–	0.06–0.125	0.06	0.125
Amoxicillin clavulanate	0.06–0.125	0.125	0.25	0.03–1	0.03	–	0.125–0.25	0.125	0.25
Clindamycin	≤0.015–0.5	0.125	0.25	0.06–0.25	0.06	–	8–>32	32	>32
Erythromycin	≤0.015–0.03	≤0.015	0.03	0.03	0.03	–	1–16	4	8
Azithromycin	≤0.015–0.06	0.03	0.06	0.06	0.06	–	0.125–1	0.5	1
Clarithromycin	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	–	2–32	4	16
Metronidazole	4–>32	16	32	1–32	4	–	1–4	2	4

	MICs (μg/ml)[a] and isolates tested								
Antimicrobial agent	*Anaerobiospirillum succiniciproducens* [3]			*Bacteroides gracilis* [11]			*Bacteroides tectum* [22]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.5–2	1	–	≤0.015–1	≤0.015	1	0.06–8	0.125	0.25
Trovafloxacin	0.5–2	1	–	≤0.015–2	0.03	0.5	0.03–0.125	0.06	0.125
Penicillin G	0.5–1	0.5	–	≤0.015–4	0.125	4	≤0.015–32	0.03	16
Amoxicillin clavulanate	0.25–0.5	0.25	–	0≤.015–2	0.5	2	0.03–0.5	0.06	0.5
Clindamycin	32	32	–	0.03–8	0.25	2	≤0.015—0.125	≤0.015	≤0.015
Erythromycin	8–16	16	–	0.125–2	1	2	0.25–1	0.5	0.5
Azithromycin	0.5–1	0.5	–	0.06–0.5	0.125	0.5	0.5–2	1	2
Clarithromycin	8–32	32	–	0.25–2	1	1	0.125	0.125	0.125
Antimicrobial agent	*Anaerobiospirillum succiniciproducens* [3]			*Bacteroides gracilis* [11]			*Bacteroides tectum* [22]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Metronidazole	4–8	8	–	0.06–>32	0.5	>32	0.125–2	0.5	0.5
Antimicrobial agent	*Bacteroides ureolyticus* [17]			*Bilophila wadsworthia* [16]			*Clostridium clostridioforme* [11]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	≤0.015–2	≤0.015	2	0.125–>8	0.25	4	0.5–>8	0.5	1

	MICs (μg/ml)[a] and isolates tested								
Trovafloxacin	≤0.015–4	0.06	4	0.125–>8	0.5	>8	1–8	4	4
Penicillin G	≤0.015–1	≤0.015	0.25	2–16	4	8	0.5–>32	1	16
Amoxicillin clavulanate	≤0.015–1	≤0.015	0.125	1–4	2	4	0.5–8	0.5	1
Clindamycin	0.03–0.5	0.06	0.25	0.25–2	0.5	2	≤0.015–2	0.06	2
Erythromycin	0.125–2	0.25	2	4–32	16	32	0.25–>32	16	>32
Azithromycin	0.06–0.25	0.06	0.25	1–16	4	16	0.125–>32	16	>32
Clarithromycin	0.125–4	0.5	2	4–32	16	32	0.125–>32	4	>32
Metronidazole	0.06–2	0.25	1	0.125	0.125	0.125	0.03–1	0.125	0.5
Antimicrobial agent	*Clostridium difficile* [14]			*Clostridium inocuum* [11]			*Clostridium ramosum* [10]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	1–>8	2	>8	0.125–>8	0.25	2	0.125–2	0.25	1
Trovafloxacin	0.5–>8	1	>8	0.25–>8	0.5	8	0.25–8	0.5	2
Penicillin G	1–4	2	4	0.25–>32	0.5	0.5	0.06–1	0.06	1
Amoxicillin clavulanate	0.5–1	1	1	0.5–2	0.5	0.5	0.06–0.25	0.06	0.25
Clindamycin	0.25–>32	0.5	>32	0.25–>32	0.5	>32	0.25–4	2	2
Erythromycin	0.25–>32	0.5	>32	0.5–>32	>32	>32	0.5–>32	1	>32
Azithromycin	1–>32	2	>32	0.125–>32	>32	>32	0.125–>32	0.25	>32
Antimicrobial agent	*Clostridium difficile* [14]			*Clostridium inocuum* [11]			*Clostridium ramosum* [10]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%

	MICs (μg/ml)[a] and isolates tested								
Clarithromycin	0.125–>32	0.5	>32	0.25–>32	>32	>32	0.25–>32	0.5	>32
Metronidazole	0.25–1	0.5	0.5	0.5–2	0.5	1	1	1	1
Antimicrobial agent	*Fusobacterium* spp group 1 [19][b]			*Fusobacterium* spp. group 2 [12][C]			*Fusobacterium russii* [12]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.06–8	0.25	8	0.125–>8	4	4	0.5–>8	>8	>8
Trovafloxacin	0.25–4	0.5	4	1–>8	4	4	0.5–4	4	4
Penicillin G	≤0.015–16	≤0.015	2	≤0.015–>32	0.25	0.5	≤0.015–0.06	0.03	0.06
Amoxicillin clavulanate	≤0.015–0.25	0.06	0.125	0.125–>4	1	2	≤0.015–0.25	0.06	0.125
Clindamycin	≤0.015–2	0.06	0.125	0.06–8	1	8	≤0.015—0.125	0.03	0.06
Erythromycin	1–>32	8	32	8–>32	>32	>32	1–>32	4	>32
Azithromycin	0.06–32	1	8	1–>32	16	32	0.03–32	0.25	32
Clarithromycin	≤0.015–32	8	32	4–>32	>32	>32	2–>32	4	>32
Metronidazole	0.125–0.5	0.25	4	0.125–1	0.5	1	≤0.015–0.25	0.125	0.25

	MICs (μg/ml)[a] and isolates tested								
Antimicrobial agent	*Fusobacterium varium* [17]			*Peptostreptococcus asaccharolyticus* [11]			*Peptostreptococcus magnus* [13]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.25–>8	>8	>8	0.125–0.25	0.25	0.25	0.03–0.06	0.03	0.06
Trovafloxacin	0.5–>8	4	>8	0.5–2	1	1	0.06–0.25	0.125	0.25
Penicillin G	0.03–>32	0.5	8	≤0.015–1	0.03	0.25	≤0.015–1	0.03	0.25
Amoxicillin clavulanate	0.125–4	2	4	0.03–1	0.03	0.125	0.03–1	0.03	0.125
Clindamycin	0.06–16	4	16	≤0.015–>32	0.06	>32	0.06–2	0.5	2
Erythromycin	32–>32	>32	>32	1–>32	4	>32	1–>32	4	>32
Azithromycin	2–>32	32	>32	0.5–>32	4	>32	2–>32	4	>32
Antimicrobial agent	*Fusobacterium varium* [17]			*Peptostreptococcus asaccharolyticus* [11]			*Peptostreptococcus magnus* [13]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Clarithromycin	32–>32	>32	>32	0.5–>32	2	>32	0.5–>32	2	>32
Metronidazole	0.125–4	1	2	0.125–2	0.5	1	0.25–2	0.5	0.5
Antimicrobial agent	*Peptostreptococcus micros* [12]			*Peptostreptococcus prevotii* [9]			*Porphyromonas asaccharolyticus* [11]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.06–0.125	0.06	0.06	0.06–0.25	0.125	–	0.06–0.125	0.06	0.125

	MICs (μg/ml)[a] and isolates tested								
Trovafloxacin	0.03–0.125	0.06	0.06	0.25–1	0.25	–	0.03–0.25	0.25	0.25
Penicillin G	≤0.015–0.03	≤0.015	0.03	0.03–0.06	0.03	–	≤0.015	≤0.015	≤0.015
Amoxicillin clavulanate	0.03–0.125	0.03	0.125	≤0.015–0.125	0.03	–	≤0.015–0.03	≤0.015	0.03
Clindamycin	0.06–0.125	0.125	0.125	0.030–32	1	–	≤0.015–>32	≤0.015	>32
Erythromycin	0.5–1	0.5	0.5	0.03–>32	>32	–	0.03–32	0.03	32
Azithromycin	0.5–1	0.5	1	0.06–>32	32	–	0.125–>32	0.25	>32
Clarithromycin	0.06	0.5	0.5	≤0.015–>32	>32	–	≤0.015–>32	0.06	>32
Metronidazole	0.03–0.25	0.25	0.25	0.125–1	0.5	–	≤0.015	≤0.015	≤0.015
Antimicrobial agent	*Porphyromonas canoris* [10]			*Porphyromonas gingivalis* [13]			*Porphyromonas macaccae* [13]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.06–0.25	0.25	0.25	≤0.015–0.125	0.06	0.125	0.03–0.125	0.06	0.125
Trovafloxacin	0.06–0.5	0.25	0.5	0.03–0.06	0.06	0.06	0.03–0.125	0.06	0.125

	MICs (μg/ml)[a] and isolates tested								
Penicillin G	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.06	≤0.015	0.03	≤0.015–1	0.5	0.5
Amoxicillin clavulanate	≤0.015–0.03	≤0.015	0.03	≤0.015–0.06	≤0.015	0.06	≤0.015–0.06	≤0.015	≤0.015
Clindamycin	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015–0.03	≤0.015	≤0.015
Erythromycin	0.03–0.25	0.06	0.125	0.06–0.5	0.125	0.5	0.06–0.25	0.125	0.25
Antimicrobial agent	*Porphyromonas canoris* [10]			*Porphyromonas gingivalis* [13]			*Porphyromonas macaccae* [13]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Azithromycin	0.125–0.5	0.25	0.25	0.125–1	0.25	0.5	0.125–1	0.5	0.5
Clarithromycin	0.06–0.125	0.06	0.125	0.06–0.125	0.06	0.125	0.06–0.125	0.125	0.125
Metronidazole	≤0.015–0.5	0.25	0.25	≤0.015–0.03	≤0.015	0.03	≤0.015–0.125	0.06	0.125
Antimicrobial agent	*Porphyromonas* spp. [11][d]			*Prevotella bivia* [21]			*Prevotella buccae-oris* group [22][e]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.06–0.125	0.06	0.125	4–>8	8	8	0.5–8	2	2
Trovafloxacin	0.06–1	0.25	1	1–4	2	2	0.25–4	1	2
Penicillin G	≤0.015–4	≤0.015	≤0.015	0.25–32	16	32	0.06–>32	8	>32

	MICs (μg/ml)[a] and isolates tested								
Amoxicillin clavulanate	≤0.015–0.06	≤0.015	≤0.015	0.06–4	0.5	4	0.125–2	0.25	1
Clindamycin	≤0.015	≤0.015	≤0.015	≤0.015–>32	≤0.015	0.03	≤0.015–0.125	≤0.015	0.03
Erythromycin	≤0.015–0.5	0.06	0.06	0.06–>32	1	2	0.5–8	1	2
Azithromycin	0.125–1	0.25	0.5	0.25–>32	0.5	1	0.125–4	0.5	1
Clarithromycin	0.06–0.125	0.06	0.125	0.06–>32	0.125	0.25	0.06–1	0.125	0.25
Metronidazole	≤0.015–0.25	0.03	0.125	0.5–4	2	4	0.5–4	1	2
Antimicrobial agent	*Prevotella heparinolytica* [16]			*Prevotella intermedia* [11]			*Prevotella melaninogenica* [12]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.25–0.5	0.5	0.5	0.06–1	0.25	0.5	0.125–>8	1	8
Trovafloxacin	0.125–0.25	0.125	0.25	0.06–1	0.5	1	0.06–8	1	4
Penicillin G	0.06–0.25	0.06	0.125	≤0.015–16	0.03	4	≤0.015–2	0.25	2
Amoxicillin clavulanate	0.06–0.25	0.125	0.25	0.03–0.5	0.03	0.125	0.03–16	2	4

	MICs (μg/ml)[a] and isolates tested								
Clindamycin	≤0.015	≤0.015	≤0.015	≤0.015—0.03	≤0.015	≤0.015	≤0.015–32	≤0.015	0.5
Antimicrobial agent	*Prevotella heparinolytica* [16]			*Prevotella intermedia* [11]			*Prevotella melaninogenica* [12]		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Erythromycin	0.25–0.5	0.25	0.25	0.03–0.5	0.06	0.25	0.06–32	1	8
Azithromycin	0.5–1	0.5	1	0.03–1	0.125	0.5	0.125–>32	0.25	32
Clarithromycin	0.06–0.125	0.125	0.125	≤0.015–0.125	≤0.015	0.125	0.06–4	0.125	4
Metronidazole	0.06–1	0.5	1	0.03–1	0.5	1	0.125–4	0.5	1
Antimicrobial agent	*Prevotella denticola/loeschii group* [6]			*Veillonella* spp. [24]					
	Range	50%	90%	Range	50%	90%			
Gemifloxacin	0.25–8	0.5	–	0.03–>8	1	8			
Trovafloxacin	0.06–4	1	–	0.125–>8	0.25	>8			
Penicillin G	≤0.015–32	4	–	≤0.015–8	1	4			
Amoxicillin clavulanate	0.03–0.5	0.06	–	≤0.015–>4	0.5	2			
Clindamycin	≤0.015–0.25	≤0.015	–	0.03–>32	0.06	2			
Erythromycin	0.125–16	0.25	–	1–>32	16	>32			

	MICs (μg/ml)[a] and isolates tested								
Azithromycin	0.06–16	0.5	–	0.125–>32	4	>32			
Clarithromycin	0.03–2	0.06	–	1–>32	16	>32			
Metronidazole	0.5–1	1	–	0.25–2	1	2			

[a]MIC_{50}, MIC_{90} : Minimal inhibitory concentration for 50% and 90% of isolates tested, respectively.

[b]*Fusobacterium gonidiaformans* (1); *Fusobacterium naviforme* (8); *Fusobacterium necrophorum* (8); *Fusobacterium nucleatum* (1); *Fusobacterium nucleatum* ss *animalis* (1).

[c]*Fusobacterium mortiferum* (2); *Fusobacterium necrogenes* (3); *Fusobacterium ulcerans* (7).

[d]*Porphyromonas cangingivalis* (4); *Porphyromonas cansulci* (2); *Porphyromonas circumdentaria* (2); *Porphyromonas levii* (3).

[e]*Prevotella buccae* (20); *Prevotella oris* (2).

Cormicon and Jones studied ten clostridial isolates and found a maximum MIC of 2 μg/ml to gemifloxacin (Cormicon MG, Jones RN. Antimicrobial activity and spectrum of LB 2030, a novel fluoronaphthyridone. *Antimicrob Agents Chemother* 1997; 41: 204–211). Marco *et al.* reported all 19 unspecieted clostridial isolates they studied to be susceptible to ≤2 μg/ml (See Marco F, Barrett MS, Jones RN. Antimicrobial activity of LB 20304, a fluoronapthyridone, tested against anaerobic bacteria).

There is marked variation in susceptibility patterns of different anaerobic genera and species to trovafloxacin and gemifloxacin. Important clinical anaerobic isolates should have individual strain susceptibilities determined. It is difficult to predict susceptibility based on a grouping of several species in a less commonly encountered or identified genus.

In view of these findings, also provided by the invention is a method of treating or preventing a bacterial infection by anaerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with anaerobic pathogenic bacteria.

While a preferred object of the invention provides a method wherein said anaerobic pathogenic bacteria is selected from the group consisting of: *Prevotella denticola/loeschii group*, *Veillonella* spp., *Prevotella heparinolytica*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Porphyromonas* spp. (*Porphyromonas cangingivalis*; *Porphyromonas cansulci*; *Porphyromonas circumdentaria*; and *Porphyromonas levii*), *Prevotella bivia*, *Prevotella buccae-oris* group, *Porphyromonas canoris*, *Porphyromonas gingivalis*, *Porphyromonas macaccae*, *Peptostreptococcus micros*, *Peptostreptococcus prevotii*, *Porphyromonas asaccharolyticus*, *Fusobacterium varium*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus magnus*, *Fusobacterium* spp group 1 (*Fusobacterium gonidiaformans*, *Fusobacterium naviforme*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum*, *and Fusobacterium nucleatum* ss *animalis*), *Fusobacterium* spp. group 2 (*Fusobacterium mortiferum*, *Fusobacterium necrogenes*, and *Fusobacterium ulcerans*), *Fusobacterium russii*, *Clostridium difficile*, *Clostridium inocuum*, *Clostridium ramosum*, *Bacteroides ureolyticus*, *Bilophila wadsworthia*, *Clostridium clostridioforme*, *Anaerobiospirillum succiniciproducens*, *Bacteroides gracilis*, *Bacteroides tectum*, *Actinomyces odontolyticus*, *Actinomyces israelii*, or *Anaerobiospirillum thomasii*.

Other anaerobic pathogenic bacteria may also be included in the methods of the invention. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention further provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Serratia* spp., *Providencia stuartii*, *Salmonella* spp., *Stenotrophomonas maltophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus* MSSA, *Staphylococcus aureus* MRSA, *Staphylococcus epidermidis* MSSE, *Staphylococcus epidermidis* MRSE, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Streptococcus lancefield* Gp B, *Streptococcus millerii*, *Streptococcus pneumoniae*, Quinolone-resistant *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Clostridium perfringens*, *Clostridium difficile*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevoti*, or *Bacteroides fragilis*.

This aspect of the instant invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various pathogens. An objective of these analyses was to determine the *in vitro* activity of gemifloxacin (GFX) (SB-265805) to that of ciprofloxacin (CIP), trovafloxacin (TRX) and levofloxacin (LEV) against 782 recent clinical isolates. GFX inhibit 90% of Enterobacteriaceae at ≤0.5 μg/ml (except *Serratia* spp., MIC_{90}1 μg/ml) and display similar activity to CIP, TRX and LEV. *Pseudomonas aeruginosa* is moderately susceptible to GFX (MIC_{90} 4 μg/ml).

Against *Streptococcus pneumoniae* GFX (MIC_{90} 0.06 μg/ml) is 2–4-fold more active than TRX and 16–32-fold more active than CIP. GFX is the most active agent against quinolone-resistant *S. pneumoniae* tested; of 16 strains resistant to CIP (MIC 4–8 μg/ml) all are susceptible to ≤0.12 μg/ml of GFX. Four strains susceptible to ≥128 μg/ml CIP are susceptible to 0.5–2 μg/ml GFX. MSSA are equally susceptible to GFX and TRX (MIC_{90} 0.06 μg/ml) but MRSA are less susceptible (GFX MIC_{90} 8 μg/ml). *Haemophilus influenzae*, *Moraxella catarrhalis* are susceptible to GFX (MIC_{90} ≤0.06 μg/ml). *Enterococcus* spp. are more susceptible to GFX than to other agents. GFX and TRX have high activity against *Bacteroides fragilis* (MIC_{90} 0.5 μg/ml) and peptostreptococci (MIC_{90} 0.25 μg/ml). A tentative breakpoint of 0.5 μg/ml was suggested following regression analysis of disk zone size plotted against MIC. A 1 μg disk is suggested and a zone diameter of >20 mm indicating susceptibility. The false sensitivity and resistance rates are 0% and 5.6% respectively.

Gemifloxacin has been reported as having enhanced activity against Gram positive cocci (See Oh JI, Paek K-S, Ahn M-J *et al. In vitro* and *in vivo* evaluation of LB20304, a

new fluoronaphthyridinone. *Antimicrob Agents Chemother* 1996; 40: 1564–1568; Comican MG, Jones RN. Antimicrobial activity and spectrum of LB20304, a novel fluoronaphthyridone. *Antimicrob Agents Chemother* 1997; 41: 204–211). In this study, the activity of gemifloxacin with commonly used antimicrobials against 782 recently isolated bacteria was compared. The techniques of the British Society for Antimicrobial Chemotherapy (BSAC) Working Party on Susceptibility Testing have been employed to establish a tentative *in vitro* breakpoint (*See*, Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27 (Suppl D)).

As described herein, Gemifloxacin has been compared to ciprofloxacin, trovafloxacin, levofloxacin, nalidixic acid and amoxicillin/clavulanic acid. Susceptibilities were performed in accordance with the techniques of the BSAC Working Party (See Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27 (Suppl D)). The final inoculum was approximately 10^4 CFU. The media was Unipath Iso-Sensitest agar (supplemented as required) or Wilkin-Chalgren agar. Incubation was performed in an appropriate atmosphere at 35°–37°C.

The MICs (as described above) and zone diameters of 1, 2 and 5 μg gemifloxacin disks were measured, growth was semi-confluent, scattergrams of zone size against MIC were performed, the BSAC formula gave a tentative breakpoint of 0.5–1 μg/ml, and false sensitive and resistance rates were measured (*See*, Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27 (Suppl D)).

The results of susceptibility testing are shown in Table 4. The activities of gemifloxacin, ciprofloxacin, trovafloxacin and levofloxacin against the Enterobacteriaceae are similar, the MICs being ± one doubling dilution of each other. Strains less susceptible to ciprofloxacin are less susceptible to the other fluoroquinolones All the fluoroquinolones display lesser activity against *Pseudomonas aeruginosa* including gemifloxacin (MIC_{90} 4 μg/ml). Against MSSA, gemifloxacin is 32-fold more active than ciprofloxacin. Similar differences are seen with *Staphylococcus epidermidis* and the enterococci. Against *Streptococcus pneumoniae* gemifloxacin is 2–4-fold more active than trovafloxacin and 16–32-fold more active than ciprofloxacin. A total of 10 strains of *S. pneumoniae* are resistant to ciprofloxacin (MIC 4–8 μg/ml); these are susceptible to 0.06–0.12 μg/ml of gemifloxacin. A total of 4 strains are highly ciprofloxacin resistant (MIC >128 μg/ml), these are susceptible to 0.5–2 μg/ml of gemifloxacin.

Table 4. *In vitro* activity (μg/ml) of gemifloxacin and other agents against recent clinical isolates

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
	E. coli (80)			*Klebsiella* spp. (48)			*Enterobacter* spp. (10)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.015	1	0.002–>128	0.05	0.25	0.008–64	0.03	0.5	0.015–4
Ciprofloxacin	0.015	1	0.002–>128	0.03	0.5	0.008–32	0.03	2	0.008–32
Levofloxacin	0.03	1	0.015–64	0.06	1	0.015–64	0.06	2	0.03–8
Trovafloxacin	0.03	2	0.004–>128	0.06	1	0.015–64	0.06	0.5	0.03–2
Nalidixic acid	4	>128	0.5–>128	8	>128	2–>128	8	>128	4–>128
Cefixime	0.12	1	0.008–32	0.03	0.12	0.008–128	1	>128	0.015–>128
Co–amoxiclav	4	16	0.5–32	2	16	1–32	32	>128	2–>128
	Proteus mirabilis (50)			*Proteus vulgaris* (20)			*Morganella morganii* (21)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Gemifloxacin	0.12	0.12	0.03–8	0.06	0.12	0.03–0.12	0.06	0.06	0.03–0.12
Ciprofloxacin	0.03	0.06	0.008–2	0.015	0.03	0.008–0.06	0.015	0.015	0.008–0.06
Levofloxacin	0.06	0.12	0.03–2	0.03	0.06	0.015–0.12	0.03	0.06	0.015–0.12
Trovafloxacin	0.25	0.25	0.06–16	0.12	0.25	0.03–0.25	0.25	0.25	0.03–0.5
Nalidixic acid	8	8	4–>128	4	4	2–8	4	4	2–8
Cefixime	0.004	0.008	0.002–0.008	0.008	0.008	0.002–0.015	0.06	2	0.03–16
Co–amoxiclav	0.5	2	0.25–32	1	2	0.5–4	32	128	8–128
	Serratia spp. (20)			*Providencia stuartii* (16)			*Salmonella* spp. (10)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.12	1	0.008–4	0.12	0.25	0.015–1	0.03	0.06	0.015–0.25
	Serratia spp. (20)			*Providencia stuartii* (16)			*Salmonella* spp. (10)		
Ciprofloxacin	0.12	1	0.008–4	0.25	0.5	0.03–1	0.03	0.06	0.03–0.25
Levofloxacin	0.12	1	0.03–4	0.25	0.5	0.06–2	0.06	0.06	0.06–0.5
Trovafloxacin	0.25	2	0.015–16	0.12	0.5	0.03–1	0.06	0.12	0.06–0.5
Nalidixic acid	4	>128	1–>128	8	>128	2–>128	8	8	8

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Cefixime	0.25	4	0.06–64	0.008	0.015	0.004–1	0.06	0.25	0.03–0.25
Co–amoxiclav	16	128	4–>128	128	128	1–>128	1	16	0.5–16
	Stenotrophomonas maltophila (10)			*Pseudomonas aeruginosa* (15)			*Acinetobacter baumannii* (11)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.5	4	0.12–4	0.25	4	0.12–32	16	16	0.03–16
Ciprofloxacin	1	16	0.25–16	0.25	32	0.25–32	64	128	0.25–>128
Levofloxacin	0.5	4	0.25–16	0.5	8	0.25–64	8	8	0.12–16
Trovafloxacin	0.5	2	0.12–4	0.5	4	0.12–128	16	16	0.03–16
Nalidixic acid	32	64	4–>128	>128	>128	32–>128	>128	>128	4–>128
Cefixime	>128	>128	16–>128	32	>128	4–>128	>128	>128	8–>128
Co–amoxiclav	128	>128	128–>128	128	>128	128–>128	8	32	8–32
	Staphylococcus aureus MSSA (39)			*Staphylococcus aureus* MRSA (20)			*Staphylococcus epidermidis* MSSE (17)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Gemifloxacin	0.03	0.06	0.015–0.25	4	8	1–16	0.015	0.03	0.015–2
Ciprofloxacin	0.5	2	0.25–4	128	128	16–128	0.25	0.25	0.25–128
Levofloxacin	0.25	0.5	0.12–1	16	16	8–32	0.25	0.25	0.12–16
Trovafloxacin	0.03	0.06	0.015–0.25	1	2	1–8	0.03	0.06	0.015–8
Nalidixic acid	>128	>128	16–>128	>128	>128	32–>128	64	128	32–>128
	Staphylococcus aureus MSSA (39)			*Staphylococcus aureus* MRSA (20)			*Staphylococcus epidermidis* MSSE (17)		
Cefixime	16	16	4–64	128	128	64–>128	4	8	2–64
Co-amoxiclav	0.25	0.5	0.12–0.5	16	16	2–32	0.12	0.25	0.06–0.5
	Staphylococcus epidermidis MRSE (10)			*Staphylococcus saprophyticus* (30)			*Enterococcus faecalis* (22)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.015	2	0.015–2	0.03	0.03	0.015–0.06	0.12	2	0.03–16
Ciprofloxacin	0.25	16	0.25–64	0.5	0.5	0.25–2	1	128	0.5–>128
Levofloxacin	0.25	16	0.12–16	0.5	0.5	0.25–0.5	1	32	0.5–128

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Trovafloxacin	0.03	4	0.015–8	0.06	0.06	0.03–0.12	0.25	8	0.12–16
Nalidixic acid	64	>128	32–>128	>128	>128	32–>128	>128	>128	>128
Cefixime	128	>128	32–>128	64	128	4–>128	128	>128	4–>128
Co–amoxiclav	2	64	0.5–64	0.25	1	0.12–64	0.5	0.5	0.5–8
	Enterococcus faecium (18)			*Streptococcus pyogenes* (18)			*Streptococcus lancefield* Gp B (20)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	2	8	0.03–64	0.03	0.06	0.015–0.06	0.06	0.12	0.03–0.12
Ciprofloxacin	8	>128	0.5–>128	0.5	1	0.5–2	1	2	0.5–2
Levofloxacin	8	64	0.5–128	1	1	0.5–1	1	2	0.5–2
Trovafloxacin	4	16	0.06–16	0.12	0.25	0.12–0.25	0.25	0.25	0.25–0.5
Nalidixic acid	>128	>128	>128	>128	>128	64–>128	>128	>128	>128
Cefixime	>128	>128	4–>128	0.06	0.12	0.06–0.5	0.25	0.5	0.06–2
Co–amoxiclav	16	32	0.25–64	0.015	0.015	0.015–0.06	0.06	0.06	0.015–0.06

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
	Streptococcus millerii (26)			*Streptococcus pneumoniae* (50)			Quinolone-resistant *Streptococcus pneumoniae* (16) (Ciprofloxacin MIC ≥ 1 μg/ml)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.06	0.06	0.008–0.12	0.03	0.06	0.015–0.25	0.06	0.5	0.03–2
Ciprofloxacin	1	2	0.03–4	1	2	0.5–16	4	128	1–>128
Levofloxacin	1	1	0.03–4	1	1	0.5–2	2	32	1–64
Trovafloxacin	0.25	0.25	0.015–0.5	0.12	0.12	0.06–0.25	0.25	8	0.12–16
Nalidixic acid	>128	>128	2–>128	>128	>128	>128	>128	>128	>128
Cefixime	2	8	0.03–>128	0.12	8	0.06–32	0.25	0.25	0.12–1
Co–amoxiclav	0.06	0.12	0.015–2	0.015	0.5	0.015–1	0.015	0.015	0.015–0.06
	Haemophilus influenzae (50)			*Neisseria gonorrhoeae* (31)			*Neisseria meningitidis* (11)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Gemifloxacin	0.002	0.008	0.001–0.03	0.001	0.002	0.001–0.03	0.001	0.002	0.001–0.002
Ciprofloxacin	0.008	0.015	0.004–0.015	0.002	0.004	0.001–0.06	0.008	0.008	0.004–0.008
Levofloxacin	0.015	0.015	0.008–0.03	0.008	0.008	0.004–0.12	0.008	0.015	0.008–0.015
Trovafloxacin	0.008	0.015	0.002–0.06	0.004	0.004	0.002–0.03	0.004	0.004	0.004–0.008
Nalidixic acid	1	2	0.25–2	1	1	0.25–>128	1	2	0.25–2
Cefixime	0.03	0.12	0.015–0.25	0.008	0.008	0.001–0.008	0.002	0.002	0.002–0.004
Co–amoxiclav	0.5	1	0.25–16	0.25	0.5	0.06–0.5	0.06	0.12	0.06–0.5
	Moraxella catarrhalis (43)			*Clostridium perfringens* (10)			*Clostridium difficile* (9)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.015	0.015	0.008–0.12	0.12	0.12	0.03–0.12	1	2	1–2
	Moraxella catarrhalis (43)			*Clostridium perfringens* (10)			*Clostridium difficile* (9)		

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Ciprofloxacin	0.06	0.06	0.004–1	0.5	0.5	0.5	16	16	8–16
Levofloxacin	0.06	0.06	0.015–0.5	0.5	0.5	0.25–0.5	4	4	4
Trovafloxacin	0.015	0.03	0.008–1	0.25	0.25	0.06–0.25	1	1	1–2
Nalidixic acid	4	8	1–>128	32	32	16–64	>128	>128	>128
Cefixime	0.12	0.5	0.03–1	8	8	2–64	64	64	32–64
Co–amoxiclav	0.06	0.12	0.015–0.5	0.25	0.25	0.03–0.5	0.5	1	0.25–2
	Peptostreptococcus anaerobius (10)			*Peptostreptococcus prevoti* (11)			*Bacteroides fragilis* (26)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.25	0.25	0.03–0.25	0.12	0.25	0.03–0.25	0.5	0.5	0.25–1
Ciprofloxacin	0.5	1	0.12–1	0.5	0.5	0.12–16	2	4	2–16
Levofloxacin	0.5	0.5	0.25–2	0.25	4	0.25–16	1	2	1–4
Trovafloxacin	0.12	0.12	0.12–0.25	0.12	1	0.06–1	0.25	0.25	0.12–0.5
Nalidixic acid	>128	>128	16–>128	>128	>128	16–>128	>128	>128	>128
Cefixime	4	32	1–128	2	64	0.5–64	128	>128	32–>128
Co–amoxiclav	0.25	8	0.12–32	0.12	0.25	0.008–2	0.5	2	0.5–8

Other respiratory pathogens (*Haemophilus influenzae* and *Moraxella catarrhalis*) are extremely susceptible to gemifloxacin. *Bacteroides fragilis* are all susceptible to ≤0.5 μg/ml of gemifloxacin.

A proposed tentative breakpoint of 0.5 μg/ml was chosen. Figure 5 shows the MICs and zone diameters for a 1 μg disk of gemifloxacin. The 2 and 5 μg disks gave zone diameters of 30–45 mm for susceptible isolates and would cause unacceptably large zones. Utilizing a zone diameter of 20 mm as breakpoint, the false resistance rate is 6% and a false susceptibility rate of around 1%. Of the 36 falsely resistant strains, 8 were enterococci (MIC 0.12–0.5 μg/ml), 17 were Enterobacteriaceae with reduced susceptibility to ciprofloxacin and 11 were *Serratia, Acinetobacter* and *Stenotrophomonas* spp with MICs close to the breakpoint. Figure 6 shows the scattergram of a 5 μg disk and *P. aeruginosa*. Tentative recommendations are shown in Table 5.

The data confirms that of others, except for the surprising result that gemifloxacin is found to be more active against *B. fragilis* (*See*, Oh JI, Paek K-S, Ahn M-J *et al. In vitro* and *in vivo* evaluation of LB20304, a new fluoronaphthyridinone. *Antimicrob Agents Chemother* 1996; 40: 1564–1568; Comican MG, Jones RN. Antimicrobial activity and spectrum of LB20304, a novel fluoronaphthyridone. *Antimicrob Agents Chemother* 1997; 41: 204–211). The high activity of gemifloxacin against *S. pneumoniae* is noteworthy – including against those strains resistant to ciprofloxacin. A tentative breakpoint of 0.5 μg/ml would appear to give reliable results.

Table 5. Tentative MIC breakpoints (BP) and zone diameter breakpoints (ZD BP) for gemifloxacin using BSAC recommendations and expected MIC and zone diameters for control strains

Type	MIC BP (μg/ml)		ZD BP (mm)			NCTC controls			ATCC controls		
	S	R	Disk content (μg)	S	R	Number	MIC (μg/ml)	ZD BP (mm)	Number	MIC (μg/ml)	ZD BP (mm)
Enterobacteriaceae	≤0.5	≥1	1	≥20	≤19	10418*	0.008	36	25922*	0.008	33
Staphylococci	≤0.5	≥1	1	≥20	≤19	6571	0.015	42	25923	0.03	22
Enterococci	≤0.5	≥1	1	≥20	≤19	–	–	–	29212	0.03	24
Haemophili	≤0.5	≥1	1	≥20	≤19	11931	0.002	28	49247	0.002	35
Pneumococci	≤0.5	≥1	1	≥20	≤19	–	–	–	49619	0.03	29
Pseudomonads	≤0.5	≥1	5	≥20	≤19	10662	0.25	25	27853	0.25	30

While a preferred object of the invention provides a method wherein said pathogenic bacteria is selected from the group consisting of: *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Serratia* spp., *Providencia stuartii*, *Salmonella* spp., *Stenotrophomonas maltophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus* MSSA, *Staphylococcus aureus* MRSA, *Staphylococcus epidermidis* MSSE, *Staphylococcus epidermidis* MRSE, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Streptococcus lancefield* Gp B, *Streptococcus millerii*, *Streptococcus pneumoniae*, Quinolone-resistant *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Clostridium perfringens*, *Clostridium difficile*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevoti*, and *Bacteroides fragilis*.. Other pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention still further provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Serratia* spp., *Providencia stuartii*, *Salmonella* spp., *Stenotrophomonas maltophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus* MSSA, *Staphylococcus aureus* MRSA, *Staphylococcus epidermidis* MSSE, *Staphylococcus epidermidis* MRSE, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Streptococcus lancefield* Gp B, *Streptococcus millerii*, *Streptococcus pneumoniae*, Quinolone-resistant *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Clostridium perfringens*, *Clostridium difficile*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevoti*, or *Bacteroides fragilis*.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various pathogens. An objective of these analyses was to determine the *in vitro* activity of gemifloxacin (GFX) to that of ciprofloxacin (CIP), trovafloxacin (TRX) and levofloxacin (LEV) against 782 recent clinical isolates. GFX inhibit 90% of Enterobacteriaceae at ≤0.5 μg/ml (except *Serratia* spp., MIC_{90}1 μg/ml) and display similar activity to CIP, TRX and LEV. *Pseudomonas aeruginosa* is moderately susceptible to GFX (MIC_{90} 4 μg/ml).

Against *Streptococcus pneumoniae* GFX (MIC_{90} 0.06 μg/ml) is 2–4-fold more active than TRX and 16–32-fold more active than CIP. GFX is the most active agent against quinolone-resistant *S. pneumoniae* tested; of 16 strains resistant to CIP (MIC 4–8 μg/ml) all

are susceptible to ≤0.12 μg/ml of GFX. Four strains susceptible to ≥128 μg/ml CIP are susceptible to 0.5–2 μg/ml GFX. MSSA are equally susceptible to GFX and TRX (MIC_{90} 0.06 μg/ml) but MRSA are less susceptible (GFX MIC_{90} 8 μg/ml). *Haemophilus influenzae*, *Moraxella catarrhalis* are susceptible to GFX (MIC_{90} ≤0.06 μg/ml). *Enterococcus* spp. are more susceptible to GFX than to other agents. GFX and TRX have high activity against *Bacteroides fragilis* (MIC_{90} 0.5 μg/ml) and peptostreptococci (MIC_{90} 0.25 μg/ml). A tentative breakpoint of 0.5 μg/ml was suggested following regression analysis of disk zone size plotted against MIC. A 1 μg disk is suggested and a zone diameter of >20 mm indicating susceptibility. The false sensitivity and resistance rates are 0% and 5.6% respectively.

In a further study, the activity of gemifloxacin was compared with commonly used antimicrobials against 782 recently isolated bacteria was compared. The techniques of the British Society for Antimicrobial Chemotherapy (BSAC) Working Party on Susceptibility Testing have been employed to establish a tentative *in vitro* breakpoint (*See*, Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27 (Suppl D)).

Gemifloxacin has been compared to ciprofloxacin, trovafloxacin, levofloxacin, nalidixic acid and amoxicillin/clavulanic acid. Susceptibilities were performed in accordance with the techniques of the BSAC Working Party (See Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27 (Suppl D)). The final inoculum was approximately 10^4 CFU. The media was Unipath Iso-Sensitest agar (supplemented as required) or Wilkin-Chalgren agar. Incubation was performed in an appropriate atmosphere at 35°–37°C.

The MICs (as described above) and zone diameters of 1, 2 and 5 μg gemifloxacin disks were measured, growth was semi-confluent, scattergrams of zone size against MIC were performed, the BSAC formula gave a tentative breakpoint of 0.5–1 μg/ml, and false sensitive and resistance rates were measured (*See*, Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; 27 (Suppl D)).

The results of susceptibility testing are shown in Table 6. The activities of gemifloxacin, ciprofloxacin, trovafloxacin and levofloxacin against the Enterobacteriaceae are similar, the MICs being ± one doubling dilution of each other. Strains less susceptible to ciprofloxacin are less susceptible to the other fluoroquinolones All the fluoroquinolones display lesser activity against *Pseudomonas aeruginosa* including gemifloxacin (MIC_{90} 4

μg/ml). Against MSSA, gemifloxacin is 32-fold more active than ciprofloxacin. Similar differences are seen with *Staphylococcus epidermidis* and the enterococci. Against *Streptococcus pneumoniae* gemifloxacin is 2–4-fold more active than trovafloxacin and 16–32-fold more active than ciprofloxacin. A total of 10 strains of *S. pneumoniae* are resistant to ciprofloxacin (MIC 4–8 μg/ml); these are susceptible to 0.06–0.12 μg/ml of gemifloxacin. A total of 4 strains are highly ciprofloxacin resistant (MIC >128 μg/ml), these are susceptible to 0.5–2 μg/ml of gemifloxacin.

Table 6. *In vitro* activity (μg/ml) of gemifloxacin and other agents against recent clinical isolates

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
	E. coli (80)			*Klebsiella* spp. (48)			*Enterobacter* spp. (10)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.015	1	0.002–>128	0.05	0.25	0.008–64	0.03	0.5	0.015–4
Ciprofloxacin	0.015	1	0.002–>128	0.03	0.5	0.008–32	0.03	2	0.008–32
Levofloxacin	0.03	1	0.015–64	0.06	1	0.015–64	0.06	2	0.03–8
Trovafloxacin	0.03	2	0.004–>128	0.06	1	0.015–64	0.06	0.5	0.03–2
Nalidixic acid	4	>128	0.5–>128	8	>128	2–>128	8	>128	4–>128
Cefixime	0.12	1	0.008–32	0.03	0.12	0.008–128	1	>128	0.015–>128
Co–amoxiclav	4	16	0.5–32	2	16	1–32	32	>128	2–>128
	Proteus mirabilis (50)			*Proteus vulgaris* (20)			*Morganella morganii* (21)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Gemifloxacin	0.12	0.12	0.03–8	0.06	0.12	0.03–0.12	0.06	0.06	0.03–0.12
Ciprofloxacin	0.03	0.06	0.008–2	0.015	0.03	0.008–0.06	0.015	0.015	0.008–0.06
Levofloxacin	0.06	0.12	0.03–2	0.03	0.06	0.015–0.12	0.03	0.06	0.015–0.12
Trovafloxacin	0.25	0.25	0.06–16	0.12	0.25	0.03–0.25	0.25	0.25	0.03–0.5
Nalidixic acid	8	8	4–>128	4	4	2–8	4	4	2–8
Cefixime	0.004	0.008	0.002–0.008	0.008	0.008	0.002–0.015	0.06	2	0.03–16
Co–amoxiclav	0.5	2	0.25–32	1	2	0.5–4	32	128	8–128
	Serratia spp. (20)			*Providencia stuartii* (16)			*Salmonella* spp. (10)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.12	1	0.008–4	0.12	0.25	0.015–1	0.03	0.06	0.015–0.25
	Serratia spp. (20)			*Providencia stuartii* (16)			*Salmonella* spp. (10)		
Ciprofloxacin	0.12	1	0.008–4	0.25	0.5	0.03–1	0.03	0.06	0.03–0.25
Levofloxacin	0.12	1	0.03–4	0.25	0.5	0.06–2	0.06	0.06	0.06–0.5
Trovafloxacin	0.25	2	0.015–16	0.12	0.5	0.03–1	0.06	0.12	0.06–0.5
Nalidixic acid	4	>128	1–>128	8	>128	2–>128	8	8	8

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)									
Cefixime	0.25	4	0.06–64	0.008	0.015	0.004–1	0.06	0.25	0.03–0.25	
Co–amoxiclav	16	128	4–>128	128	128	1–>128	1	16	0.5–16	
	Stenotrophomonas maltophila (10)			*Pseudomonas aeruginosa* (15)			*Acinetobacter baumannii* (11)			
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
Gemifloxacin	0.5	4	0.12–4	0.25	4	0.12–32	16	16	0.03–16	
Ciprofloxacin	1	16	0.25–16	0.25	32	0.25–32	64	128	0.25–>128	
Levofloxacin	0.5	4	0.25–16	0.5	8	0.25–64	8	8	0.12–16	
Trovafloxacin	0.5	2	0.12–4	0.5	4	0.12–128	16	16	0.03–16	
Nalidixic acid	32	64	4–>128	>128	>128	32–>128	>128	>128	4–>128	
Cefixime	>128	>128	16–>128	32	>128	4–>128	>128	>128	8–>128	
Co–amoxiclav	128	>128	128–>128	128	>128	128–>128	8	32	8–32	
	Staphylococcus aureus MSSA (39)			*Staphylococcus aureus* MRSA (20)			*Staphylococcus epidermidis* MSSE (17)			
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	

Antimicrobial agent	MIC (µg/ml) and isolate (number tested)								
Gemifloxacin	0.03	0.06	0.015–0.25	4	8	1–16	0.015	0.03	0.015–2
Ciprofloxacin	0.5	2	0.25–4	128	128	16–128	0.25	0.25	0.25–128
Levofloxacin	0.25	0.5	0.12–1	16	16	8–32	0.25	0.25	0.12–16
Trovafloxacin	0.03	0.06	0.015–0.25	1	2	1–8	0.03	0.06	0.015–8
Nalidixic acid	>128	>128	16–>128	>128	>128	32–>128	64	128	32–>128
	Staphylococcus aureus MSSA (39)			*Staphylococcus aureus* MRSA (20)			*Staphylococcus epidermidis* MSSE (17)		
Cefixime	16	16	4–64	128	128	64–>128	4	8	2–64
Co–amoxiclav	0.25	0.5	0.12–0.5	16	16	2–32	0.12	0.25	0.06–0.5
	Staphylococcus epidermidis MRSE (10)			*Staphylococcus saprophyticus* (30)			*Enterococcus faecalis* (22)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.015	2	0.015–2	0.03	0.03	0.015–0.06	0.12	2	0.03–16
Ciprofloxacin	0.25	16	0.25–64	0.5	0.5	0.25–2	1	128	0.5–>128
Levofloxacin	0.25	16	0.12–16	0.5	0.5	0.25–0.5	1	32	0.5–128

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Trovafloxacin	0.03	4	0.015–8	0.06	0.06	0.03–0.12	0.25	8	0.12–16
Nalidixic acid	64	>128	32–>128	>128	>128	32–>128	>128	>128	>128
Cefixime	128	>128	32–>128	64	128	4–>128	128	>128	4–>128
Co–amoxiclav	2	64	0.5–64	0.25	1	0.12–64	0.5	0.5	0.5–8
	Enterococcus faecium (18)			*Streptococcus pyogenes* (18)			*Streptococcus lancefield* Gp B (20)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	2	8	0.03–64	0.03	0.06	0.015–0.06	0.06	0.12	0.03–0.12
Ciprofloxacin	8	>128	0.5–>128	0.5	1	0.5–2	1	2	0.5–2
Levofloxacin	8	64	0.5–128	1	1	0.5–1	1	2	0.5–2
Trovafloxacin	4	16	0.06–16	0.12	0.25	0.12–0.25	0.25	0.25	0.25–0.5
Nalidixic acid	>128	>128	>128	>128	>128	64–>128	>128	>128	>128
Cefixime	>128	>128	4–>128	0.06	0.12	0.06–0.5	0.25	0.5	0.06–2
Co–amoxiclav	16	32	0.25–64	0.015	0.015	0.015–0.06	0.06	0.06	0.015–0.06

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
	Streptococcus millerii (26)			*Streptococcus pneumoniae* (50)			Quinolone-resistant *Streptococcus pneumoniae* (16) (Ciprofloxacin MIC ≥ 1 μg/ml)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.06	0.06	0.008–0.12	0.03	0.06	0.015–0.25	0.06	0.5	0.03–2
Ciprofloxacin	1	2	0.03–4	1	2	0.5–16	4	128	1–>128
Levofloxacin	1	1	0.03–4	1	1	0.5–2	2	32	1–64
Trovafloxacin	0.25	0.25	0.015–0.5	0.12	0.12	0.06–0.25	0.25	8	0.12–16
Nalidixic acid	>128	>128	2–>128	>128	>128	>128	>128	>128	>128
Cefixime	2	8	0.03–>128	0.12	8	0.06–32	0.25	0.25	0.12–1
Co–amoxiclav	0.06	0.12	0.015–2	0.015	0.5	0.015–1	0.015	0.015	0.015–0.06
	Haemophilus influenzae (50)			*Neisseria gonorrhoeae* (31)			*Neisseria meningitidis* (11)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)								
Gemifloxacin	0.002	0.008	0.001–0.03	0.001	0.002	0.001–0.03	0.001	0.002	0.001–0.002
Ciprofloxacin	0.008	0.015	0.004–0.015	0.002	0.004	0.001–0.06	0.008	0.008	0.004–0.008
Levofloxacin	0.015	0.015	0.008–0.03	0.008	0.008	0.004–0.12	0.008	0.015	0.008–0.015
Trovafloxacin	0.008	0.015	0.002–0.06	0.004	0.004	0.002–0.03	0.004	0.004	0.004–0.008
Nalidixic acid	1	2	0.25–2	1	1	0.25–>128	1	2	0.25–2
Cefixime	0.03	0.12	0.015–0.25	0.008	0.008	0.001–0.008	0.002	0.002	0.002–0.004
Co–amoxiclav	0.5	1	0.25–16	0.25	0.5	0.06–0.5	0.06	0.12	0.06–0.5
	Moraxella catarrhalis (43)			*Clostridium perfringens* (10)			*Clostridium difficile* (9)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.015	0.015	0.008–0.12	0.12	0.12	0.03–0.12	1	2	1–2
	Moraxella catarrhalis (43)			*Clostridium perfringens* (10)			*Clostridium difficile* (9)		

Antimicrobial agent	MIC (µg/ml) and isolate (number tested)								
Ciprofloxacin	0.06	0.06	0.004–1	0.5	0.5	0.5	16	16	8–16
Levofloxacin	0.06	0.06	0.015–0.5	0.5	0.5	0.25–0.5	4	4	4
Trovafloxacin	0.015	0.03	0.008–1	0.25	0.25	0.06–0.25	1	1	1–2
Nalidixic acid	4	8	1–>128	32	32	16–64	>128	>128	>128
Cefixime	0.12	0.5	0.03–1	8	8	2–64	64	64	32–64
Co–amoxiclav	0.06	0.12	0.015–0.5	0.25	0.25	0.03–0.5	0.5	1	0.25–2
	Peptostreptococcus anaerobius (10)			*Peptostreptococcus prevoti* (11)			*Bacteroides fragilis* (26)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Gemifloxacin	0.25	0.25	0.03–0.25	0.12	0.25	0.03–0.25	0.5	0.5	0.25–1
Ciprofloxacin	0.5	1	0.12–1	0.5	0.5	0.12–16	2	4	2–16
Levofloxacin	0.5	0.5	0.25–2	0.25	4	0.25–16	1	2	1–4
Trovafloxacin	0.12	0.12	0.12–0.25	0.12	1	0.06–1	0.25	0.25	0.12–0.5
Nalidixic acid	>128	>128	16–>128	>128	>128	16–>128	>128	>128	>128
Cefixime	4	32	1–128	2	64	0.5–64	128	>128	32–>128
Co–amoxiclav	0.25	8	0.12–32	0.12	0.25	0.008–2	0.5	2	0.5–8

Certain other respiratory pathogens (*Haemophilus influenzae* and *Moraxella catarrhalis*) are extremely susceptible to gemifloxacin. *Bacteroides fragilis* are all susceptible to ≤0.5 μg/ml of gemifloxacin.

A proposed tentative breakpoint of 0.5 μg/ml was chosen. Figure 1 shows the MICs and zone diameters for a 1 μg disk of gemifloxacin. The 2 and 5 μg disks gave zone diameters of 30–45 mm for susceptible isolates and would cause unacceptably large zones. Utilizing a zone diameter of 20 mm as breakpoint, the false resistance rate is 6% and a false susceptibility rate of around 1%. Of the 36 falsely resistant strains, 8 were enterococci (MIC 0.12–0.5 μg/ml), 17 were Enterobacteriaceae with reduced susceptibility to ciprofloxacin and 11 were *Serratia, Acinetobacter* and *Stenotrophomonas* spp with MICs close to the breakpoint. Figure 2 shows the scattergram of a 5 μg disk and *P. aeruginosa*. Tentative recommendations are shown in Table 7.

The data confirms that of others, except that gemifloxacin is found to be more active against *B. fragilis* (*See*, Oh JI, Paek K-S, Ahn M-J *et al. In vitro* and *in vivo* evaluation of LB20304, a new fluoronaphthyridinone. *Antimicrob Agents Chemother* 1996; 40: 1564–1568; Comican MG, Jones RN. Antimicrobial activity and spectrum of LB20304, a novel fluoronaphthyridone. *Antimicrob Agents Chemother* 1997; 41: 204–211). The high activity of gemifloxacin against *S. pneumoniae* is noteworthy – including against those strains resistant to ciprofloxacin. A tentative breakpoint of 0.5 μg/ml would appear to give reliable results.

Table 7. Tentative MIC breakpoints (BP) and zone diameter breakpoints (ZD BP) for gemifloxacin using BSAC recommendations and expected MIC and zone diameters for control strains

Type	MIC BP (μg/ml)		ZD BP (mm)			NCTC controls			ATCC controls		
	S	R	Disk content (μg)	S	R	Number	MIC (μg/ml)	ZD BP (mm)	Number	MIC (μg/ml)	ZD BP (mm)
Enterobacteriaceae	≤0.5	≥1	1	≥20	≤19	10418*	0.008	36	25922*	0.008	33
Staphylococci	≤0.5	≥1	1	≥20	≤19	6571	0.015	42	25923	0.03	22
Enterococci	≤0.5	≥1	1	≥20	≤19	–	–	–	29212	0.03	24
Haemophili	≤0.5	≥1	1	≥20	≤19	11931	0.002	28	49247	0.002	35
Pneumococci	≤0.5	≥1	1	≥20	≤19	–	–	–	49619	0.03	29
Pseudomonads	≤0.5	≥1	5	≥20	≤19	10662	0.25	25	27853	0.25	30

While a preferred object of the invention provides a method wherein said pathogenic bacteria is selected from the group consisting of: *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Serratia* spp., *Providencia stuartii*, *Salmonella* spp., *Stenotrophomonas maltophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus* MSSA, *Staphylococcus aureus* MRSA, *Staphylococcus epidermidis* MSSE, *Staphylococcus epidermidis* MRSE, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Streptococcus lancefield* Gp B, *Streptococcus millerii*, *Streptococcus pneumoniae*, Quinolone-resistant *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Clostridium perfringens*, *Clostridium difficile*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevoti*, and *Bacteroides fragilis*.. Other pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against pneumococcal bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various pneumococci pathogens. An objective of these analyses was to determine the activities of gemifloxacin (SB-265805), ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin, trovafloxacin, amoxicillin, cefuroxime, azithromycin and clarithromycin against 4 penicillin-susceptible, 4 -intermediate and 4 -resistant (including 2 ciprofloxacin-resistant) pneumococci by time-kill. . Broth MICs (μg/ml) are: gemifloxacin: 0.016–0.5; ciprofloxacin: 0.5–32; levofloxacin: 1–32, sparfloxacin: 0.125–32; grepafloxacin: 0.06–16; trovafloxacin: 0.06–8; amoxicillin: 0.016–2; cefuroxime: 0.016–4; azithromycin: 0.008–>64; clarithromycin: 0.008–64. Results are shown below at MIC/2 x MIC (number of strains with -1, -2, -3 $\log_{10}$ decrease in count compared to 0 hours):

Antimicrobial	3 hours			6 hours			12 hours			24 hours		
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3
Gemifloxacin	4/6	1/0	0/0	11/12	2/7	0/0	12/12	8/11	3/8	12/12	10/12	8/12
Ciprofloxacin	4/9	0/4	0/0	8/12	3/8	0/2	10/12	9/12	3/6	11/12	10/12	6/11
Levofloxacin	9/9	1/2	0/0	12/12	6/9	0/1	12/12	11/12	7/9	12/12	12/12	12/12
Sparfloxacin	4/8	0/1	0/0	8/12	2/4	0/0	11/12	9/10	4/5	11/12	11/12	10/12
Grepafloxacin	1/3	0/0	0/0	4/9	1/1	0/0	7/10	3/8	0/1	8/11	5/10	3/9
Trovafloxacin	4/5	0/1	0/0	6/11	2/4	0/0	7/12	4/11	1/7	6/11	1/11	1/11
Amoxicillin	4/6	0/2	0/0	6/11	1/5	0/1	7/12	6/11	2/8	10/12	9/12	7/12
Cefuroxime	4/7	0/1	0/0	7/12	2/8	0/1	9/12	7/11	1/8	9/12	9/12	9/11

Azithromycin[a]	2/3	0/1	0/0	3/4	2/2	2/2	5/5	5/5	2/4	7/7	6/7	5/5
Clarithromycin[a]	3/3	1/2	0/0	5/5	0/2	1/2	5/5	5/5	2/4	7/7	7/7	5/7

[a]Only seven strains with MICs ≤0.25 μg/ml were tested.

Gemifloxacin has the lowest MICs and, at twice the MIC, is uniformly bactericidal, irrespective of penicillin or ciprofloxacin MICs. Gemifloxacin is the only quinolone active against ciprofloxacin-resistant strains at achievable MICs. Other quinolones and β-lactams had similar kinetics relative to MIC and macrolides show slower kinetics.

The incidence of pneumococci resistant to penicillin G and other β-lactam and non-β-lactam compounds has increased worldwide at an alarming rate, including in the USA. Major foci of infections currently include South Africa, Spain, Central and Eastern Europe, and parts of Asia. (See Friedland IR, McCracken GH, Jr. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. *N Engl J Med* 1994; 331: 377–382; and Jacobs MR, Appelbaum PC. Antibiotic-resistant pneumococci. *Rev Med Microbiol* 1995; 6: 77–93). In the USA, a recent survey has shown an increase in resistance to penicillin from <5% before 1989 (including <0.02% of isolates with MICs ≥2.0 μg/ml) to 6.6% in 1991–1992 (with 1.3% of isolates with MICs ≥2.0 μg/ml) (See Breiman RF, Butler JC, Tenover FC, Elliott JA, Facklam RR. Emergence of drug-resistant pneumococcal infections in the United States. *JAMA* 1994; 271: 1831–1835). In another more recent survey, 23.6% (360) of 1527 clinically significant pneumococcal isolates were not susceptible to penicillin.(See Doern GV, Brueggemann A, Holley HP, Rauch AM. Antimicrobial resistance of *Streptococcus pneumoniae* isolated from outpatients in the United States during the winter months of 1994 to 1995: results of a 30-center national surveillance study. *Antimicrob Agents Chemother* 1996; 40: 1208–1213). It is also important to note the high rates of isolation of penicillin-intermediate and -resistant pneumococci (approximately 30%) in middle ear fluids from patients with refractory otitis media, compared to other isolation sites (*See*, Block S, Harrison CJ, Hedrick JA, *et al.* Penicillin-resistant *Streptococcus pneumoniae* in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. *Pediatr Infect Dis J* 1995; 14: 751–759). The problem of drug-resistant pneumococci is compounded by the ability of resistant clones to spread from country to country, and from continent to continent (*See*, Munoz R, Musser JM, Crain M, *et al.* Geographic distribution of penicillin-resistant clones of *Streptococcus pneumoniae*: characterization by penicillin-binding protein profile, surface protein A typing, and multilocus enzyme analysis. *Clin Infect Dis* 1992; 15: 112–118).

There is an urgent need of oral compounds for out-patient treatment of otitis media

and respiratory tract infections caused by penicillin-intermediate and -resistant pneumococci (*See*, Breiman RF, Butler JC, Tenover FC, Elliott JA, Facklam RR. Emergence of drug-resistant pneumococcal infections in the United States. *JAMA* 1994; 271: 1831–1835). Available quinolones such as ciprofloxacin and ofloxacin yield moderate *in vitro* activity against pneumococci, with MICs clustering around the breakpoints (*See*, Pankuch GA, Jacobs MR, Appelbaum PC. Activity of CP 99,219 compared to DU-6859a, ciprofloxacin, ofloxacin, levofloxacin, lomefloxacin, tosufloxacin, sparfloxacin and grepafloxacin against penicillin-susceptible and -resistant pneumococci. *J Antimicrob Chemother* 1995; 35: 230–232.; Spangler SK, Jacobs MR, Appelbaum PC. Susceptibilities of penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae* to RP 59500, vancomycin, erythromycin, PD 131628, sparfloxacin, temafloxacin, Win 57273, ofloxacin and ciprofloxacin. *Antimicrob Agents Chemother* 1992; 36: 856–859; and Spangler SK, Jacobs MR, Pankuch GA, Appelbaum PC. Susceptibility of 170 penicillin-susceptible and -resistant pneumococci to six oral cephalosporins, four quinolones, desacetylcefotaxime, Ro 23-9424 and RP 67829. *J Antimicrob Chemother* 1993; 31: 273–280.).

Previous preliminary studies. have shown that gemifloxacin is very active against pneumococci (*See*, Cormican MG, Jones RN. Antimicrobial activity and spectrum of LB 20304, a novel fluoronaphthyridone. *Antimicrob Agents Chemother* 1997; 41: 204–211; Hohl AF, Frei R, Pünter V, *et al.* International multicenter investigation of LB 20304, a new fluoronaphthyridone. *Clin Microbiol Infect* 1998; 4: 280–284; and Oh J-I, Paek K-S, Ahn M-J, *et al. In vitro* and *in vivo* evaluations of LB 20304, a new fluoronaphthyridone. *Antimicrob Agents Chemother* 1996; 40: 1564–1568).

A further study examined the anti-pneumococcal activity of gemifloxacin compared to ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin, trovafloxacin, amoxicillin, cefuroxime, azithromycin and clarithromycin by time-kill testing of 12 pneumococcal strains of varying quinolone and penicillin susceptibilities. For time-kill studies, 4 penicillin-susceptible, 4 -intermediate and 4 -resistant strains (2 quinolone resistant, obtained courtesy of David Felmingham from the Alexander Project collection) are tested.

Gemifloxacin susceptibility powder was obtained from SmithKline Beecham Laboratories, Harlow, UK; other antimicrobials are obtained from their respective manufacturers. Broth MICs for 12 strains tested by time-kill and 6 tested by PAE have been performed according to NCCLS recommendations using cation-adjusted Mueller-Hinton broth with 5% lysed defibrinated horse blood (*See*, National Committee for Clinical Laboratory Standards. In: *Methods for dilution antimicrobial susceptibility tests for bacteria*

that grow aerobically (3rd edition; approved standard. NCCLS publication no. M7-A4) National Committee for Clinical Laboratory Standards:Villanova, PA, 1997). Standard quality control strains, including *Streptococcus pneumoniae* ATCC 49619, were included in each run of agar and broth dilution MICs.

For time-kill studies, glass tubes containing 5 ml cation-adjusted Mueller-Hinton broth (Difco) + 5% lysed horse blood with doubling antimicrobial concentrations are inoculated with 5 x 10^5 to 5 x 10^6 CFU/ml and incubated at 35°C in a shaking water bath. Antimicrobial concentrations were chosen to comprise 3 doubling dilutions above and 3 dilutions below the agar dilution MIC. Growth controls with inoculum but no antimicrobial agent have been included with each study (*See*, Pankuch GA, Jacobs MR, Appelbaum PC. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin and vancomycin by using time-kill methodology. *Antimicrob Agents Chemother* 1994; 38: 2065–2072; and Pankuch GA, Lichtenberger C, Jacobs MR, Appelbaum PC. Antipneumococcal activities of RP 59500 (quinupristin/dalfopristin), penicillin G, erythromycin, and sparfloxacin determined by MIC and rapid time-kill methodologies. *Antimicrob Agents Chemother* 1996; 40: 1653–1656). Lysed horse blood was prepared as described previously.(*See*, Pankuch , Jacobs et al and Pankuch, Lichtenberger et al, supra). The bacterial inoculum was prepared by suspending growth from an overnight blood agar plate in Mueller-Hinton broth until turbidity matched a no. 1 McFarland standard. Dilutions required to obtain the correct inoculum (5 x 10^5–5 x 10^6 CFU/ml) were determined by prior viability studies using each strain.(See See Pankuch , Jacobs et al and Pankuch, Lichtenberger et al, supra). To inoculate each tube of serially diluted antimicrobial, 50 µl of diluted inoculum was delivered by pipette beneath the surface of the broth. Tubes were then vortexed and plated for viability counts within 10 minutes (approximately 0.2 hours). The original inoculum was determined by using the untreated growth control. Only tubes containing an initial inoculum within the range of 5 x 10^5 to 5 x 10^6 CFU/ml are acceptable (*See*, Pankuch , Jacobs et al and Pankuch, Lichtenberger et al, supra).

Viability counts of antimicrobial-containing suspensions were performed by plating 10-fold dilutions of 0.1 ml aliquots from each tube in sterile Mueller-Hinton broth onto trypticase soy agar 5% sheep blood agar plates (BBL). Recovery plates were incubated for up to 72 hours. Colony counts were performed on plates yielding 30–300 colonies. The lower limit of sensitivity of colony counts was 300 CFU/ml.(See See Pankuch , Jacobs et al and Pankuch, Lichtenberger et al, supra).

Time-kill assays were analyzed by determining the number of strains which yielded a

$\Delta\log_{10}$ CFU/ml of -1, -2 and -3 at 0, 3, 6, 12 and 24 hours, compared to counts at time 0 hours. Antimicrobial agents are considered bactericidal at the lowest concentration that reduced the original inoculum by ≥ 3 $\log_{10}$ CFU/ml (99.9%) at each of the time periods, and bacteriostatic if the inoculum is reduced by 0–<3 $\log_{10}$ CFU/ml. With the sensitivity threshold and inocula used in these studies, no problems are encountered in delineating 99.9% killing, when present. The problem of bacterial carryover is addressed by dilution as described previously (*See*, Pankuch , Jacobs et al and Pankuch, Lichtenberger et al, supra). For macrolide time-kill testing, only strains with MICs ≤ 4.0 µg/ml were tested.

Microbroth dilution MIC results of the 12 strains tested by time-kill are presented in Table 8. Microdilution MICs are all within one dilution of agar MICs. For the two quinolone resistant strains (both penicillin susceptible), gemifloxacin microbroth MICs are 0.5 and 0.25 µg/ml, respectively. Time-kill results (Table 9) show that levofloxacin at the MIC, gemifloxacin and sparfloxacin at 2 x MIC and ciprofloxacin, grepafloxacin and trovafloxacin at 4 x MIC, are bactericidal after 24 hours. Various degrees of 90% and 99% killing by all quinolones is detected after 3 hours. Gemifloxacin and trovafloxacin are both bactericidal at 2 x MIC for the two quinolone resistant pneumococcal strains. Gemifloxacin is uniformly bactericidal after 24 hours at ≤ 0.5 µg/ml. Amoxicillin, at 2 x MIC and cefuroxime at 4 x MIC, are bactericidal after 24 hours, with some degree of killing at earlier time periods. By contrast, macrolides gave slower killing against the 7 susceptible strains tested, with 99.9% killing of all strains at 2–4 x MIC after 24 hours. Time-kill kinetics of gemifloxacin against a penicillin-resistant pneumococcal strain is depicted graphically in Figure 7.

Certain previous studies have shown gemifloxacin to be 32–64-fold more active than ciprofloxacin, ofloxacin, sparfloxacin and trovafloxacin against methicillin-susceptible and -resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis* and *S. pneumoniae*. Gemifloxacin is also highly active against most members of the family *Enterobacteriaceae*, with activity more potent than those of sparfloxacin and ofloxacin and comparable to that of ciprofloxacin. Gemifloxacin is the most active agent against Gram positive species resistant to other quinolones and glycopeptides. Gemifloxacin has variable activity against anaerobes, and is very active against the Gram positive group (*See*, Cormican MG, Jones RN. Antimicrobial activity and spectrum of LB 20304, a novel fluoronaphthyridone. *Antimicrob Agents Chemother* 1997; 41: 204; Hohl AF, Frei R, Pünter V, *et al.* International multicenter investigation of LB 20304, a new fluoronaphthyridone. *Clin Microbiol Infect* 1998; 4: 280–284; and Oh J-I, Paek K-S, Ahn M-J, *et al. In vitro* and *in vivo* evaluations of LB 20304, a new fluoronaphthyridone. *Antimicrob Agents Chemother* 1996;

40: 1564–1568).

Gemifloxacin gives the lowest quinolone MICs against all pneumococcal strains tested followed by trovafloxacin, grepafloxacin, sparfloxacin, levofloxacin and ciprofloxacin. MICs are similar to those described previously (*See*, Pankuch GA, Jacobs MR, Appelbaum PC. Activity of CP 99,219 compared to DU-6859a, ciprofloxacin, ofloxacin, levofloxacin, lomefloxacin, tosufloxacin, sparfloxacin and grepafloxacin against penicillin-susceptible and -resistant pneumococci. *J Antimicrob Chemother* 1995; 35: 230–232; Spangler SK, Jacobs MR, Appelbaum PC. Susceptibilities of penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae* to RP 59500, vancomycin, erythromycin, PD 131628, sparfloxacin, temafloxacin, Win 57273, ofloxacin and ciprofloxacin. *Antimicrob Agents Chemother* 1992; 36: 856–859; and Spangler SK, Jacobs MR, Pankuch GA, Appelbaum PC. Susceptibility of 170 penicillin-susceptible and -resistant pneumococci to six oral cephalosporins, four quinolones, desacetylcefotaxime, Ro 23-9424 and RP 67829. *J Antimicrob Chemother* 1993; 31: 273–280).

Gemifloxacin also shows killing against the 12 strains tested, including the two quinolone resistant strains. At ≤0.5 μg/ml, gemifloxacin is bactericidal against all 12 strains. Killing rates relative to MICs are similar to those of other quinolones, with significant killing occurring earlier than with β-lactams and macrolides (*See*, Pankuch GA, Jacobs MR, Appelbaum PC supra; Pankuch GA, Jacobs MR, Appelbaum PC. Comparative activity of ampicillin, amoxycillin, amoxycillin/clavulanate and cefotaxime against 189 penicillin-susceptible and -resistant pneumococci. *J Antimicrob Chemother* 1995; 35: 883–888.; Visalli MA, Jacobs MR, Appelbaum PC. Activity of CP 99,219 (trovafloxacin) compared with ciprofloxacin, sparfloxacin, clinafloxacin, lomefloxacin and cefuroxime against ten penicillin-susceptible and penicillin-resistant pneumococci by time-kill methodology. *J Antimicrob Chemother* 1996; 37: 77–84; Visalli MA, Jacobs MR, Appelbaum PC. MIC and time-kill study of activities of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, cefotaxime, imipenem and vancomycin against nine penicillin-susceptible and -resistant pneumococci. *Antimicrob Agents Chemother* 1996; 40: 362–366). Kill kinetics of quinolone and non-quinolone compounds are similar to those described previously (14, 16-18) Pankuch GA, Jacobs MR, Appelbaum PC. supra; Pankuch GA, Jacobs MR, Appelbaum PC., supra; Visalli MA, Jacobs MR, Appelbaum PC. Activity of CP 99,219 (trovafloxacin) compared with ciprofloxacin, sparfloxacin, clinafloxacin, lomefloxacin and cefuroxime against ten penicillin-susceptible and penicillin-resistant pneumococci by time-kill methodology. *J Antimicrob Chemother* 1996; 37: 77–84; Visalli MA, Jacobs MR, Appelbaum PC. supra).

As demonstrated, gemifloxacin is the most potent quinolone tested by MIC and time-kill against both quinolone-susceptible and -resistant pneumococci. While the incidence of quinolone-resistant pneumococci is currently very low, the introduction of broad-spectrum quinolones into clinical practice, particularly in the pediatric population, can lead to the selection of quinolone- resistant strains. Quinolones such as gemifloxacin may be used in the pediatric environment using appropriate doses determined by a physician. Additionally, for quinolone-resistant pneumococci , gemifloxacin can be employed as a theraputic option. Gemifloxacin is a promising new anti-pneumococcal agent, irrespective of the strains' susceptibility to quinolones and other agents.

Table 8. Microdilution MICs of 12 strains tested by time-kill

Antimicrobial agent	1 (S)[a]	2 (S)	3 (S)[b]	4 (S)[b]	5 (I)	6 (I)	7 (I)	8 (I)	9 (R)	10 (I)	11 (R)	12 (R)
Penicillin G	0.06	0.03	0.016	0.016	0.25	0.25	1	0.5	4	2	4	4
Gemifloxacin	0.016	0.016	0.5	0.25	0.03	0.016	0.016	0.016	0.03	0.016	0.016	0.03
Ciprofloxacin	1	0.5	32	32	2	1	4	0.5	1	1	2	1
Levofloxacin	2	1	32	32	1	2	1	1	2	2	1	2
Sparfloxacin	0.125	0.25	32	16	0.5	0.25	0.25	0.25	0.5	0.25	0.25	0.5
Grepafloxacin	0.06	0.06	16	8	0.125	0.125	0.125	0.125	0.25	0.125	0.125	0.25
Trovafloxacin	0.06	0.06	8	4	0.06	0.06	0.06	0.125	0.125	0.06	0.06	0.125
Amoxicillin	0.016	0.016	0.008	0.008	0.03	0.125	0.125	0.06	1	1	2	2
Cefuroxime	0.5	0.25	0.016	0.016	0.5	0.5	0.5	0.25	2	0.5	4	2
Azithromycin	0.008	0.06	>64	0.125	>64	0.03	0.125	0.125	>64	>64	0.125	>64
Clarithromycin	0.008	0.03	>64	0.03	32	0.008	0.016	0.03	>64	>64	0.03	>64

[a]S = penicillin susceptible; I = penicillin intermediate; R = penicillin resistant.
[b]Quinolone resistant.

Table 9. Time-kill results of 12 pneumococcal strains

Antimicrobial agent	3 hours			6 hours			12 hours			24 hours		
	-1[a]	-2[a]	-3[a]	-1	-2	-3	-1	-2	-3	-1	-2	-3
Gemifloxacin												
8 x MIC	10[b]	2	0	12	8	2	12	12	9	12	12	12
4 x MIC	9	1	0	12	8	0	12	12	8	12	12	12
2 x MIC	6	0	0	12	7	0	12	11	8	12	12	12
MIC	4	1	0	11	2	0	12	8	3	12	10	8
0.5 x MIC	1	0	0	4	0	0	3	0	0	2	2	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Ciprofloxacin												
8 x MIC	10	8	2	12	11	6	12	12	10	12	12	12
4 x MIC	9	6	1	12	10	5	12	12	10	12	12	12
2 x MIC	9	4	0	12	8	2	12	12	6	12	12	11
MIC	4	0	0	8	3	0	10	9	3	11	10	6
0.5 x MIC	0	0	0	1	1	0	2	1	0	2	1	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Levofloxacin												
8 x MIC	11	3	0	12	9	4	12	12	10	12	12	12
4 x MIC	10	4	0	12	9	1	12	12	8	12	12	12
2 x MIC	10	2	0	12	9	1	12	12	9	12	12	12
MIC	9	1	0	12	6	0	12	11	7	12	12	12
0.5 x MIC	4	1	0	8	1	0	7	3	0	8	7	5
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0

Antimicrobial agent	3 hours			6 hours			12 hours			24 hours		
	-1[a]	-2[a]	-3[a]	-1	-2	-3	-1	-2	-3	-1	-2	-3
Sparfloxacin												
8 x MIC	10	2	0	12	9	4	12	12	9	12	12	12
4 x MIC	9	1	0	12	8	0	12	11	8	12	12	12
2 x MIC	8	1	0	12	4	0	12	10	5	12	12	12
MIC	4	0	0	8	2	0	11	9	4	11	11	10
0.5 x MIC	1	0	0	5	1	0	4	0	0	6	4	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	1	1	1
Grepafloxacin												
8 x MIC	8	2	1	12	5	2	12	11	7	12	12	12
4 x MIC	6	0	0	12	4	0	12	10	5	12	12	12
2 x MIC	3	0	0	9	1	0	10	8	1	11	10	9
MIC	1	0	0	4	1	0	7	3	0	8	5	3
0.5 x MIC	0	0	0	0	0	0	1	0	0	1	1	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Trovafloxacin												
8 x MIC	12	3	0	12	10	1	12	12	9	12	12	12
4 x MIC	9	2	0	12	9	1	12	10	8	12	12	12
2 x MIC	5	1	0	11	4	0	12	11	7	11	11	11
MIC	4	0	0	6	2	0	7	4	1	6	1	1
0.5 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0

Antimicrobial agent	3 hours			6 hours			12 hours			24 hours		
	-1[a]	-2[a]	-3[a]	-1	-2	-3	-1	-2	-3	-1	-2	-3
Amoxicillin												
8 x MIC	9	4	0	11	8	3	12	12	10	12	12	12
4 x MIC	7	2	0	12	7	0	12	12	9	12	12	12
2 x MIC	6	2	0	11	5	1	12	11	8	12	12	12
MIC	4	0	0	6	1	0	7	6	2	10	9	7
0.5 x MIC	0	0	0	0	0	0	1	1	0	3	2	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	1	0	0
Cefuroxime												
8 x MIC	9	5	0	12	12	4	12	12	12	12	12	12
4 x MIC	9	3	0	12	11	1	12	12	11	12	12	12
2 x MIC	7	1	0	12	8	1	12	11	8	12	12	11
MIC	4	0	0	7	2	0	9	7	1	9	9	9
0.5 x MIC	3	0	0	1	0	0	0	0	0	1	1	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Azithromycin[c]												
8 x MIC	3	1	0	6	4	2	7	5	5	7	7	7
4 x MIC	4	1	0	6	3	2	6	5	4	7	7	7
2 x MIC	3	1	0	4	2	2	5	5	4	7	7	5
MIC	2	0	0	3	2	2	5	5	2	7	6	5
0.5 x MIC	1	0	0	1	1	1	1	1	1	1	1	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0

Antimicrobial agent	3 hours			6 hours			12 hours			24 hours		
	-1[a]	-2[a]	-3[a]	-1	-2	-3	-1	-2	-3	-1	-2	-3
Clarithromycin[c]												
8 x MIC	4	2	0	7	2	2	5	5	5	7	7	7
4 x MIC	3	2	0	7	2	2	5	5	5	7	7	7
2 x MIC	3	2	0	5	2	2	5	5	4	7	7	7
MIC	3	1	0	5	0	1	5	5	2	7	7	5
0.5 x MIC	1	0	0	3	0	0	1	1	1	4	3	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0

[a]ΔLog_{10} CFU/ml lower than 0 hours; [b]Number of strains tested; [c]Only 7 strains with macrolide MICs ≤0.125 μg/ml were tested.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: penicillin-susceptible, intermediate and resistant (including ciprofloxacin-resistant) pneumococci. Other pneumococcal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against maxillary sinus.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various bacterial pathogens. An objective of these analyses was to determine the efficacy of the novel fluoroquinolone, gemifloxacin, (SB-265805) compared with that of ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, grepafloxacin, moxifloxacin, sparfloxacin, amoxicillin and amoxicillin/clavulanic acid against a total of more than 250 strains isolated from recently acute or chronic maxillary sinusitis. The MICs were determined by agar dilution technique. The activity of gemifloxacin (MIC_{90} 0.06 μg/ml) is superior to that of ciprofloxacin, ofloxacin, levofloxacin, grepafloxacin, moxifloxacin and sparfloxacin (MIC_{90} ≥0.25 μg/ml) against the *Streptococcus pneumoniae* isolates. Against *Moraxella catarrhalis* and *Haemophilus influenzae,* gemifloxacin and grepafloxacin (MIC_{90} ≤0.02 μg/ml) are the most active antimicrobial agents tested. Against *Staphylococcus aureus*, gemifloxacin, trovafloxacin and moxifloxacin are more effective (MIC_{90} 0.06 μg/ml) than ciprofloxacin, amoxicillin and amoxicillin/clavulanic acid (MIC_{90} ≥1 μg/ml). A similar activity (MIC_{90} 0.25 μg/ml) was observed with gemifloxacin and moxifloxacin against anaerobic strains tested. The activity of gemifloxacin is similar to that of ofloxacin, trovafloxacin, moxifloxacin and sparfloxacin (MIC_{90} 0.5 μg/ml) against various other strains, such as some *Enterobacteriaceae* or non-fermentative Gram negative *bacilli*. Combined with favorable pharmacokinetics in humans, gemifloxacin can be a valuable oral compound for the treatment of acute or chronic sinusitis caused by isolates resistant to usual oral therapies.

Like other quinolones, gemifloxacin works by inhibiting the normal function of the

A subunit of DNA gyrase, a bacterial DNA topoisomerase. In susceptibility studies, gemifloxacin is appreciably more potent than most fluoroquinolones against many Gram positive organisms, including *Streptococcus pneumoniae*, *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus* spp. Gemifloxacin retains activity against a range of resistant Gram negative bacilli. It has potent activity against various anaerobic and atypical respiratory pathogens like *Legionella pneumophila*, *Mycoplasma* spp. and *Chlamydia* spp.

The objective of a study described herein was to determine the MIC of gemifloxacin, ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, grepafloxacin, moxifloxacin, sparfloxacin, amoxicillin and amoxicillin/clavulanic acid against a variety of strains such as *Haemophilus* spp., *S. pneumoniae* and *Moraxella catarrhalis*, isolated recently from acute or chronic maxillary sinus infections.

A variety of strains were obtained from recently maxillary sinus aspiration. Fresh isolates from specimens were cultured to produce pure culture. Multiple cultures from the same patient or source were excluded unless a change in organism or antibiogram was noted. Identification of organisms was done by standard methods (*See*, Murray et al., In: *Manual of Clinical Microbiology* (6th edition), American Society of Microbiology, 1995: pp 282–620). Antimicrobial activity was tested against 250 selected isolates (Table 10). Emphasis was placed on testing as many isolates as possible which contain commonly isolated sinusitis organisms or organisms that have demontrated resistance to common oral therapy.

The agar dilution method using replicate plating of the organisms onto a series of agar plates of increasing concentrations was used (*See,* National Committee for Clinical Laboratory Standards. Methods for antimicrobial susceptibility tests for bacteria that growth aerobically, Approved standards M 7-A4. National Committee for Laboratory Standards, Villanova, PA, 1997). A series of doubling dilutions have been done from 256–0.02 μg/ml.

Antibiotic was dissolved in water or corresponding dissolution solutions for the preparation of stock solutions. Antimicrobial stock solutions were prepared at concentrations of 1000 μg/ml or ten times the highest concentration tested whichever was greater. Small volumes of the sterile stock solutions were dispensed in sterile polypropylene vials, sealed carefully and were stored at -20°C or below -60°C. Vials were removed as needed and were used the same day. Any unused drug was discarded .

Mueller-Hinton (M-H) agar was used for routine susceptibility testing of aerobic and facultative anaerobic bacteria. M-H agar was supplemented with 5% defibrinated sheep blood

for testing those organisms that do not grow on the unsupplemented medium. The medium used for testing *Haemophilus* was *Haemophilus* Test Medium (HTM). The medium used for testing anaerobes is Wilkins-Chalgren agar. Mueller-Hinton agar, HTM or Wilkins-Chalgren agar were prepared from dehydrated base according to the manufacturer's recommendations. After it was autoclaved, the agar was allowed to cool to 48–50°C in a water bath before addition of antimicrobial solutions. The pH of each batch of agar was checked after autoclaving. The pH of the medium was 6.9–6.95 at 25°C. The measurement is done with a standard combination pH electrode or with a surface electrode.

Appropriate dilutions of antimicrobial solution were added to M-H agar, HTM or Wilkins-Chalgren which were allowed to equilibrate to 48–50°C in a water bath. The agar and antimicrobial solution was mixed thoroughly and poured into petri dishes on a level surface. The petri were poured as quickly as possible after the mixing to prevent cooling and partial solidification in the container. The agar depth was between 3 and 4 mm. The agar was allowed to solidify at room temperature. The plates were used immediately or were stored in plastic freezer bags at 4°C for up to 4 weeks. Following storage, the plates were allowed to equilibrate to room temperature before use. The surface was dried before inoculation.

The standardized inoculum was prepared by inoculating 4-5 colonies of a single type into a tube containing 5.0 ml of M-H broth. This bacterial suspension was incubated at 35°C until it was visibly turbid. The density of this inoculum was adjusted to a turbidity of 0.5 McFarland standard by addition of M-H broth. The adjusted suspension was diluted 1:10 in sterile sodium chloride solution to obtain the desired inoculum concentration of 10^7 CFU/ml. With the replicating device, the final inoculum on the agar contained 10^4 CFU in an area of 5–8 mm.

The surface of the agar was dried before inoculation. The plates were placed in an incubator with the lids open. The tubes containing the adjusted and diluted bacterial suspensions (10^7 CFU/ml) were arranged in order in a rack. An aliquot of each suspension was placed into the corresponding well in the replicator block. The inocula was applied to the agar surface with inocula replicating device. A control plate first and a second plate last were inoculated to insure that no contamination or antimicrobial carryover occurred during the inoculating process.

The inoculated plate was left at room temperature until the moisture in the inoculum was absorbed into agar. The plates were inverted and were incubated at 35°C for 24 hours in aerobic atmosphere for aerobes or facultative anaerobes, in atmosphere containing 5–7% CO_2 for *Haemophilus* and in anaerobic atmospheres for anaerobes .

The susceptibility results are presented in Tables 11-14. The susceptibility of Gram positive *cocci* to gemifloxacin compared with those of oral compounds is shown in Table 11. The activity of gemifloxacin against Gram positive cocci is significantly superior ($p < 0.05$) to some common oral compounds tested such as levofloxacin, ofloxacin, amoxicillin or amoxicillin/ clavulanic acid. Gemifloxacin inhibits 90% of Gram positive cocci at a concentration of 0.06 μg/ml. Against *S. pneumoniae*, the activity of gemifloxacin (MIC_{90} 0.06 μg/ml) is similar to trovafloxacin, but it is superior to ciprofloxacin, ofloxacin, levofloxacin, and sparfloxacin (MIC_{90} ≥0.5 μg/ml). Against *S. aureus* sinus pathogens, gemifloxacin, moxifloxacin, trovafloxacin (MIC_{90} 0.06 μg/ml) and sparfloxacin (MIC_{90} 0.12 μg/ml) are the most active compounds tested. The activity of gemifloxacin is more important than the activity of ciprofloxacin, amoxicillin (MIC_{90}1 μg/ml) and amoxicillin/clavulanic acid (MIC_{90} 2 μg/ml) against *S. aureus*.

Table 12 shows the susceptibility of *Haemophilus* spp. to gemifloxacin. The strains of *H. influenzae* are susceptible to gemifloxacin at a MIC_{90} of <0.02 μg/ml. This activity is significantly superior to ofloxacin, moxifloxacin, sparfloxacin, amoxicillin and amoxicillin/clavulanic acid. Against *Haemophilus parainfluenzae*, gemifloxacin (MIC_{90} 0.12 μg/ml) is still superior to ofloxacin (MIC_{90} 0.5 μg/ml), moxifloxacin (MIC_{90} 0.5 μg/ml), sparfloxacin (MIC_{90}1 μg/ml), amoxicillin (MIC_{90} 1 μg/ml) and amoxicillin/clavulanic acid (MIC_{90} 0.5 μg/ml).

Table 13 shows the susceptibility of strains of *M. catarrhalis* to gemifloxacin. Against *M. catarrhalis*, gemifloxacin and grepafloxacin (MIC_{90} ≤0.02 μg/ml) are the most active compounds tested. The activity of gemifloxacin is significantly superior to sparfloxacin, amoxicillin/clavulanic acid (MIC_{90} 0.5 μg/ml) and amoxicillin (MIC_{90} 8 μg/ml).

The susceptibility of various strains isolated from maxillary sinus is shown in Table 14. Against anaerobic strains, gemifloxacin (MIC_{90} 0.25 μg/ml) and moxifloxacin (MIC_{90} 0.25 μg/ml) are the most active agents tested. However the activity of gemifloxacin was significantly superior to ofloxacin (MIC_{90} 2 μg/ml), trovafloxacin (MIC_{90} 4 μg/ml), grepafloxacin (MIC_{90} 8 μg/ml), and sparfloxacin (MIC_{90} 16 μg/ml). Against other various strains, gemifloxacin is as active as ofloxacin, trovafloxacin, moxifloxacin and sparfloxacin (MIC_{90} 0.5 μg/ml).

Gemifloxacin shows a broad spectrum of antimicrobial activity against most strains isolated from acute or chronic maxillary sinusitis. Moreover, gemifloxacin appears to have a

better and constant activity than most beta-lactams such as amoxicillin and amoxicillin/clavulanic acid against many maxillary sinus isolates such as *S. aureus, Haemophilus* spp., *M. catarrhalis* and anaerobic strains. The overall *in vitro* activity of gemifloxacin is significantly greater than ciprofloxacin, ofloxacin, levofloxacin and sparfloxacin against strains of *S. pneumoniae*. Another attractive attribute of gemifloxacin is that the drug has significant activity against *Haemophilus* spp., *M. catarrhalis*, some anaerobic strains and other various strains tested such as non-fermentative Gram negative bacilli, *Neisseria meningitidis* and beta-hemolytic *Streptococcus*. Gemifloxacin, ciprofloxacin, grepafloxacin and trovafloxacin were the most active agents tested against *Haemophilus* spp. and *Moraxella* sinus isolates. This particular activity is markedly important against beta-lactamase producing strains of *H. influenzae* and also in patients who are allergic to penicillins. Combined with favorable pharmacokinetics in humans, gemifloxacin could be a valuable oral compound for the treatment of acute or chronic sinusitis caused by microrganisms resistant to usual oral therapy.

Table 10. Tested strains isolated from maxillary sinus pathogens

Isolates	Number of strains tested
Streptococcus pneumoniae	85
Haemophilus influenzae	45
Haemophilus parainfluenzae	10
Moraxella catarrhalis	45
Staphylococcus aureus	31
Anaerobes[a]	22
Other spp[b]	15

[a]Including *Peptostreptococcus* and *Bacteroides* spp.

[b]Including beta-hemolytic *Streptococcus* and Gram negative rods

Table 11. Susceptibility of Gram positive cocci

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)					
	Streptococcus pneumoniae (85)			*Staphylococcus aureus* (31)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Gemifloxacin	≤0.02–0.06	0.03	0.06	0.03–1	0.06	0.06
Moxifloxacin	≤0.02–0.25	0.12	0.25	0.03–0.12	0.06	0.06
Trovafloxacin	≤0.02–0.12	0.06	0.12	≤0.02–0.06	0.03	0.03
Grepafloxacin	0.03–0.5	0.25	0.25	0.06–0.25	0.12	0.12
Levofloxacin	0.12–2	1	1	0.12–0.5	0.25	0.25
Ofloxacin	0.25–4	2	2	0.25–1	0.5	0.5
Sparfloxacin	0.03–0.5	0.25	0.5	0.3–0.12	0.06	0.12
Ciprofloxacin	0.06–2	0.5	1	0.12–1	0.5	1
Amoxicillin	≤0.02–1	0.03	0.03	0.06–2	1	1
Amoxicillin/clavulanic acid	≤0.02–1	≤0.02	0.03	0.03–2	1	1

Table 12. Susceptibility of *Haemophilus* spp.

Antimicrobial agent	MIC (μg/ml) and isolate (No. tested)					
	Haemophilus influenzae (45)			*Haemophilus parainfluenzae* (10)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Gemifloxacin	≤0.02–0.03	≤0.02	≤0.02	≤0.02–0.12	0.06	0.12
Moxifloxacin	≤0.02–0.12	0.03	0.06	0.06–0.5	0.25	0.5
Trovafloxacin	≤0.02–0.06	≤0.02	0.03	≤0.02–0.12	0.03	0.12
Grepafloxacin	≤0.02–0.03	≤0.02	≤0.02	≤0.02–0.12	0.06	0.12
Levofloxacin	≤0.02–0.03	0.03	0.03	0.03–0.25	0.06	0.25
Ofloxacin	≤0.02–0.06	0.03	0.06	0.03–0.5	0.12	0.5
Sparfloxacin	0.03–1	0.25	0.25	0.12–1	0.5	1
Ciprofloxacin	≤0.02	≤0.02	≤0.02	≤0.02–0.06	0.03	0.06
Amoxicillin	0.06–64	0.25	2	0.03–1	0.06	1
Amoxicillin/clavulanic acid	≤0.02–1	0.25	0.5	0.03–0.5	0.25	0.5

Table13. Susceptibility of *Moraxella catarrhalis*

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)		
	M. catarrhalis (45)		
	Range	MIC_{50}	MIC_{90}
Gemifloxacin	≤0.02–0.03	≤0.02	≤0.02
Moxifloxacin	0.03–0.12	0.06	0.06
Trovafloxacin	≤0.02–0.06	≤0.02	0.03
Grepafloxacin	≤0.02–0.25	≤0.02	≤0.02
Levofloxacin	≤0.02–0.12	0.03	0.06
Ofloxacin	≤0.02–0.25	0.06	0.06
Sparfloxacin	≤0.02–1	≤0.02	0.5
Ciprofloxacin	≤0.02–0.25	0.03	0.03
Amoxicillin	≤0.02–16	1	8
Amoxicillin/clavulanic acid	≤0.02–2	0.12	0.5

Table 14. Susceptibility of various anaerobic and *Streptococcus* strains

Antimicrobial agent	MIC (μg/ml) and isolate (number tested)					
	Anaerobic strains (22)[a]			*Streptococcus* strains (15)[b]		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Gemifloxacin	0.03–0.25	0.12	0.25	≤0.02–0.5	0.12	0.5
Moxifloxacin	0.03–0.25	0.03	0.25	≤0.02–0.5	0.06	0.5
Trovafloxacin	0.06–4	1	4	≤0.02–0.5	0.06	0.5
Grepafloxacin	0.25–8	0.25	8	≤0.02–1	0.06	1
Levofloxacin	0.12–1	0.25	1	0.03–0.25	0.12	0.25
Ofloxacin	0.25–2	0.5	2	0.06–0.5	0.25	0.5
Sparfloxacin	0.25–16	4	16	≤0.02–0.5	0.03	0.5
Ciprofloxacin	0.06–1	0.5	1	≤0.02–0.12	0.12	0.12
Amoxicillin	0.25–8	0.25	8	0.03–≥256	2	4
Amoxicillin/clavulanic acid	0.25–1	0.25	1	0.03–≥256	2	16

[a]Including 12 strains of *Bacteroides* spp., 7 strains of *Peptostreptococcus* spp. and 3 strains of *Bacteroides urealyticus*.
[b]Including 5 strains of Enterobacteriaceae, 6 strains of non-fermentative Gram negative bacilli, 2 strains of *Neisseria meningitidis* and 2 strains of beta-hemolytic *Streptococcus*.

Also provided by the invention is a method of treating or preventing a bacterial infection by maxillary sinus pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with maxillary sinus pathogenic bacteria.

A preferred object of the invention provides a method wherein said maxillary sinus pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae, Haemophilus influenzae, Haemophilus parainfluenzae, Moraxella catarrhalis, Staphylococcus aureus, Peptostreptococcus spp., Bacteroides urealyticus, Enterobacteriaceae,* non-fermentative Gram negative *bacilli, Neisseria meningitidis, Bacteroides spp., beta-hemolytic Streptococcus* and Gram negative rods. Other maxillary sinus pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various Gram negative, non-fermenting bacterial pathogens, especially especially *Pseudomonas. Aeruginosa, Acinetobacter* spp., *Stenotrophomonas maltophilia.*

The *in vitro* activity of the fluoroquinolone gemifloxacin (SB-265805) against 178 Gram negative, non-fermenting clinical isolates was compared with that of other selected antimicrobials. An objective of these analyses was to determine the MICs of gemifloxacin, ciprofloxacin, ofloxacin, trovafloxacin, grepafloxacin, levofloxacin, gentamicin and co-trimoxazole, which was accomplished by the broth microdilution technique in cation-adjusted Mueller–Hinton broth against 50 isolates each of *Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Acinetobacter* spp. and against 28 strains of *Burkholderia cepacia*. The MIC_{50}/MIC_{90} values obtained are shown in Table 15.

Gemifloxacin is highly potent activity against a wide range of Gram negative and Gram positive bacteria. An aspect of the invention is based, in part, on a further analysis examined the susceptibility of clinically relevant, non-fermentative, Gram negative bacilli to gemifloxacin in comparison with other fluoroquinolones, gentamicin and co-trimoxazole.

A total of 178 clinical isolates of Gram negative, non-fermentative bacteria were investigated: *Pseudomonas aeruginosa* (n = 50), *Acinetobacter* spp. (n = 50),

Stenotrophomonas maltophilia (n = 50) and *Burkholderia cepacia* (n = 28). The MICs of gemifloxacin, ciprofloxacin, ofloxacin, trovafloxacin, grepafloxacin, levofloxacin, gentamicin and co-trimoxazole were determined using the broth microdilution technique in cation-adjusted Mueller–Hinton broth according to NCCLS guidelines. The MIC endpoint was interpreted as the lowest concentration of drug to prevent visible bacterial growth.

Table 15 shows the *in vitro* antibacterial activities of gemifloxacin and other antimicrobials against Gram negative, non-fermentative bacteria. Tables 16 and 17 present the MIC distributions of gemifloxacin and ciprofloxacin, respectively. MIC distributions of gemifloxacin, ciprofloxacin, levofloxacin and trovafloxacin against *P. aeruginosa*, *Acinetobacter* spp. and *S. maltophilia* are illustrated in Figures 8 and 9, respectively.

Gemifloxacin and ciprofloxacin are more active than trovafloxacin and levofloxacin against *P. aeruginosa*. *Acinetobacter* isolates are most susceptible to trovafloxacin and gemifloxacin, and 80% or more of the strains are inhibited by 1 μg/ml of all fluoroquinolones tested. Gemifloxacin and trovafloxacin are the most active compounds tested against *S. maltophilia*. Ciprofloxacin was the least active agent, although more than 50% of *S. maltophilia* strains had MICs of ≥2 μg/ml. All the quinolones tested demonstrate poor activity against *B. cepacia*.

Table 15. *In Vitro* Antibacterial Activities of Gemifloxacin and Other Antimicrobials Against Gram Negative, Non Fermentative Bacteria

Microrganism	**Antimicrobial**	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
Pseudomonas aeruginosa (n = 50)	Gemifloxacin	0.12–64	0.5	8
	Ciprofloxacin	0.5–32	0.5	8
	Ofloxacin	0.5–128	4	16
	Trovafloxacin	0.12–32	1	16
	Grepafloxacin	0.12–32	1	16
	Levofloxacin	0.12–32	1	8
	Gentamicin	0.5–128	4	16
	Co-trimoxazole	0.5–128	64	128

Microrganism	**Antimicrobial**	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
Acinetobacter spp. (n = 50)	Gemifloxacin	0.004–8	0.06	1
	Ciprofloxacin	0.03–32	0.06	4
	Ofloxacin	0.12–32	0.25	4
	Trovafloxacin	0.015–8	0.03	1
	Grepafloxacin	0.015–16	0.06	2
	Levofloxacin	0.06–8	0.12	2
	Gentamicin	0.06–128	0.5	32
	Co-trimoxazole	0.06–128	0.12	2
Stenotrophomonas maltophilia (n = 50)	Gemifloxacin	0.06–32	2	8
	Ciprofloxacin	0.25–32	4	32
	Ofloxacin	0.25–64	8	16
	Trovafloxacin	0.06–32	2	8
	Grepafloxacin	0.06–16	2	8
	Levofloxacin	0.12–32	2	8
	Gentamicin	2–128	128	>128
	Co-trimoxazole	0.12–128	4	32
Burkholderia cepacia (n = 50)	Gemifloxacin	2–16	16	16
	Ciprofloxacin	4–32	16	16
	Ofloxacin	8–128	32	128
	Trovafloxacin	8–32	32	32
	Grepafloxacin	4–32	16	16
	Levofloxacin	4–32	16	16
	Gentamicin	16–128	128	128
	Co-trimoxazole	0.5–128	2	4

Table 16. MIC Distribution of Gemifloxacin

Microrganism	% of isolates inhibited at MIC (μg/ml)												
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Pseudomonas. Aeruginosa	0	0	0	10	40	62	64	80	86	92	94	98	100
Acinetobacter spp.	14	48	74	80	82	82	90	94	98	100			
Stenotrophomonas maltophilia	0	0	2	4	6	10	44	80	82	94	96	100	
Burkholderia cepacia	0	0	0	0	0	0	0	4	4	33	100		

Table 17. MIC Distribution of Ciprofloxacin

Microrganism	% of isolates inhibited at MIC (μg/ml)											
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Pseudomonas aeruginosa	0	0	2	24	40	64	74	86	90	92	94	100
Acinetobacter spp.	0	4	14	54	70	80	86	88	94	94	94	100
Stenotrophomonas maltophilia	0	0	0	0	4	4	6	14	50	84	96	100
Burkholderia cepacia	0	0	0	0	0	0	0	0	4	7	100	

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram negative, non-fermenting pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with Gram negative, non-fermenting pathogenic bacteria.

While a preferred object of the invention provides a method wherein said Gram negative, non-fermenting pathogenic bacteria is selected from the group consisting of: *Pseudomonas. Aeruginosa, Acinetobacter* spp., *Stenotrophomonas maltophilia* and *Burkholderia cepacia* bacteria. Other Gram negative, non-fermenting pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Chlamydia pneumoniae* or respiratory tract bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Chlamydia pneumoniae* or respiratory tract pathogens. An objective of these analyses was to determine the *in vitro* activity of gemifloxacin as compared with that of levofloxacin, moxifloxacin, trovafloxacin, erythromycin and doxycycline against 20 isolates of *C. pneumoniae*, including recent clinical isolates.

Gemifloxacin has potent activity against respiratory tract pathogens, including *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae* and *Legionella* spp. The activity of gemifloxacin, levofloxacin, moxifloxacin, trovafloxacin, erythromycin and doxycycline was compared against 20 isolates of *Chlamydia pneumoniae,* including the prototype strain TW183, four other laboratory strains from the US and Japan plus 15 recent US clinical isolates from adults with community-acquired pneumonia. Testing was carried out in cycloheximide-treated HEp-2 cells. The results are summarized in Table 18.

Of the compounds studied, Gemifloxacin is the most active quinolone against *C. pneumoniae*, but is less active than erythromycin and doxycycline. The potent, broad-spectrum activity of gemifloxacin indicates that it has a role in the treatment of respiratory tract infections, including those caused by *C. pneumoniae.*

Chlamydia pneumoniae is a frequent cause of community-acquired respiratory tract infection (RTI), including pneumonia and bronchitis in adults and children. Quinolones have attracted interest as potential therapy for community-acquired RTI because they are active against a wide range of pathogens responsible for these infections, such as *Mycoplasma pneumoniae, Streptococcus pneumoniae* (including penicillin-resistant strains) and *C. pneumoniae*.

C. pneumoniae isolates tested were: TW-183 (Washington Research Foundation, Seattle, WA, USA), CM-1 (ATCC 1360), J21 (from Japan, ATCC VR1435), W6805, T2219 and 15 recent clinical isolates from adults enrolled in a multicenter community-acquired pneumonia treatment study.

Testing was performed in HEp-2 cells grown in 96-well microtiter plates. Each well was inoculated with 0.1 ml of the test strain diluted to yield 10^3–10^4 inclusion-forming units (IFU) per ml, centrifuged at 1700 x g for 1 h and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with 0.2 ml of medium containing 1 μg of cycloheximide per ml and serial twofold dilutions of the test drug. After incubation at 35°C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen (Pathfinder, Kallestad Diagnostics, Chaska, MN, USA).

Herien "MIC" means the lowest antimicrobial concentration at which no inclusions are seen. The minimal bactericidal concentration ("MBC") is defined as the lowest antimicrobial concentration that resulted in no inclusions after passage in antimicrobial-free cells. All tests were run in triplicate.

The MICs and MBCs for *C. pneumoniae* are given in Table 1

Of the compounds tested, gemifloxacin is the most active quinolone. The MICs obtained for gemifloxacin against *C. pneumoniae* are consistent from strain to strain. Data on the activity of gemifloxacin against *C. pneumoniae* are limited, in part due to the relatively small number of clinical isolates available for testing. When Ridgway *et al.* tested five isolates of *C. pneumoniae*, a MIC range of 0.06–0.12 μg/ml was obtained. (*See*, Ridgway GL, Salman N, *et al. In: Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC, USA: American Society for Microbiology, 1998: p 256, abstract F-97.) This is within the standard error of the test or secondary to the methods used.

The activity of the other quinolones tested in this study, levofloxacin, moxifloxacin and trovafloxacin, is the same as those previously reported using different selections of isolates. (*See*, Block S, Hedrick J, *et al. Mycoplasma pneumoniae* and *Chlamydia*

pneumoniae in community acquired pneumonia in children: comparative safety and efficacy of clarithromycin and erythromycin suspensions. *Pediatr Infect Dis J* 1995; 14: 471–477; Cormican MG, Jones RN. Antimicrobial activity and spectrum of LB20304, a novel fluoronaphthyridone. *Antimicrob Agents Chemother* 1997; 41: 204–211; Grayston JT, Campbell LA, *et al.* A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. *J Infect Dis* 1990; 161: 618–625;Hammerschlag MR. Community-acquired pneumonia due to atypical organisms in adults: diagnosis and treatment. *Infect Dis Clin Pract* 1999; 8: 232–240; Moore T, Niconovich N, *et al. In: Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC, USA: American Society for Microbiology, 1998: p 257, abstract F-98; Oh J-I, Paek K-S, *et al. In vitro* and *in vivo* evaluation of LB20304, a new fluoronaphthyridone. *Antimicrob Agents Chemother* 1996; 40: 1564–1568; Roblin PM, Dumornay W, *et al.* Use of HEp-2 cells for improved isolation and passage of *Chlamydia pneumoniae*. *J Clin Microbiol* 1992; 30: 1968–1971). The broad-spectrum activity of gemifloxacin *in vitro* indicates that it may have a role in the treatment of RTI, including *C. pneumoniae* infections.

An aspect of the invention provides a method for modulating metabolism of *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria. Skilled artisans can readily choose *Chlamydia pneumoniae* or respiratory tract pathogenic pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Table 18. Activity of Gemifloxacin and Other Antimicrobials Against 20 Isolates of *C. pneumoniae* (50% and 90% MIC or MBC for 50% and 90% of Strains, Respectively)

Antimicrobial	MIC (μg/ml)		MBC (μg/ml)	
	Range	90%	Range	90%
Gemifloxacin	0.125–0.25	0.25	0.125–0.25	0.25
Levofloxacin	0.25–1	1	0.25–1	1
Moxifloxacin	0.125–1	1	0.125–1	1
Trovafloxacin	0.5–1	1	0.5–1	1
Erythromycin	0.008–0.06	0.06	0.008–0.06	0.06
Doxycycline	0.016–0.06	0.06	0.016–0.06	0.06

A quinolone, particularly a gemifloxacin compound or composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria, preferably a *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria, shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections caused by or related to *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria.

In addition to the therapy described above, a gemifloxacin compound or composition used in the methods of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins, particularly *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria, exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, a quinolone, particularly a gemifloxacin compound or composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1μg/ml to 10mg/ml for bathing of wounds or indwelling devices.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria is selected from the group consisting of: *Chlamydia pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae* and *Legionella* spp. Other *Chlamydia pneumoniae* or respiratory tract pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against respiratory tract bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various respiratory tract pathogens. An objective of these analyses was to determine the effect of gemifloxacin against ciprofloxacin-resistant (cip^r) strains of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Gemifloxacin was compared to amoxycillin/clavulanate, ciprofloxacin, cefuroxime, grepafloxacin, levofloxacin, azithromycin, trovafloxacin or tosufloxacin. Gemifloxacin, a fluoroquinolone agent with a broad spectrum of antibacterial activity, has excellent *in vivo* efficacy against ciprofloxacin-susceptible (cip^s) strains of *Streptococcus pneumoniae* and *Haemophilus influenzae*. Animals were infected via intrabronchial instillation to produce pneumonia and oral therapy was initiated at 1 h or 24 h with amoxycillin/clavulanate, ciprofloxacin, cefuroxime, grepafloxacin, levofloxacin, azithromycin, trovafloxacin or tosufloxacin. The doses were chosen to approximate, in the rat serum or tissue, concentrations measured in man following therapeutic dosing. Therapy continued o.d. or b.i.d. for 3 days, and at 96 h post-infection the lungs were excised for the recovery and enumeration of viable bacterial numbers.

Following infection with cip^r strains of *S. pneumoniae*, gemifloxacin produced a marked response, reducing bacterial numbers significantly ($p = <0.01$) compared with no treatment. None of the other fluoroquinolone antimicrobials were better at reducing bacterial numbers than gemifloxacin, and ciprofloxacin, trovafloxacin and levofloxacin were significantly ($p = <0.05$) less efficacious. Against infection caused by cip^r *H. influenzae*, gemifloxacin reduced bacterial numbers significantly ($p = <0.01$) compared with no treatment and was as effective as amoxycillin/clavulanate. In contrast, ciprofloxacin, grepafloxacin, trovafloxacin and tosufloxacin were ineffective and bacterial numbers were not significantly different ($p = >0.05$) to those in untreated animals. This data indicates a high potential benefit for the use of gemifloxacin in the treatment of respiratory tract infections caused by cip^r *S. pneumoniae* and *H. influenzae*

Respiratory tract infections (RTI), including pneumonia, bronchitis, sinusitis and otitis media, are common causes of morbidity and mortality worldwide. *Streptococcus pneumoniae* is an important pathogen in upper and lower RTI. β-Lactam and macrolide antimicrobials have been the treatment of choice for RTI, but recently new quinolones have become available for clinical and/or investigational use. These agents have a broad spectrum of antibacterial activity, including against penicillin-resistant *S. pneumoniae*. However, their use may be limited by the increasing incidence of resistance against them found in strains of *S. pneumoniae*. Gemifloxacin is a fluoroquinolone antimicrobial

currently under development for the treatment of community-acquired infections and has excellent *in vivo* efficacy against ciprofloxacin-sensitive strains of *S. pneumoniae* and *H. influenzae*. The studies described here demonstrate the efficacy of gemifloxacin mesylate in comparison with ciprofloxacin, grepafloxacin, levofloxacin, trovafloxacin, amoxycillin/clavulanate, cefuroxime and azithromycin against experimental RTI in rats caused by ciprofloxacin-resistant *S. pneumoniae* and *H. influenzae*.

Bacterial inocula for *S. pneumoniae* were prepared by harvesting growth from blood agar plates and suspending it in phosphate-buffered saline (PBS). *H. influenzae* was grown as an overnight culture in Mueller–Hinton broth with the addition of 5% Fildes extract. Tenfold dilutions of each organism were prepared into cooled (41°C) molten nutrient agar. Animals were anesthetized and infected by intrabronchial instillation of a 50 µl inoculum for *S. pneumoniae* (6.0–6.7 $\log_{10}$ CFU) and 100 µl for *H. influenzae* (5.2 $\log_{10}$ CFU) via intratracheal intubation (Smith, *et al.*, Antimicrob. Agents Chemother., 38: 608-610 (1994)). For *S. pneumoniae* infections, oral therapy was initiated 1 h post-infection with a further dose at 5 h and continued o.d. or b.i.d for 3 days. Treatment for *H. influenzae* infection commenced 24 h post-infection and continued o.d. or b.i.d. for 3 days. Further groups of rats received distilled water and served as infected control animals. Approximately 17 h after cessation of therapy the animals were killed, and the lungs excised and homogenized in 1 ml of PBS to enable the recovery and enumeration of viable bacteria.

The MICs of these agents to the infecting organisms are shown in Table 19. Doses administered were chosen to approximate in the rat the serum or tissue AUCs achieved in man following therapeutic dosing (Table 20) (Wise, *et al.*, *J. Antimicrob. Chemother.*, 18 (Suppl D): 71-81 (1986); Harding, *et al.*, *Antimicrob. Agents Chemother.*, 25: 78-82 (1984); Foulds, *et al.*, *J. Antimicrob. Chemother.*, 25 (Suppl. A): 73-82 (1990); Teng, *et al.*, *J. Antimicrob. Chemother.*, 39 (Suppl. B): 87-92 (1997); Chien, *et al.*, *Antimicrob. Agents. Chemother.*, 41: 2256-2260 (1997); Minami, *et al.*, *Antimicrob. Agents Chemother.*, 42: 453-455 (1998); Efthymiopoulous, *J. Antimicrob. Chemother.*, 40 (Suppl. A): 35-43 (1997). In order to extend the period of exposure while maintaining the same AUC, gemifloxacin and selected agents were administered twice daily at half the dose in specific studies.

Subsequent to infection with *S. pneumoniae* 622286 (Figure 10), gemifloxacin produces a response and reduces bacterial counts significantly compared with untreated animals (2.4 ± 1.1 and 6.4 ± 1.3 $\log_{10}$ CFU/lungs; $p = <0.01$). Amoxycillin/clavulanate and

cefuroxime have a potent effect (≤1.69 $\log_{10}$ CFU/lungs; p = <0.01), while azithromycin produces a variable response and mean bacterial counts are similar to those in untreated animals (6.4 ± 2.0 $\log_{10}$ CFU/lungs; p = >0.05). Of the comparator quinolones tested, tosufloxacin demonstrates the best effect, similar to that of gemifloxacin (3.9 ± 2.3 $\log_{10}$ CFU/lungs p = >0.05). Gemifloxacin is significantly better (p = <0.01) at reducing bacterial numbers than azithromycin and the fluoroquinolones ciprofloxacin, trovafloxacin, grepafloxacin and levofloxacin.

Following infection with *S. pneumoniae* 305313, bacterial numbers in untreated animals are 7.1 ± 1.4 $\log_{10}$ CFU/lungs (Figure 11). Gemifloxacin produces a response, similar to that seen with amoxycillin/clavulanate, cefuroxime and azithromycin (2.1 ± 1.4, ≤1.69, ≤1.69, ≤1.69 $\log_{10}$ CFU/lungs, respectively; p = >0.05). Ciprofloxacin, tosufloxacin and levofloxacin are ineffective (6.7 ± 1.0, 6.1 ± 0.7 and 6.7 ± 0.21 $\log_{10}$ CFU/lungs, respectively; p = >0.05). The response obtained with grepafloxacin and trovafloxacin is significantly different compared with that in untreated controls (2.6 ± 1.0 and 4.3 ± 1.0 $\log_{10}$ CFU/lungs; p = <0.01); the reduction in bacterial numbers with grepafloxacin is similar (p = >0.05) to that obtained with gemifloxacin and trovafloxacin is significantly less effective (p = <0.01). Of interest, the effect obtained with grepafloxacin in this study is better than would be expected from the *in vitro* activity against this strain.

The effect of extending the period of exposure of gemifloxacin while maintaining the same AUC was also examined in direct comparison with grepafloxacin, levofloxacin and trovafloxacin using twice-daily dosing of 150, 100, 62.5 and 20 mg/kg, respectively (Figure 12). Bacterial numbers isolated from animals infected with *S. pneumoniae* 305313 are 7.9 ± 0.4, and they are significantly higher (p = <0.01) than those obtained from treated animals. However, gemifloxacin produces the greatest response and is significantly more effective than grepafloxacin, levofloxacin and trovafloxacin (3.3 ± 1.3, 6.9 ± 0.5, 5.7 ± 1.3 and 5.3 ± 1.2 $\log_{10}$ CFU/lungs, respectively; p = <0.01).

Following infection with *H. influenzae* HI43 (ciprofloxacin resistant), bacterial numbers in untreated animals are 5.1 ± 1.2 $\log_{10}$ CFU/lungs and are similar to those obtained from ciprofloxacin, grepafloxacin, trovafloxacin and tosufloxacin treated animals (4.3 ± 1.2, 3.7 ± 1.4, 3.9 ± 1.8 and 3.8 ± 1.4 $\log_{10}$ CFU/lungs, respectively; p = >0.05) (Figure 13). In contrast, gemifloxacin produces a response which compared favorably with that of amoxycillin/clavulanate, azithromycin, cefuroxime and levofloxacin (2.5 ± 1.5, 1.8± 0.3, <1.69, 2.8 ± 1.3 and 3.1± 1.4 $\log_{10}$ CFU/lungs, respectively; p = >0.05).

Gemifloxacin affords protection against ciprofloxacin-resistant strains of *S.*

pneumoniae and *H. influenzae*, and bacterial numbers are reduced significantly compared with those in untreated animals. Overall, gemifloxacin demonstrates a superior effect compared with comparator quinolones against ciprofloxacin-resistant *S. pneumoniae* strains. Against ciprofloxacin-resistant strains of *H. influenzae* gemifloxacin has equivalent efficacy to amoxycillin/clavulanate, azithromycin, cefuroxime and levofloxacin, whereas ciprofloxacin, grepafloxacin, trovafloxacin and tosufloxacin are ineffective. The data presented here indicate a high benefit for the use of gemifloxacin in the treatment of RTI caused by ciprofloxacin-resistant strains of *S. pneumoniae* and *H. influenzae*.

Table 19. Susceptibility of the Bacterial Strains to the Antimicrobials Tested (MICs)

Microrganism	MIC (μg/ml)							
	Gemifloxacin	Ciprofloxacin	Grepafloxacin	Levofloxacin	Trovafloxacin	Tosufloxacin	Azithromycin	Cefuroxime	Amoxycillin/ clavulanate
Streptococcus pneumoniae 622286	0.125	32.0	4.0	4.0	4.0	2.0	≥64.0	≤0.06	0.125/0.06
Streptococcus pneumoniae 305313	0.125	16.0	0.5	1.0	2.0	<0.008	<0.06	16.0	≤0.06/0.03
Haemophilus influenzae HI43	0.125	1.0	*	2.0	1.0	*	0.125	4.0	2/1.0

Table 20. Doses Chosen to Approximate in the Rat the Serum or Tissue AUCs Achieved in Man Following Therapeutic Dosing

Treatment	Rat		Man		Reference
	Dose (mg/kg)	AUC (μg.h/ml)	Dose (mg)	AUC (μg.h/ml)	
Gemifloxacin mesylate o.d.	300	6.9	400	8.0	
Gemifloxacin mesylate b.i.d.	150	3.5			
Ciprofloxacin b.i.d.	200	13.8	750	14.9	2
Cefuroxime b.i.d.	70	14.3	250	14.0	3
Azithromycin o.d.	40/20	3.4	1000/500	4.1	4
Trovafloxacin o.d.	40	36.1	200	30.4	5
Trovafloxacin b.i.d.	20	18.0			
Levofloxacin o.d.	125	48.0	500	47.5	6
Levofloxacin b.i.d.	62.5	24.0			
Tosufloxacin b.i.d.	25	7.7	600	5.6	7
Grepafloxacin o.d.	200	17.0	600	19.7	8
Grepafloxacin b.i.d.	100	8.5			

Also provided by the invention is a method of treating or preventing a bacterial infection by respiratory tract pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with respiratory tract pathogenic bacteria.

While a preferred object of the invention provides a method wherein said respiratory tract pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae* and *Haemophilus influenzae*. Other respiratory tract pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against bacterial meningitis bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various bacterial meningitis pathogens. An objective of these analyses was to determine the efficacy of gemifloxacin in comparison with vancomycin, cefotaxime, cefuroxime, ciprofloxacin, levofloxacin and trovafloxacin against infant rat meningitis caused by *S. pneumoniae* 1629..

Gemifloxacin (100 mg/kg) is highly effective against *S. pneumoniae* meningitis and resulted in 83% survival compared with no treatment ($p = \leq 0.01$; no animal survived until day 7). No comparator agent at corresponding doses afforded significantly ($p = \geq 0.05$) greater protection against *S. pneumoniae* meningitis than gemifloxacin. This data indicates the benefit of gemifloxacin in the treatment of bacterial meningitis.

Bacterial meningitis in children results in substantial morbidity and mortality.(Baraff, *et al. Pediatric Infectious Disease Journal*, 12: 389-394 (1993)). Neurologic effects, such as paralysis, seizures or hearing loss, can result from this serious infection. One of the key pathogens involved in bacterial meningitis in children is *Streptococcus pneumoniae*. (Kornelisse, *et al., Clin. Infec. Dis.*, 21: 1390-1397 (1995)). Gemifloxacin is a broad-spectrum fluoroquinolone with excellent potency *in vitro* against strains of *S. pneumoniae*. The studies described here demonstrate the efficacy of gemifloxacin mesylate in comparison with vancomycin, cefotaxime, cefuroxime, ciprofloxacin, levofloxacin and trovafloxacin against infant rat meningitis caused by *S. pneumoniae* 1629.

The *in vivo* activity of gemifloxacin was evaluated in an experimental meningitis model in infant rats caused by *Streptococcus pneumoniae*. Cerebral spinal fluid was obtained from the rats 15 h after infection by puncture of the cisterna magna for confirmation of meningitis. Therapy (50 and/or 100 mg/kg i.p.) commenced 15 h post-infection with gemifloxacin, vancomycin, cefotaxime, cefuroxime, ciprofloxacin, levofloxacin, trovafloxacin or ceftriaxone. Treatment continued o.d. or b.i.d. for a further 3 days and survival was monitored for up to 7 days post-infection.

Female Sprague-Dawley (CD) rats (Taconic Farms, New York, NY, USA) were housed one per cage and fed lab chow and water *ad libitum*. The rats were allowed to give birth naturally and the infant rats were infected with *S. pneumoniae* at 5 days of age. The litter remained with the dam for the duration of the study. *S. pneumoniae* 1629 was grown on chocolate II agar plates for 18 h in the presence of 5% CO_2 at 37°C and the growth was harvested to prepare a stock suspension. The concentration of the stock was determined by an absorbance (560 nm) assay and infecting inocula were confirmed by viable counts (100 µl spread) onto trypticase soy agar (TSA) with 5% sheep blood.

Meningitis was established based on a method described previously. (Delaplane, *et al., J. Antimicrob. Chemother.*, 11: 69-73 (1983)). Briefly, *S. pneumoniae* 1629 (20–80 CFU/rat) was injected i.p. into 5-day-old rats. At 15 h after infection, a sample was taken from the cerebrospinal fluid (CSF) of each rat to determine meningitic status. Therapy was initiated immediately after the 15 h sample and was continued o.d. or b.i.d for 3 days. Further groups of animals received phosphate-buffered saline (PBS) and served as infected controls. The rats were monitored for survival for the duration of the experiment (7 days). On days 4 and 7 after infection, CSF and blood samples were taken from surviving animals. CSF samples (10 µl) were obtained by puncture of the cisterna magna and blood samples (10 µl) were taken from the tail vein for the enumeration of viable bacterial numbers. CSF samples were visually inspected and any CSF samples found to contain blood were excluded from the study. Samples were plated onto TSA with 5% sheep blood and incubated overnight with 5% CO_2 at 37°C. Survival data were compared using Fisher's exact test.

The MICs of the agents to *S. pneumoniae* 1629 are shown in Table 21. Concentrations of the agents in blood and CSF were measured to determine the AUC in infant rats (Table 22).

On day 7 after infection there were no survivors (0%) in the PBS-treated group (Figure 14). All therapies increased survival rates significantly ($p = \leq 0.03$) compared with

untreated animals. Gemifloxacin at 100 mg/kg and 50 mg/kg afforded good protection, and survival rates of 83% and 80%, respectively, were achieved ($p = \leq 0.01$). Ciprofloxacin (100 mg/kg) and levofloxacin (100 mg/kg) were also highly effective, with survival rates of 100% and 88%, respectively ($p = \leq 0.01$). Trovafloxacin, tested at 50 mg/kg because toxicity was seen previously at 100 mg/kg, produced a slightly lower survival rate of 56% ($p = \leq 0.03$), but this was not significantly different to that attained with a corresponding dose of gemifloxacin or to the therapeutic agents tested at 100 mg/kg ($p = \geq 0.05$). Vancomycin, included as a standard agent, produced a survival rate of 83% ($p = \leq 0.01$).

Gemifloxacin significantly increases the survival rate of infant rats compared with PBS-treated controls (83% and 0%, respectively [Figure 15]; $p = \leq 0.01$) and compares favorably ($p = \geq 0.05$) with cefuroxime and cefotaxime survival rates (100%; $p = \leq 0.01$). Vancomycin, included as an antimicrobial control, produces 80% survival ($p = \leq 0.01$). Bacterial numbers in blood and CSF samples taken from surviving rats on day 4 and day 7 post-infection were below the level of detection (≤ 2 $\log_{10}$ CFU/ml).

Gemifloxacin affords protection against an experimental meningitis infection caused by a penicillin-sensitive strain of *S. pneumoniae* in infant rats. The survival rates for rats administered gemifloxacin compares favorably with those obtained with the competitor quinolones tested. Gemifloxacin is as effective as the cephalosporin antimicrobials cefuroxime and cefotaxime against *S. pneumoniae* meningitis. No comparator agent at corresponding doses was shown to result in significantly greater protection against *S. pneumoniae* meningitis in infant rats than gemifloxacin. The efficacy demonstrated in these studies with *S. pneumoniae* indicates a benefit for the use of gemifloxacin in the treatment of bacterial meningitis.

The invention provides a method for modulating metabolism of bacterial meningitis pathogenic bacteria. Skilled artisans can readily choose bacterial meningitis pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention can be those described herein.

Table 21. MICs of the Test Antimicrobials to *S. pneumoniae* 1629

Microrganism	Antimicrobial (μg/ml)					
	Gemifloxacin	Ciprofloxacin	Levofloxacin	Trovafloxacin	Cefotaxime	Cefuroxime	Vancomycin
S. pneumoniae 1629	0.008	0.5	0.5	0.06	≤0.016	≤0.06	0.125

Table 22. Pharmacokinetics and Percentage Penetration into CSFFor Test Antimicrobials in Infant Rats

Antimicrobial	Dose (mg/kg)	Blood		CSF		
		Mean peak concentration (μg/ml)	AUC (μg.h/ml)	Mean peak concentration (μg/ml)	AUC (μg.h/ml)	Penetration (%)
Gemifloxacin	50	11.5	46.4	1.3	6.1	13
Gemifloxacin	100	27.8	121.9	2.1	10.6	9
Ciprofloxacin	100	39.4	124.4	1.9	6.8	5
Trovafloxacin	50	16.9	71.3	8.4	32.3	45
Levofloxacin	100	54.4	192.9	20.6	86.3	45
Cefuroxime	100	89.8	273.5	9.1	29.3	11
Cefotaxime	100	71.6	196.4	7.8	22.2	11
Vancomycin	100	71.8	255.5	9	46.4	18

Also provided by the invention is a method of treating or preventing a bacterial infection by bacterial meningitis pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with bacterial meningitis pathogenic bacteria.

While a preferred object of the invention provides a method wherein said bacterial meningitis pathogenic bacteria is *Streptococcus pneumoniae.* Other bacterial meningitis pathogenic bacteria can also be included in the methods. The skilled artisan can identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against Gram negative bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various Gram negative pathogens. An objective of these analyses was to determine the MIC of the fluoroquinolone gemifloxacin against a panel of 895 recent clinical isolates consisting of both Enterobacteriaceae and non-Enterobacteriaceae.

Gemifloxacin was compared to trovafloxacin, grepafloxacin, levofloxacin,

ciprofloxacin, ofloxacin, nalidixic acid, gentamicin, cefuroxime, amoxycillin/clavulanate, penicillin, ampicillin, clarithromycin, azithromycin and trimethoprim/sulfamethoxazole. Isolates were collected in hospital laboratories from three geographically distributed regions in the US. MICs for gemifloxacin and comparator compounds consisting of six quinolones, four β-lactams, two macrolides, one aminoglycoside and trimethoprim/sulfamethoxazole were determined by broth microdilution using NCCLS recommended procedures. With the exception of gentamicin, the quinolones were consistently more active than the other compounds tested. The antibacterial activity of gemifloxacin was equal to that of levofloxacin and ciprofloxacin, and generally better than that of ofloxacin, grepafloxacin, trovafloxacin and naladixic acid. MIC_{90}s (μg/ml) obtained for gemifloxacin were: *Escherichia coli*, 0.016; *Klebsiella pneumoniae*, 0.25; *Enterobacter aerogenes*, 0.25; *Enterobacter cloacae*, 1; *Pseudomonas aeruginosa*, 8; *Morganella morganii*, 0.12; *Proteus* spp., 4; *Stenotrophomonas maltophilia*, 4; *Klebsiella oxytoca*, 0.25; *Citrobacter freundii*, 2; *Acinetobacter* sp., 32; and *Serratia* spp., 1. These results demonstrate that gemifloxacin has antibacterial activity that is comparable to or better than that of quinolones currently available for the treatment of infections caused by Gram negative organisms.

Previous studies have shown that gemifloxacin has antibacterial activity against most of these pathogens. (Oh, *et al.*, *Antimicrob. Agents Chemother.*, 40: 1564-1568 (1996): Cormican, *et al.*, *Antimicrob. Agents Chemother.*, 41: 204-211 (1997)).To confirm these findings, the antimicrobial activity of gemifloxacin was determined against a panel of 895 recent clinical bacterial isolates consisting of Enterobacteriaceae and non-Enterobacteriaceae. The antibacterial activities of the following 14 comparator compounds were also determined: trovafloxacin, grepafloxacin, levofloxacin, ciprofloxacin, ofloxacin, nalidixic acid, gentamicin, cefuroxime, amoxycillin/clavulanate, penicillin, ampicillin, clarithromycin, azithromycin and trimethoprim/sulfamethoxazole.

A total of 895 recent Gram negative clinical isolates were obtained from three hospital laboratories located in geographically distributed regions within the US. As part of the collection process, isolates were checked for purity and frozen at -70°C in 10% glycerol. Before testing, each isolate was passaged from the frozen stock for 2 consecutive days on trypticase soy agar containing 5% sheep blood. NCCLS recommended quality control organisms were obtained from the SmithKline Beecham Anti-infectives Research Culture Collection, Collegeville, PA, USA.

Microtiter susceptibility plates were obtained from Sensititre (AccuMed International Ltd, Westlake, OH, USA; lot # CMP5ASMK-8342, -8423, -8331 and

CMP4BSMK-8334A, -8451, -8351). Plates contained the following compounds in serial twofold doubling dilutions (concentration range, μg/ml): gemifloxacin, 0.001–256; trovafloxacin, 0.016–16; grepafloxacin, 0.016–16; levofloxacin, 0.016–16; ciprofloxacin, 0.016–16; ofloxacin, 0.06–64; nalidixic acid, 0.06–64; gentamicin, 0.06–64; cefuroxime, 0.06–64; amoxycillin/clavulanate, 0.03/0.016–32/16; penicillin, 0.016–16; ampicillin, 0.06–64; clarithromycin, 0.016–16; azithromycin, 0.06–64; and trimethoprim/sulfamethoxazole, 0.06/1.14–64/1216.

Following the recommended procedure of the manufacturer, each plate was inoculated with 100 μl of a single test isolate, which resulted in a final inoculum density of 5×10^5 CFU/ml. (AccuMed International Ltd., Westlake, OH, USA; lot #CMP5ASMK-8342, -8423, -8331 and CMP4BSMK-8334A, -8451, -8351). Colony counts were performed at random to ensure the appropriate inoculum density was obtained. The Microlab AT Plus 2 (Hamilton Co., Reno, NV, USA) was used to add the inoculum to the microtiter plate. A 10 μl aliquot of the inoculum was plated on trypticase soy agar containing 5% sheep blood to determine the purity of the final test inoculum.

The plates were covered with a microtiter plate lid and incubated at 35°C in air for 20–24 h. The MIC was determined as the lowest concentration of drug that inhibited visible growth of the test isolates. For trimethoprim/sulfamethoxazole, the MIC was determined as the concentration that produced an 80–90% decrease in growth compared with the positive growth control. Individual MIC data were summarized and reported using the following parameters: MIC_{range}, MIC_{50} and MIC_{90}.

The following American Type Culture Collection (ATCC) quality control strains were tested: *Staphylococcus aureus* 29213, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, *E. coli* 35218 and *S. aureus* 25923. These NCCLS recommended quality control organisms were obtained from the SmithKline Beecham Anti-infectives Research Culture Collection, Collegeville, PA, USA. Quality control organisms were included on each day of testing. The results for that day were only accepted if the quality control values were within the acceptable limits as established by the NCCLS. (National Committee for Clinical Laboratory Standards 1999. Approved Standard M100-S9, ninth ed., Wayne, PA, USA). Quality control limits, tentatively approved by the NCCLS in January 1999 were used for gemifloxacin.

A summary of the broth microdilution results is shown in Table 23. The quinolones are consistently more active than all other compounds tested except gentamicin. The antibacterial activity of gemifloxacin is equal to that of levofloxacin and ciprofloxacin

and generally better than that of ofloxacin, grepafloxacin, trovafloxacin and naladixic acid.

Gemifloxacin has *in vitro* antibacterial activity that is comparable to or better than that of quinolones currently available for the treatment of infections caused by Gram negative pathogens.

Table 23. Antibacterial Activity of Gemifloxacin and 14 Comparator Compounds Against 895 Recent Gram Negative Clinical Isolates

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Escherichia coli (n = 150)			
Gemifloxacin	0.004–32	0.008	0.016
Trovafloxacin	≤0.016–>16	≤0.016	0.06
Grepafloxacin	≤0.016–>16	≤0.016	0.03
Levofloxacin	≤0.016–>16	0.03	0.06
Ciprofloxacin	≤0.016–>16	≤0.016	0.03
Ofloxacin	≤0.06–>64	≤0.06	0.12
Nalidixic acid	1–>64	4	8
Gentamicin	0.12–>64	0.25	1
Cefuroxime	1–>64	4	16
Amoxycillin/clavulanate*	1–>32	4	16
Penicillin	4–>16	>16	>16
Ampicillin	1–>64	4	>64
Clarithromycin	16–>16	>16	>16
Azithromycin	0.5–>64	2	4
Trimethoprim/sulfamethoxazole	≤0.06–>64	0.12	>64
Klebsiella pneumoniae (n = 149)			
Gemifloxacin	0.008–32	0.03	0.25
Trovafloxacin	≤0.016–>16	0.06	0.5
Grepafloxacin	≤0.016–>16	0.03	0.5
Levofloxacin	0.03–>16	0.06	0.5
Ciprofloxacin	≤0.016–>16	0.03	0.5
Ofloxacin	≤0.06–>64	0.12	2
Nalidixic acid	2–>64	4	>64
Gentamicin	≤0.06–64	0.25	0.5

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Cefuroxime	1–>64	2	16
Amoxycillin/clavulanate*	1–>32	2	8
Penicillin	8–>16	>16	>16
Ampicillin	2–>64	32	>64
Clarithromycin	>16	>16	>16
Azithromycin	2–64	4	8
Trimethoprim/sulfamethoxazole	≤0.06–>64	0.25	2
Klebsiella oxytoca (n = 59)			
Gemifloxacin	0.008–8	0.016	0.25
Trovafloxacin	≤0.016–16	0.03	0.5
Grepafloxacin	≤0.016–8	0.03	0.25
Levofloxacin	0.03–4	0.03	0.5
Ciprofloxacin	≤0.016–8	≤0.016	0.25
Ofloxacin	≤0.06–16	≤0.06	1
Nalidixic acid	2–>64	2	>64
Gentamicin	0.12–64	0.25	0.5
Cefuroxime	1–>64	4	64
Amoxycillin/clavulanate*	1–32	2	16
Penicillin	16–>16	>16	>16
Ampicillin	4–>64	64	>64
Clarithromycin	16–>16	>16	>16
Azithromycin	2–32	8	8
Trimethoprim/sulfamethoxazole	≤0.06–>64	0.25	1
Enterobacter cloacae (n = 76)			
Gemifloxacin	0.008–16	0.016	1
Trovafloxacin	≤0.016–>16	0.03	4
Grepafloxacin	≤0.016–>16	0.03	2
Levofloxacin	≤0.016–>16	0.03	0.5
Ciprofloxacin	≤0.016–16	≤0.016	2
Ofloxacin	≤0.06–32	≤0.06	4
Nalidixic acid	2–>64	4	>64

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Gentamicin	0.12–>64	0.25	0.5
Cefuroxime	1–>64	16	>64
Amoxycillin/clavulanate*	1–>32	>32	>32
Penicillin	>16	>16	>16
Ampicillin	2–>64	>64	>64
Clarithromycin	>16	>16	>16
Azithromycin	2–>64	8	16
Trimethoprim/sulfamethoxazole	≤0.06–>64	0.25	4
Enterobacter aerogenes (n = 60)			
Gemifloxacin	0.008–2	0.016	0.25
Trovafloxacin	≤0.016–4	0.06	0.5
Grepafloxacin	≤0.016–4	0.03	0.5
Levofloxacin	≤0.016–2	0.06	0.5
Ciprofloxacin	≤0.016–4	≤0.016	0.25
Ofloxacin	≤0.06–4	0.12	1
Nalidixic acid	2–>64	4	64
Gentamicin	≤0.06–32	0.25	0.5
Cefuroxime	2–>64	8	>64
Amoxycillin/clavulanate*	1–>32	32	>32
Penicillin	>16	>16	>16
Ampicillin	16–>64	>64	>64
Clarithromycin	>16	>16	>16
Azithromycin	4–16	4	8
Trimethoprim/sulfamethoxazole	0.12–>64	0.25	1
Proteus sp. (n = 86)			
Gemifloxacin	0.03–128	0.12	4
Trovafloxacin	0.06–>16	0.25	16
Grepafloxacin	0.06–>16	0.25	8
Levofloxacin	0.03–>16	0.06	1
Ciprofloxacin	≤0.016–>16	0.03	1
Ofloxacin	≤0.06–>64	0.12	4

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Nalidixic acid	2–>64	4	>64
Gentamicin	0.12–32	0.5	1
Cefuroxime	0.5–>64	2	>64
Amoxycillin/clavulanate*	0.5–8	1	8
Penicillin	1–>16	4	>16
Ampicillin	0.5–>64	1	>64
Clarithromycin	>16	>16	>16
Azithromycin	16–>64	64	>64
Trimethoprim/sulfamethoxazole	0.12–>64	0.25	2
Serratia spp. (n = 63)			
Gemifloxacin	0.008–4	0.12	1
Trovafloxacin	0.03–16	0.5	4
Grepafloxacin	≤0.016–8	0.25	2
Levofloxacin	≤0.016–4	0.12	1
Ciprofloxacin	≤0.016–8	0.06	1
Ofloxacin	≤0.06–16	0.25	4
Nalidixic acid	1–>64	2	>64
Gentamicin	0.25–>64	0.5	1
Cefuroxime	2–>64	>64	>64
Amoxycillin/clavulanate*	2–>32	>32	>32
Penicillin	>16	>16	>16
Ampicillin	2–>64	>64	>64
Clarithromycin	>16	>16	>16
Azithromycin	4–>64	16	32
Trimethoprim/sulfamethoxazole	0.25–>64	0.5	2
Citrobacter freundii (n = 52)			
Gemifloxacin	0.004–16	0.12	2
Trovafloxacin	≤0.016–>16	0.25	4
Grepafloxacin	≤0.016–>16	0.25	4
Levofloxacin	≤0.016–16	0.12	2
Ciprofloxacin	≤0.016–>16	0.03	2

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Ofloxacin	≤0.06–64	0.25	8
Nalidixic acid	1–>64	4	>64
Gentamicin	0.25–64	0.25	1
Cefuroxime	2–>64	8	>64
Amoxycillin/clavulanate*	1–>32	32	>32
Penicillin	>16	>16	>16
Ampicillin	4–>64	>64	>64
Clarithromycin	16–>16	>16	>16
Azithromycin	1–64	4	16
Trimethoprim/sulfamethoxazole	≤0.06>64	0.25	>64
Morganella morganii (n = 32)			
Gemifloxacin	0.016–8	0.06	0.12
Trovafloxacin	0.06–16	0.25	0.25
Grepafloxacin	0.06–16	0.12	0.25
Levofloxacin	≤0.016–4	0.03	0.12
Ciprofloxacin	≤0.016–2	≤0.016	0.03
Ofloxacin	≤0.06–8	≤0.06	0.25
Nalidixic acid	1–>64	2	4
Gentamicin	0.25–2	0.5	1
Cefuroxime	16–>64	32	>64
Amoxycillin/clavulanate*	>32	>32	>32
Penicillin	>16	>16	>16
Ampicillin	32–>64	>64	>64
Clarithromycin	>16	>16	>16
Azithromycin	16–>64	32	64
Trimethoprim/sulfamethoxazole	0.12–>64	0.25	>64
Providencia sp. (n = 11)			
Gemifloxacin	0.016–16	–**	–
Trovafloxacin	0.03–16	–	–
Grepafloxacin	0.03–16	–	–
Levofloxacin	0.03–16	–	–

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Ciprofloxacin	≤0.016–>16	–	–
Ofloxacin	0.12–32	–	–
Nalidixic acid	2–>64	–	–
Gentamicin	0.25–8	–	–
Cefuroxime	0.12–32	–	–
Amoxycillin/clavulanate*	0.5–>32	–	–
Penicillin	1–>16	–	–
Ampicillin	1–>64	–	–
Clarithromycin	>16	–	–
Azithromycin	32–>64	–	–
Trimethoprim/sulfamethoxazole	<0.06–>64	–	–
Pseudomonas aeruginosa (n = 72)			
Gemifloxacin	0.03–256	0.25	8
Trovafloxacin	0.03–>16	0.5	16
Grepafloxacin	0.03–>16	0.5	16
Levofloxacin	0.06–>16	0.5	8
Ciprofloxacin	0.03–>16	0.25	8
Ofloxacin	0.12–>64	1	32
Nalidixic acid	8–>64	>64	>64
Gentamicin	0.25–32	2	4
Cefuroxime	16–>64	>64	>64
Amoxycillin/clavulanate*	>32	>32	>32
Penicillin	>16	>16	>16
Ampicillin	>64	>64	>64
Clarithromycin	16–>16	>16	>16
Azithromycin	16–>64	>64	>64
Trimethoprim/sulfamethoxazole	0.5–>64	8	32
Stenotrophomonas maltophilia (n = 54)			
Gemifloxacin	0.016–16	0.5	4
Trovafloxacin	0.03–16	0.5	4

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Grepafloxacin	0.03–16	0.5	4
Levofloxacin	0.06–16	1	8
Ciprofloxacin	0.12–>16	2	16
Ofloxacin	0.25–64	2	16
Nalidixic acid	4–>64	8	32
Gentamicin	0.25–>64	32	>64
Cefuroxime	32–>64	>64	>64
Amoxycillin/clavulanate*	8–>32	32	>32
Penicillin	16–>16	>16	>16
Ampicillin	4–>64	>64	>64
Clarithromycin	8–>16	>16	>16
Azithromycin	0.5–>64	32	64
Trimethoprim/sulfamethoxazole	0.12–16	1	8
Acinetobacter sp. (n = 27)			
Gemifloxacin	0.008–32	0.016	32
Trovafloxacin	≤0.016–>16	0.03	>16
Grepafloxacin	≤0.016–>16	0.03	>16
Levofloxacin	0.06–>16	0.12	>16
Ciprofloxacin	0.06–>16	0.12	>16
Ofloxacin	0.12–>64	0.25	64
Nalidixic acid	2–>64	4	>64
Gentamicin	≤0.06–>64	0.5	>64
Cefuroxime	2–>64	32	>64
Amoxycillin/clavulanate*	0.12–>32	16	>32
Penicillin	2–>16	>16	>16
Ampicillin	1–>64	32	>64
Clarithromycin	4–>16	16	>16
Azithromycin	0.25–64	1	32
Trimethoprim/sulfamethoxazole	0.12–64	0.25	64
B. cepacia (n = 4)			
Gemifloxacin	0.016–1	–[b]	–

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Trovafloxacin	0.12–1	–	–
Grepafloxacin	0.06–1	–	–
Levofloxacin	0.06–1	–	–
Ciprofloxacin	≤0.016–1	–	–
Ofloxacin	0.12–4	–	–
Nalidixic acid	4–32	–	–
Gentamicin	0.12–32	–	–
Cefuroxime	32–>64	–	–
Amoxycillin/clavulanate*	2–>32	–	–
Penicillin	8–>16	–	–
Ampicillin	4–>64	–	–
Clarithromycin	2–>16	–	–
Azithromycin	2–32	–	–
Trimethoprim/sulfamethoxazole	1–4	–	–

*Amoxycillin/clavulanate (2:1). Concentration listed refers to the amoxycillin component

**MIC_{50} and MIC_{90} calculations were not performed with fewer than 25 isolates

The invention provides a method for modulating metabolism of Gram negative pathogenic bacteria. Skilled artisans can readily choose Gram negative pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram negative pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with Gram negative pathogenic bacteria.

While a preferred object of the invention provides a method wherein said Gram negative pathogenic bacteria is selected from the group consisting of: *Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, Pseudomonas*

aeruginosa, Morganella morganii, Proteus spp., Stenotrophomonas maltophilia, Klebsiella oxytoca, Citrobacter freundii, Acinetobacter sp., and *Serratia spp.* Other Gram negative pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against Gram positive or Gram negative aerobic bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various Gram positive or Gram negative aerobic pathogens. An objective of these analyses was to determine the post-antibiotic effect (PAE) of gemifloxacin compared with that of ciprofloxacin against 10 isolates comprising *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Enterococcus faecalis, Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Proteus vulgaris*. Isolates were incubated in the presence of 2 and 4 x MIC. After 1 h of exposure, the antimicrobial was removed by ultrafiltration and viable counts were made for 6 h. The PAE was defined as $T - C$, where T is the time for the antimicrobial-exposed culture to increase in viable count by 1 $\log_{10}$ and C is the time for the growth control to increase in viable count by 1 $\log_{10}$.

A measurable PAE was observed with both compounds at 2 and 4x MIC. The PAEs of gemifloxacin are comparable to those of ciprofloxacin, and for both compounds exposure to 2 x MIC results in shorter PAEs than exposure to 4 x MIC. The PAE of gemifloxacin at 4 x MIC is >6 h against *P. aeruginosa*, *P. vulgaris* and *H. influenzae*, and ranges from 0.1 to 2.5 h against the other isolates tested. These results indicate that gemifloxacin has a pronounced PAE against a broad spectrum of organisms, which, in combination with gemifloxacin's pharmacokinetic profile, indicate a less frequent dosing schedule for this compound in clinical use.

The post-antibiotic effect (PAE) is defined as the persistent suppression of bacterial growth after brief exposure of a bacterial culture to an antimicrobial. (Craig, *et al., In: Antibiotics in laboratory medicine 4th edition*, The Williams and Wilkins Co., 296-329 (1986). The measurement of a PAE can be useful in the design of an appropriate dosing regimen for an antimicrobial. An antimicrobial with a long PAE would require a less frequent dosing regimen than one with a short PAE. In this study, the PAE was determined for gemifloxacin and ciprofloxacin against 10 isolates representing a broad spectrum of

Gram positive or Gram negative aerobic organisms.

Gemifloxacin, batch # 03R1P2-1-1, potency 73.8%, was obtained from SmithKline Beecham Pharmaceuticals, Harlow, UK. Ciprofloxacin, lot G, potency 100%, was obtained as a USP reference standard (Rockville, MD, USA).

The following isolates were obtained from the SmithKline Beecham Anti-infectives Research Culture Collection: *Staphylococcus aureus* ATCC 29213, *Staphylococcus saprophyticus* 662, *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247, *Enterococcus faecalis* ATCC 29212, *Moraxella catarrhalis* MC2, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* KP2 and *Proteus vulgaris* ATCC 13315.

Trypticase soy agar containing 5% sheep blood (BBL, Cockeysville, MD, USA) was used to subculture frozen isolate stocks and for *S. pneumoniae* colony counts. Mueller–Hinton II agar (BBL) was used for colony counts of the non-fastidious organisms. Chocolate II agar (BBL) was used for colony counts of the *H. influenzae* isolate. Cation-adjusted Mueller–Hinton broth (CAMHB) (BBL) was used to test the non-fastidious organisms. CAMHB supplemented with 5% lyzed horse blood (BBL) was used to test the *S. pneumoniae* isolate. *Haemophilus* Test Medium (BBL) was used to test the *H. influenzae* isolate.

Organisms were subcultured from a frozen stock (-80°C) onto a trypticase agar plate containing 5% sheep blood and incubated for 20–24 h at 35°C. A 5 ml tube of saline was inoculated with a sufficient number of colonies from the overnight culture to obtain a turbidity equivalent to a 0.5 McFarland standard. This inoculum was diluted 100-fold into 18 ml of the appropriate media (contained in a 50 ml flask) to produce a final test inoculum of approximately 1 x 10^6 CFU/ml.

The MICs of gemifloxacin and ciprofloxacin were determined using the NCCLS recommended procedure for broth microdilution. (National Committee for Clinical Laboratory Standards, Approved Standard M2-A6, sixth ed., Wayne, PA, USA). Both compounds were tested in serial twofold dilutions ranging in concentration from 0.0001 to 32 μg/ml. A positive growth control (antimicrobial free) was included on each microtiter plate. Following inoculation, plates were incubated at 35°C in air for 18–24 h. A 10 μl aliquot of the inoculum was plated onto trypticase soy agar containing 5% sheep blood to determine the purity of the final test inoculum.

The PAE effect was determined using a filtration method as previously

described.(Thornburn, et al. *Antimicrob. Agents Chemother.*, 40: 2796-2801 (1996)).Each test isolate was added to a 50 ml flask containing the antimicrobial (at 2 or 4 x MIC) in 20 ml of the appropriate broth. After addition of the isolate, a colony count was performed to determine the density (CFU/ml) of the starting inoculum. Flasks were incubated on a shaker at 35°C.

After 2 h hours of antimicrobial exposure a further colony count was performed. The contents of each flask were then filtered using a 0.2 micron filter to remove the antimicrobial. The filtrate was washed twice with 10 ml of pre-warmed broth and the filter was resuspended into 20 ml of pre-warmed media. A colony count was performed immediately after resuspension of the test isolate. Flasks were returned to the incubator and colony counts were performed at 1 h intervals for 6 h. Due to filtration problems associated with the media supplements used for *S. pneumoniae* and *H. influenzae*, a 1:100 dilution of the inoculum was made into pre-warmed broth to remove the antimicrobial. The control for determination of the PAE included incubation of the test isolate for 2 h in drug-free media followed by filtration and incubation of the isolate in drug-free media.

Five 10-fold dilutions were made for each isolate/antimicrobial and control at every time interval. Using a 10 µl disposable loop, 50 µl from each well were spread onto the appropriate agar media. The plates were incubated overnight and colony counts were made at the dilution that provided 30–300 colonies.

The MIC was determined as the lowest concentration of compound that inhibited visible growth of the isolate. A microtiter mirror reader was used to assist in determining the MIC endpoint.

A semi-logarithmic graph with number of colonies on the *y*-axis and time on the *x*-axis was prepared for each isolate. The PAE effect was determined using the equation:

$$PAE = T - C$$

where T is the time required for the test isolate count to increase by 10-fold ($1 \times \log_{10}$) above the count observed immediately after removal of the antimicrobial and C is the time required for the control to increase by 10-fold above the count observed immediately after removal of the antimicrobial.

The MICs of gemifloxacin and ciprofloxacin are shown in Table 24. A summary of the PAE results are shown in Table 25.

A measurable PAE is observed for both compounds against all of the isolates

tested. In general, a shorter PAE is observed at 2 x MIC as opposed to 4 x MIC. At 4 x MIC, the gemifloxacin PAE is >6 h against *H. influenzae*, *P. aeruginosa* and *P. vulgaris*, and ranges from 0.1 to 2.5 h against the other isolates tested. The ciprofloxacin PAE values obtained at 4 x MIC range from 0.3 to 5.1 h, with the exception of *E. faecalis* ATCC 29212, where no effect is observed.

The PAE of gemifloxacin is comparable to that of ciprofloxacin against all isolates tested. A shorter PAE is observed for both compounds at 2 x MIC compared with 4 x MIC. At 4 x MIC, a longer PAE for gemifloxacin (>6 h) than ciprofloxacin is observed against *H. influenzae*, *P. aeruginosa* and *P. vulgaris*. Overall, the results of this study indicate that gemifloxacin has a pronounced PAE against a broad spectrum of organisms.

Table 24. MICs of Gemifloxacin and Ciprofloxacin Against A Broad Spectrum of Bacterial Isolates

Microrganism	MIC (μg/ml)	
	Gemifloxacin	Ciprofloxacin
Staphylococcus aureus ATCC 29213	0.016	0.25
Staphylococcus saprophyticus 662	0.016	0.25
Streptococcus pneumoniae ATCC 49619	0.016	0.5
Haemophilus influenzae ATCC 49247	0.004	0.008
Enterococcus faecalis ATCC 29212	0.03	0.25
Moraxella catarrhalis MC2	0.004	0.008
Escherichia coli ATCC 25922	0.008	0.008
Pseudomonas aeruginosa ATCC 27853	0.5	0.5
Klebsiella pneumoniae KP2	0.5	0.5
Proteus vulgaris ATCC13315	0.06	0.008

Table 25. PAE of Gemifloxacin and Ciprofloxacin at 2 and 4 x MIC

Microrganism	PAE (h)			
	Gemifloxacin		Ciprofloxacin	
	2 x MIC	4 x MIC	2 x MIC	4 x
Staphylococcus aureus ATCC 29213	1.1	1.0	1.3	1.5
Staphylococcus saprophyticus 662	1.9	2.5	2.4	3.9

Streptococcus pneumoniae ATCC 49619	0.7	1.5	0.9	1.5
Haemophilus influenza ATCC 49247	2.4	>6	0.6	2.4
Enterococcus faecalis ATCC 29212	0.1	0.6	0	0.3
Moraxella catarrhalis MC2	0.6	0.5	1.1	3.7
Escherichia coli ATCC 25922	1.1	1.9	1.2	3.7
Pseudomonas aeruginosa ATCC 27853	2.4	6.6	4.5	5.1
Klebsiella pneumoniae KP2	0.2	0.1	0.2	0.5
Proteus vulgaris ATCC 13315	3.9	>6	1.3	3.8

The invention provides a method for modulating metabolism of Gram positive or Gram negative aerobic pathogenic bacteria. Skilled artisans can readily choose Gram positive or Gram negative aerobic pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention can be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive or Gram negative aerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with Gram positive or Gram negative aerobic pathogenic bacteria.

While a preferred object of the invention provides a method wherein said Gram positive or Gram negative aerobic pathogenic bacteria is selected from the group consisting of: *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Proteus vulgaris*. Other Gram positive or Gram negative aerobic pathogenic bacteria can also be included in the methods. The skilled artisan can identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Streptococci* bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Streptococci* bacteria pathogens. An objective of these analyses was to determine the *in vitro* activity of gemifloxacin compared with that of 11 other antimicrobials against 400 *Streptococci* isolates.

Gemifloxacin was compared to gemifloxacin, ciprofloxacin, levofloxacin, sparfloxacin, trovafloxacin, grepafloxacin, penicillin, ampicillin, erythromycin, clarithromycin, tetracycline and vancomycin.

The activities of gemifloxacin and 11 other antimicrobials were determined by agar dilution technique (BSAC methodology) against various species of *streptococci*. The species selected were considered as primary pathogens or 'passenger organisms'. Passenger organisms are defined as streptococci that are part of normal flora, and can be affected by antibiotic therapy. A total of 400 clinical isolates were studied: Lancefield groups A and C (n = 50), groups B and G (n = 51), 'mitis' group and 'anginosus/milleri' group (n = 51), 'mutans' group (n = 50) and 'bovis' group (n = 46). The MIC_{90}s (μg/ml) of all *streptococci* to various quinolones were: gemifloxacin 0.008–0.06, ciprofloxacin 0.125–2.0, levofloxacin 0.5–4, sparfloxacin 0.06–1.0, trovafloxacin ≤0.016–0.25 and grepafloxacin ≤0.016–1. The MIC ranges for other standard antibiotics were: penicillin ≤0.008–4, ampicillin ≤0.008–8, erythromycin ≤0.016–≥32, clarithromycin ≤0.016–≥32, tetracycline 0.06–≥64 and vancomycin 0.125–2. In conclusion, gemifloxacin was the most potent quinolone tested. Gemifloxacin has enhanced activity against *streptococci* compared with other quinolones and is a promising agent for use against Gram positive infections.

Quinolones are used to treat a variety of clinical indications including respiratory, abdominal, skin and soft tissue, and genito-urinary infections. Gemifloxacin (SB-265805) is a broad spectrum 6-fluoronaphthyridone with an oxime-derivatized (aminomethyl) pyrrolidinyl substituent at position 7. Compared with quinolones in current use, gemifloxacin displays potent activity against *Streptococcus pneumoniae* and other Gram positive pathogens.

The Streptococci species selected in this study represent primary pathogens or 'passenger organisms'. In this context, passenger organisms are *streptococci* present as normal flora, and thus are affected by antimicrobial therapy. The use of antimicrobials can influence the normal flora directly via eradication and/or by super colonization, or indirectly through the selection of resistant organisms, which can lead to difficulties in the treatment of *Streptococci* infections.

A total of 400 distinct clinical isolates of *Streptococcus* spp. were studied. The isolates consisted of Lancefield groups A and C (n = 50), groups B and G (n = 51), 'mitis' group and 'anginosus/milleri' group (n = 51), 'mutans' group (n = 50) and 'bovis' group (n = 46). The majority of the strains tested were recent isolates from the last three years. The control strains used were *Staphylococcus aureus* NCTC 6571, *S. aureus* ATCC 29213,

Streptococcus pneumoniae ATCC 49619.

The minimum inhibitory concentration (MIC) of the strains was determined by agar dilution based on British Society for Antimicrobial Chemotherapy methods,[1] (Working Party of the British Society for Antimicrobial Chemotherapy, *Journal of Antimicrobial Chemotherapy*, 27(Suppl. D): 1-50 (1991)).using Iso-Sensitest agar (CM471 Oxoid, Basingstoke, UK) supplemented with lysed horse blood (5% v/v). Colonies were taken directly from the plate into broth to match 0.5 McFarland standard and diluted to 1:10. The antimicrobial-containing agar was inoculated with 1 µl of diluted broth culture to give a final inoculum of 10^4 CFU/spot. Plates were incubated at 35–37°C in 5% CO_2 for 18–20 hours, as some strains of

Table 26. Antimicrobial Susceptibility of Streptococci Lancefield Group A (n = 50)

Antimicrobial agents	No. of strains inhibited at the MIC (µg/ml):														MIC (µg/ml l)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	32	18	0	0	0	0	0	0	0	0	0	0	0	0	≤0.008–0.016	≤0.008	0.016
Erythromycin	0	0	0	20	24	3	0	0	0	0	1	0	2	0	0.06–≥32	0.125	0.25
Clarithromycin	0	1	37	9	0	0	0	1	0	0	2	0	0	0	≤0.016-8	0.03	0.06
Tetracycline	0	0	0	0	1	18	21	1	0	0	0	0	0	9	0.125–≥64	0.5	≥64
Ampicillin	19	21	9	1	0	0	0	0	0	0	0	0	0	0	≤0.008–0.06	0.016	0.03
Vancomycin	0	0	0	0	15	35	0	0	0	0	0	0	0	0	0.125–0.25	0.25	0.25

Ciprofloxacin	0	0	0	0	0	12	15	17	3	3	0	0	0	0	0.25–4	0.5	2
Levofloxacin	0	0	0	0	0	0	15	19	11	4	1	0	0	0	0.5–8	1	2
Trovafloxacin	0	0	2	21	15	7	4	1	0	0	0	0	0	0	0.03–1	0.125	0.25
Grepafloxacin	0	0	0	2	5	19	13	7	3	1	0	0	0	0	0.06–1	0.25	1
Sparfloxacin	0	0	0	0	1	25	17	5	2	0	0	0	0	0	0.125–2	0.25	1
Gemifloxacin	2	21	18	6	3	0	0	0	0	0	0	0	0	0	≤0.008–0.125	0.03	0.06

Table 27. Antimicrobial Susceptibility of Streptococci Lancefield Group B (n = 51)

Antimicrobial agents	No. of strains inhibited at the MIC (μg/ml):														MIC (μg/ml)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	1	0	37	13	0	0	0	0	0	0	0	0	0	0	≤0.008–0.06	0.03	0.06
Erythromycin	0	0	0	14	25	9	0	0	0	3	0	0	0	0	0.06–4	0.125	0.25
Clarithromycin	0	1	24	20	3	0	1	2	0	0	0	0	0	0	≤0.016-1	0.06	0.125
Tetracycline	0	0	0	0	1	5	5	0	0	0	0	1	2	37	0.125–≥64	≥64	≥64
Ampicillin	0	0	15	20	16	0	0	0	0	0	0	0	0	0	0.03–0.125	0.06	0.125
Vancomycin	0	0	0	0	5	38	8	0	0	0	0	0	0	0	0.125–8	0.25	0.5
Ciprofloxacin	0	0	0	0	0	0	8	29	14	0	0	0	0	0	0.5–2	1	2
Levofloxacin	0	0	0	0	0	0	0	14	30	6	1	0	0	0	1–8	2	4
Trovafloxacin	0	0	0	2	11	31	7	0	0	0	0	0	0	0	0.06–0.5	0.25	0.5

Grepafloxacin	0	0	0	0	7	21	14	7	2	0	0	0	0	0.125–2	0.25	1
Sparfloxacin	0	0	0	0	1	26	22	2	0	0	0	0	0	0.125–1	0.25	0.5
Gemifloxacin	0	9	24	9	9	0	0	0	0	0	0	0	0	0.016–0.125	0.03	0.12 5

Table 28. Antimicrobial Susceptibility of Streptococci Lancefield Group C (n = 50)

Antimicrobial agents	No. of strains inhibited at the MIC (μg/ml):														MIC (μg/ml)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	32	16	2	0	0	0	0	0	0	0	0	0	0	0	≤0.008–0.03	≤0.008	0.016
Erythromycin	0	0	0	2	28	16	2	0	0	2	0	0	0	0	0.06–4	0.125	0.25
Clarithromycin	0	0	21	24	3	0	0	1	1	0	0	0	0	0	0.03-2	0.06	0.125
Tetracycline	0	0	0	0	0	17	9	6	1	2	1	3	3	8	0.25–≥64	0.5	≥64
Ampicillin	19	14	15	1	1	0	0	0	0	0	0	0	0	0	≤0.008–0.125	0.016	0.03
Vancomycin	0	0	0	0	12	37	1	0	0	0	0	0	0	0	0.125–0.5	0.25	0.25

Ciprofloxacin	0	0	0	0	13	14	23	0	0	0	0	0	0.25–1	0.5	1	
Levofloxacin	0	0	0	0	0	13	18	19	0	0	0	0	0.5–2	1	2	
Trovafloxacin	0	0	0	17	21	11	1	0	0	0	0	0	0.06–0.5	0.12	0.25	5
Grepafloxacin	0	0	0	0	12	16	13	9	0	0	0	0	0.125–1	0.25	1	
Sparfloxacin	0	0	0	0	5	20	21	4	0	0	0	0	0.125–1	0.25	0.5	
Gemifloxacin	6	16	18	9	1	0	0	0	0	0	0	0	≤0.008–0.125	0.03	0.06	

Table 29. Antimicrobial Susceptibility of Streptococci Lancefield Group G (n = 51)

Antimicrobial agents	No. of strains inhibited at the MIC (µg/ml):														MIC (µg/ml)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	≥32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	44	6	1	0	0	0	0	0	0	0	0	0	0	0	≤0.008–0.03	≤0.008	0.016
Erythromycin	0	0	0	3	25	10	1	1	4	3	1	1	2	0	0.06–≥32	0.125	4
Clarithromycin	0	0	19	18	1	5	2	2	1	1	0	0	2	0	0.03 - ≥32	0.06	1
Tetracycline	0	0	0	0	0	15	9	1	0	2	4	2	0	18	0.25–≥64	4	≥64
Ampicillin	20	18	12	1	0	0	0	0	0	0	0	0	0	0	≤0.008–0.06	0.016	0.03
Vancomycin	0	0	0	0	20	31	0	0	0	0	0	0	0	0	0.125–0.25	0.25	0.25
Ciprofloxacin	0	0	0	0	0	11	23	12	5	0	0	0	0	0	0.25–2	0.5	1

Levofloxacin	0	0	0	0	0	17	21	8	3	2	0	0	0	0.5–8	1	2
Trovafloxacin	0	0	6	19	11	12	0	0	0	0	0	0	0	0.03–0.5	0.12	0.25
Grepafloxacin	0	0	1	3	13	16	6	0	0	0	0	0	0	0.03–1	0.25	1
Sparfloxacin	0	0	0	0	6	33	4	0	0	0	0	0	0	0.125–1	0.25	0.5
Gemifloxacin	5	20	16	8	2	0	0	0	0	0	0	0	0	≤0.008–0.125	0.03	0.06

Table 30. Antimicrobial Susceptibility of *'Mitis* Group' of Streptococci (n = 51)

Antimicrobial agents	No. of strains inhibited at the MIC (µg/ml):														MIC ((µg/ml l)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	5	5	11	8	7	4	7	1	1	2	0	0	0	0	≤0.008–4	0.06	0.5
Erythromycin	0	1	8	12	5	3	1	2	4	6	8	0	1	0	0.016–32	0.125	8
Clarithromycin	0	16	10	3	0	3	2	9	4	2	1	0	1	0	≤0.016-≥32	0.03	2
Tetracycline	0	0	0	0	1	6	19	5	2	2	2	2	1	11	0.125–≥64	0.5	≥64
Ampicillin	3	9	8	6	4	4	5	5	4	2	1	0	0	0	≤0.008–8	0.06	2
Vancomycin	0	0	0	0	3	28	19	1	0	0	0	0	0	0	0.125–1	0.25	0.5
Ciprofloxacin	0	0	0	0	0	2	3	22	17	5	2	0	0	0	0.25–8	1	4
Levofloxacin	0	0	0	0	0	0	3	15	23	8	2	0	0	0	0.5–8	2	4

Trovafloxacin	0	0	0	16	25	9	1	0	0	0	0	0	0	0.06–0.5	0.12	0.25
Grepafloxacin	0	0	0	0	13	21	10	7	0	0	0	0	0	0.125–1	0.12	1
Sparfloxacin	0	0	0	2	9	24	14	2	0	0	0	0	0	0.06–1	0.25	0.5
Gemifloxacin	2	16	18	9	6	0	0	0	0	0	0	0	0	≤0.008–0.125	0.03	0.12

Table 31. Antimicrobial Susceptibility of *'Mutans* Group' of Streptococci (n = 50)

Antimicrobial agents	No. of strains inhibited at the MIC (μg/ml):														MIC ((μg/ml)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	7	10	28	3	1	1	0	0	0	0	0	0	0	0	≤0.008–0.25	0.03	0.03
Erythromycin	0	0	9	35	6	0	0	0	0	0	0	0	0	0	0.03–0.125	0.06	0.125
Clarithromycin	0	18	26	6	0	0	0	0	0	0	0	0	0	0	≤0.016-0.06	0.03	0.06
Tetracycline	0	0	0	2	1	5	25	15	1	0	0	0	0	1	0.06–≥64	0.5	1
Ampicillin	0	5	19	24	1	1	0	0	0	0	0	0	0	0	0.016–0.25	0.06	0.06
Vancomycin	0	0	0	0	0	3	30	17	0	0	0	0	0	0	0.25–1	0.5	1
Ciprofloxacin	0	0	0	0	1	0	1	26	18	2	1	1	0	0	0.125–16	1	2
Levofloxacin	0	0	0	0	0	0	1	9	36	2	1	1	0	0	0.5–16	2	2

Trovafloxacin	1	0	0	4	14	31	0	0	0	0	0	0	0	0.06–0.25	0.25	0.25
Grepafloxacin	0	0	0	0	6	7	24	13	0	0	0	0	0	0.125–1	0.5	1
Sparfloxacin	0	0	0	1	1	4	32	12	0	0	0	0	0	0.06–1	0.5	1
Gemifloxacin	2	5	19	14	10	0	0	0	0	0	0	0	0	≤0.008–0.125	0.03	0.12 5

Table 32. Antimicrobial Susceptibility of *Streptococcus bovis* (n = 46)

Antimicrobial agents	No. of strains inhibited at the MIC (µg/ml):														MIC (µg/ml)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16	≥32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	0	0	15	28	3	0	0	0	0	0	0	0	0	0	0.03–0.125	0.06	0.06
Erythromycin	0	0	4	18	12	4	0	0	2	1	0	0	5	0	0.03–≥32	0.125	≥32
Clarithromycin	0	9	23	6	0	1	2	0	0	2	0	0	3	0	≤0.016-≥32	0.03	0.5
Tetracycline	0	0	0	0	0	4	9	3	0	1	0	7	4	18	0.25–≥64	≥16	≥64
Ampicillin	0	0	5	19	21	1	0	0	0	0	0	0	0	0	0.03–0.25	0.06	0.125
Vancomycin	0	0	0	0	11	27	8	0	0	0	0	0	0	0	0.125–0.5	0.25	0.5
Ciprofloxacin	0	0	0	0	0	0	0	21	16	8	0	0	1	0	1- ≥32	2	4
Levofloxacin	0	0	0	0	0	0	0	5	22	17	1	0	1	0	1–≥32	2	4

Trovafloxacin	0	0	0	2	10	22	11	0	0	0	1	0	0	0.06–≥16	0.25	0.5
Grepafloxacin	0	0	0	0	1	14	14	9	7	0	1	0	0	0.125–≥16	0.5	2
Sparfloxacin	0	0	0	0	0	23	11	11	0	0	1	0	0	0.25–≥16	0.25	1
Gemifloxacin	0	7	18	10	10	0	0	0	1	0	0	0	0	0.016–2	0.03	0.12

Table 33. Antimicrobial Susceptibility of *'Anginosus / Milleri'* Group of Streptococci (n = 51)

Antimicrobial agents	No. of strains inhibited at the MIC (µg/ml):														MIC (µg/ml)		
	≤0.008	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	≥32	≥64	Range	MIC_{50}s	MIC_{90}s
Penicillin	2	3	26	19	1	0	0	0	0	0	0	0	0	0	≤0.008–0.125	0.03	0.06
Erythromycin	0	6	30	12	2	0	0	0	0	0	0	0	1	0	≤0.016–≥32	0.03	0.06
Clarithromycin	0	34	15	1	0	0	0	0	0	0	0	0	1	0	≤0.016–≥32	≤0.016	0.03
Tetracycline	0	0	0	0	2	9	15	4	1	1	1	3	3	12	0.125–≥64	0.5	≥64
Ampicillin	1	2	17	8	16	7	0	0	0	0	0	0	0	0	≤0.008–0.25	0.06	0.25
Vancomycin	0	0	0	0	0	18	28	4	1	0	0	0	0	0	0.25–2	0.5	0.5
Ciprofloxacin	0	0	0	0	0	6	24	20	1	0	0	0	0	0	0.25–2	0.5	1

Levofloxacin	0	0	0	0	0	16	23	11	1	0	0	0	0	0.5–4	1	2
Trovafloxacin	0	0	4	21	20	0	0	0	0	0	0	0	0	0.03–0.25	0.06	0.12 5
Grepafloxacin	0	0	0	0	15	10	0	0	0	0	0	0	0	0.125–0.5	0.25	0.5
Sparfloxacin	0	0	0	1	9	8	0	0	0	0	0	0	0	0.06–0.5	0.25	0.5
Gemifloxacin	3	30	17	1	0	0	0	0	0	0	0	0	0	≤0.008– 0.06	≤0.0 16	0.03

streptococci were CO_2-dependent. MIC end point was read as the lowest concentration of no visible growth.

The susceptibility results are expressed as MIC ranges, MIC_{50}s and MIC_{90}s (Tables 26-33). *Streptococci* of Lancefield groups A, B, C and G are important human pathogens and are the main etiological agents in many clinically significant infections. Gemifloxacin is the most potent quinolone against streptococci of Lancefield groups A, B, C and G; including erythromycin- and tetracycline-resistant strains followed by trovafloxacin, sparfloxacin, grepafloxacin, ciprofloxacin and levofloxacin (Tables 26-29). The modal MIC_{90}s of Lancefield groups A, B, C and G is 0.06, 0.25, 0.5, 1, 1 and 2 (μg/ml) for gemifloxacin, trovafloxacin, sparfloxacin, grepafloxacin, ciprofloxacin and levofloxacin, respectively. All *streptococci* of Lancefield groups A, B, C and G were susceptible to penicillin (MIC ≤0.125 μg/ml), ampicillin (MIC ≤0.25 μg/ml) and vancomycin (MIC ≤1 μg/ml). Erythromycin, clarithromycin and tetracycline showed variable results; clarithromycin was two-fold more active than erythromycin. Tetracycline showed poor activity against Lancefield groups B and G.

The 'mitis' and 'mutans' groups of streptococci, (Hardie, *et al.*, *Journal of Applied Microbiology*, 83 (Suppl.): 1S-11S (1997)).are present as normal oral flora. However, these *streptococci* have emerged as an important cause of bacteremia causing serious morbidity and mortality in neutropenic patients. Gemifloxacin is the most potent quinolone tested against the passenger *streptococci* ('mitis' and 'mutans' groups) followed by trovafloxacin, sparfloxacin and grepafloxacin. Ciprofloxacin and levofloxacin demonstrate similar activity, reflected in their identical MIC_{50}s and MIC_{90}s values (Tables 30-31). MIC values of penicillin and ampicillin are higher for the 'mitis' group than to Lancefield groups A, C and G. MICs of erythromycin and tetracycline were also higher in 'mitis' group compared to Lancefield groups A, C and G. The MIC_{90}s of clarithromycin (2 μg/ml) are comparably lower than erythromycin (8 μg/ml). A single isolate shows intermediate susceptibility to penicillin (MIC_{90} 0.25 μg/ml). All 'mitis' and 'mutans' *streptococci* tested are susceptible to erythromycin (MIC_{90} ≤0.5 μg/ml)

Streptococci belonging to 'bovis' and 'anginosus/milleri' group,[2] are part of the normal gut flora, and have been implicated in abdominal sepsis, empyema and bacterial endocarditis associated with carcinoma of the bowel. Gemifloxacin was shown to be extremely effective against the 'bovis' and 'anginosus/milleri' groups of *streptococci* followed by trovafloxacin, sparfloxacin, grepafloxacin, ciprofloxacin and levofloxacin

(Tables 32-33). Elevated MICs to ciprofloxacin and levofloxacin were observed in 'bovis' group of the *streptococci* tested compared with 'anginosus/milleri' group. All 'bovis' and 'anginosus/milleri' group *streptococci*, while being susceptible to penicillin (MIC_{90} ≤0.125 μg/ml) and ampicillin (MIC_{90} ≤0.25 μg/ml), showed higher MIC values than Lancefield groups A, C and G. All 'bovis' group samples were sensitive to vancomycin (MIC_{90} ≤1 μg/ml); one strain of 'anginosus/milleri' has a high MIC to vancomycin (MIC_{90} 2 μg/ml). Variable results were obtained with 'bovis' group to erythromycin, clarithromycin and tetracycline. The MIC_{90}s are significantly lower for clarithromycin compared to erythromycin. Only one isolate of 'anginosus/milleri' group was resistant to erythromycin and clarithromycin (MIC_{90} ≥32 μg/ml), while tetracycline showed poor activity for the 'bovis' and 'anginosus/milleri' groups of *streptococci*.

The spectrum of pathogens causing nosocomial infections is changing. Gram positive infections are increasing, and there is a requirement for the antimicrobial armamentarium to be strengthened to cover these infections. The new fluoroquinolones provide a therapeutic alternative in this area. This data shows gemifloxacin to be the most potent fluoroquinolone tested against all the groups of *streptococci*. It is highly active against known pathogens as well as passenger organisms. In immunocompromised patients, passenger organisms have been identified as potential pathogens and under appropriate conditions can cause disease in immunocompetent patients. The *in vitro* data indicates gemifloxacin can act as an agent for the treatment of Streptococci infections. Passenger organisms could play a predominant role in the future and pose a new therapeutic problem as antimicrobial resistance emerges within a population of 'difficult-to-treat' organisms.

Penicillin remains highly active against *streptococci* of Lancefield groups A, C and G. Penicillin shows reduced activity against the other *streptococci* tested (Lancefield group B, 'mitis', 'mutans', 'bovis' and 'anginosus/milleri' groups); alternative agents (macrolides, tetracycline and vancomycin) show lower activity than penicillin against these *streptococci*. Gemifloxacin is the most potent quinolone compound tested against various groups of *streptococci*. Gemifloxacin exhibited low MICs to all strains of *streptococci* including those with intermediate or resistant susceptibility to penicillin, erythromycin, tetracycline or ciprofloxacin. The potent anti-Streptococci activity of gemifloxacin makes it a promising agent for use against Gram positive infections.

The invention provides a method for modulating metabolism of *Streptococci* pathogenic bacteria. Skilled artisans can readily choose *Streptococci* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods

of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

A quinolone, particularly a gemifloxacin compound or composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria, preferably a *Streptococci* pathogenic bacteria, shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections caused by or related to *Streptococci* pathogenic bacteria.

In addition to the therapy described above, a gemifloxacin compound or composition used in the methods of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins, particularly *Streptococci* pathogenic bacteria, exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococci* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Streptococci* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Streptococci* pathogenic bacteria is selected from the group consisting of: *Streptococcus* spp. Other *Streptococci* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Mycoplasma* bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Mycoplasma* bacterial pathogens. An objective of these analyses was to determine the *in vitro* activity of gemifloxacin, other new quinolones and macrolides using low-passaged clinical isolates and type strains of *Mycoplasma* species commonly found in the respiratory and urogenital tract of humans.

Gemifloxacin was compared to levofloxacin, trovafloxacin, grepafloxacin, azithromycin, clarithromycin, tetracycline and clindamycin. The *in vitro* activity of quinolone gemifloxacin, other new quinolones and macrolides was determined using low-

passaged clinical isolates and type strains of *Mycoplasma* species commonly found in the respiratory and urogenital tract of humans. Organisms used in the analyses included *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma penetrans* and *Ureaplasma urealyticum*. MICs were determined using a microbroth dilution method. Assays for *U. urealyticum* were performed in 10B media and all other mycoplasma assays were carried out in SP4 medium. Comparator drugs include levofloxacin, trovafloxacin, grepafloxacin, azithromycin, clarithromycin, tetracycline and clindamycin.

Data provided herein suggets that depending on the species tested, gemifloxacin shows variable results when compared with the macrolides. *In vitro*, gemifloxacin was as active as or more active than tetracycline, clindamycin and the other quinolones.

Previous studies have shown that gemifloxacin is highly active against the common respiratory tract pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* (Moore, *et al.*, ICAAC, Abstract F-098 (1998); Kim, *et al.*, ICAAC, Abstract F-093 (1998)) and the atypical organisms *Legionella pneumophila* (Critchley, *et al.*, ICAAC, Abstract F-100 (1998); Dubois, *et al.*, ICAAC, Abstract F-105 (1998)) and *Chlamydia pneumoniae* (Ridgeway, *et al*, ICAAC, Abstract F-097 (1998); Kim, *et al.*, ICAAC, Abstract F-093 (1998)). Gemifloxacin also showed antimicrobial activity in one study in which a limited number of *Mycoplasma* species were analyzed (Hannan, *et al.*, ICAAC, Abstract F-101 (1998)). To confirm the activity of gemifloxacin against *Mycoplasma* species, the MIC was determined for a large number of clinical isolates and type strains, and the *in vitro* effectiveness of gemifloxacin was compared with that of other new quinolones, macrolides, tetracycline and clindamycin.

Isolates of mycoplasmas, including low-passaged clinical isolates and type strains, were tested for antimicrobial susceptibility. Most of these isolates had been passaged only a few times in artificial media. *Mycoplasma pneumoniae* isolates included 130 strains collected from six different countries (representing isolates collected over a 10 year period). All the isolates originated from the respiratory tract of individuals with proven respiratory disease. The isolates were identified by the polymerase chain reaction as *M. pneumoniae* and were shown not to be cross-reactive with *Mycoplasma genitalium* or any other *Mycoplasma* species known to occur in humans in either the respiratory or genital tract: ATCC, n = 2; Denmark, n = 6; Australia, n = 5; Japan, n = 4; England, n = 9; China, n = 11; US, n = 93.

Mycoplasma hominis isolates (n = 50) included seven type strains and 43 low-

passaged clinical isolates. The origins of the clinical isolates are shown in Table 34. *Mycoplasma fermentans* isolates (n = 18) included one type strain, two clinical isolates (from endometrial biopsy and urethra) and 15 well-characterized strains derived from cell culture, the respiratory tract, bone marrow or synovium (provided by J. Tully, Mycoplasma Section, NIAID). *Ureaplasma urealyticum* isolates (n = 100) included 14 serotypes and 86 clinical isolates (Table 35). *M. genitalium* strains consisted of two isolates (provided by J. Tully at NIAID), and two isolates of *Mycoplasma penetrans* were tested (provided by J. Tully at NIAID and A. Yanez, Puebla, Mexico).

Antimicrobial powders were obtained from their respective manufacturers except for tetracycline and clindamycin, which were purchased from Sigma, St Louis, MO, USA. The following antimicrobials were used: gemifloxacin, trovafloxacin, levofloxacin, grepafloxacin, clarithromycin, azithromycin, tetracycline and clindamycin. Powders were dissolved according to the manufacturers' recommendations. Stock solutions of each drug containing 2048 μg/ml were prepared fresh for each assay.

A microbroth dilution method (Cassell, *et al., In: Clinical and pathogenic microbiology*, St. Louis, MO, USA, 1994) was used to determine MICs. Serial twofold dilutions of antimicrobials in *Mycoplasma* media (10B for *U. urealyticum* and SP4 for all other *Mycoplasma* species) were carried out to give a range of concentrations from 0.008 to 256 μg/ml for each drug tested. An inoculum of 10^4–10^5 organisms, as measured by color changing units (CCU)/ml, was used. Plates were incubated aerobically at 37°C. All plates were examined daily until a color change was detected in the drug-free (growth) control. Due to the rapid growth of *U. urealyticum*, the first reading should occur after 16 h of incubation followed by multiple readings per day. The MIC for each drug was determined for all isolates. The initial MIC was defined as the lowest concentration of antimicrobial in which the metabolism of the organism was inhibited, as evidenced by lack of a color change in the medium at the time the drug-free control first showed a color change (time points varied for each species). Any mycoplasmal isolate in which resistance was suspected from the test was re-tested on another day to ensure reproducibility of results. Detailed descriptions of this method have been previously reported (Cassell, *et al., In: Clinical and pathogenic microbiology*, St. Louis, MO, USA, 1994).

The microtiter susceptibility testing method employed allowed excellent reproducibility of MIC results within the simultaneous duplicate runs and between assays carried out on different days. Comparative *in vitro* activities of gemifloxacin and other antimicrobials against strains of mycoplasmas and ureaplasmas except for *M. genitalium*

and *M. penetrans* are shown in Table 36. MIC results for isolates of *M. genitalium* and *M. penetrans* are given in Table 37. Gemifloxacin demonstrates excellent *in vitro* activity against the species tested. The overall MIC range is ≤0.008–0.5 μg/ml, which is well within the range of expected clinical susceptibility.

For *M. pneumoniae* and *M. genitalium*, MICs for gemifloxacin are comparable to those of grepafloxacin, but they are two- to four-fold lower than those of the other quinolones and tetracycline. Azithromycin and clarithromycin show the best activity towards these two species, inhibiting all strains at ≤0.008 μg/ml. *M. fermentans* and *M. hominis* have a high degree of susceptibility to gemifloxacin (MIC_{90} ≤0.008 μg/ml). For *M. hominis*, the activity of gemifloxacin is comparable to that of clindamycin and grepafloxacin and is superior to that of tetracycline and the other quinolones. Macrolides were not tested because of known resistance. *M. fermentans* is resistant to clarithromycin, but showed some susceptibility to azithromycin.

U. urealyticum is equally susceptible to gemifloxacin and trovafloxacin, but the other quinolones are two- to four-fold less active. Gemifloxacin is also more effective than tetracycline or azithromycin against this microrganism. However, clarithromycin has the highest activity against *U. urealyticum*. Against *M. penetrans*, gemifloxacin is at least as active as the other compounds tested.

In vitro, gemifloxacin is as active as or more active than tetracycline, clindamycin and the other quinolones against the mycoplasmas and ureaplasmas tested. Even though the strains were collected over a 10 year period and widely differing geographic areas were analyzed, no resistance to gemifloxacin was observed. The results from this study and other recent studies (Hannan, *et al.*, ICAAC, Abstract F-101 (1998)) indicate that gemifloxacin is a promising drug for the treatment of respiratory and urogenital tract infections caused by Mycoplasma and Ureaplasma species. In addition, gemifloxacin has been reported to be a very active fluoroquinolone against a wide range of Gram negative and Gram positive pathogens, (Moore, *et al.*, ICAAC, Abstract F-098 (1998); Kim, *et al.*, ICAAC, Abstract F-093 (1998); Critchley, *et al.*, ICAAC, Abstract F-100 (1998); Dubois, *et al.*, ICAAC, Abstract F-105 (1998); Ridgeway, *et al.*, ICAAC, Abstract F-097 (1998)) making it an effective new drug in the treatment of infectious diseases.

Table 34. Origin of *M. hominis* Clinical Isolates Investigated

No. of isolates	Specimen cultured	Diagnosis
17	Endometrial Biopsy	Pelvic inflammatory disease
13	Urethra	Non-gonococcal urethritis

No. of isolates	Specimen cultured	Diagnosis
12	Endometrial Biopsy	Endometritis
1	Wound	?

Table 35. Origin of *U. urealyticum* Clinical Isolates Investigated

No. of isolates	Specimen cultured	Diagnosis
28	Endometrial Biopsy	Pelvic inflammatory disease
26	Urethra	Non-gonococcal urethritis
20	Endometrial Biopsy	Endometritis
12	Endotracheal aspirates	Infant respiratory distress syndrome

5

Table 36. *In Vitro* Activities of Gemifloxacin and Other Antimicrobials Against Mycoplasmas

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Mycoplasma pneumoniae (n = 130)			
Gemifloxacin	≤0.008–0.125	0.063	0.125
Trovafloxacin	≤0.008–1.0	0.125	0.25
Grepafloxacin	0.063–0.25	0.063	0.125
Levofloxacin	0.031–8.0	0.5	0.5
Tetracycline	0.008–1.0	0.125	0.5
Clarithromycin	≤0.008	≤0.008	≤0.008
Azithromycin	≤0.008	≤0.008	≤0.008
Mycoplasma fermentans (n = 18)			
Gemifloxacin	≤0.008	≤0.008	0.008
Trovafloxacin	≤0.008–0.031	≤0.008	0.016
Grepafloxacin	≤0.008–0.5	≤0.008	0.016
Levofloxacin	≤0.008–0.063	0.016	0.063
Tetracycline	≤0.008–0.125	0.031	0.063
Clarithromycin	≤0.008–128	16	64
Azithromycin	0.063–4.0	0.5	2.0
Clindamycin	0.016–0.125	0.016	0.031
Mycoplasma hominis (n = 30)			
Gemifloxacin	≤0.008	≤0.008	≤0.008
Trovafloxacin	≤0.008–0.031	≤0.008	0.031
Grepafloxacin	≤0.008–0.031	≤0.008	0.016
Levofloxacin	≤0.008–0.25	0.125	0.25
Tetracycline	≤0.008–128	0.063	32
Clindamycin	≤0.008–0.016	≤0.008	0.016
Ureaplasma urealyticum (n = 100)			
Gemifloxacin	≤0.008–0.5	0.125	0.250
Trovafloxacin	≤0.008–0.5	0.063	0.125
Grepafloxacin	0.031–2.0	0.25	1.0
Levofloxacin	0.125–2.0	0.5	1.0

Microrganism and antimicrobial	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Tetracycline	0.031–128	0.125	16
Clarithromycin	≤0.008–0.25	0.031	0.063
Azithromycin	0.25–4	1	4

Table 37. MIC Results for Isolates of *M. genitalium* and *M. penetrans*

Isolate	MIC_{90} (μg/ml)							
	GEM	TRO	GRE	LEV	TET	CLA	AZI	CLI
M. genitalium *(n = 2)*								
JB	0.063	0.063	0.125	1.0	0.125	≤0.008	≤0.008	0.25
G37	0.063	0.063	0.125	0.5	0.063	≤0.008	≤0.008	0.25
M. penetrans *(n = 2)*								
MP	≤0.008	≤0.008	0.016	0.031	0.125	≤0.008	≤0.008	≤0.008
MFDEB	≤0.008	≤0.008	0.5	0.016	0.016	32	0.125	0.031

The invention provides a method for modulating metabolism of *Mycoplasma* pathogenic bacteria. Skilled artisans can readily choose *Mycoplasma* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention can be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Mycoplasma* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Mycoplasma* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Mycoplasma* pathogenic bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma penetrans* and *Ureaplasma urealyticum*. Other *Mycoplasma* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these

organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Legionella* spp. .

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Legionella* spp. pathogens. An objective of these analyses was to determine the *in vitro* activity and postantibiotic effect (PAE) of gemifloxacin (SB-265805), trovafloxacin, moxifloxacin, grepafloxacin, levofloxacin, ofloxacin, ciprofloxacin, azithromycin, clarithromycin, erythromycin and rifampicin against a panel of *Legionella* spp. Test isolates included *Legionella pneumophila* serogroup 1–12, *Legionella dumoffii*, *Legionella micdadei* and *Legionella longbeacheae*. Minimum inhibitory concentrations (MICs) were determined by standard two-fold agar dilution, while PAE was determined by exposing the isolates to the test agents at 4 × MIC for 1 h. The drug was removed by three consecutive centrifugations into fresh broth, and PAE calculated by measuring bacterial growth kinetics in similar drug-free cultures. Trovafloxacin and rifampicin were the most active agents tested against all isolates ($MIC_{90} \leq 0.008$ μg/mL). Gemifloxacin displayed a high potency against all *L. pneumophila* isolates ($MIC_{90} \leq 0.03$ μg/mL) which was comparable to grepafloxacin and ciprofloxacin ($MIC_{90} \leq 0.03$ μg/mL) and more active than ofloxacin (MIC_{90} 0.03 μg/mL). Against *L. dumoffii* and *L. longbeacheae*, gemifloxacin was as active as grepafloxacin (MIC_{90} 0.06 μg/mL), and against L. micdadei gemifloxacin was equally active to moxifloxacin, ofloxacin and ciprofloxacin (MIC_{90} 0.03 μg/mL). Gemifloxacin showed the longest PAE against erythromycin-resistant *L. pneumophila* (4.65 h), compared with 4.18 h for grepafloxacin, 3.38 h for moxifloxacin and 2.83 h for trovafloxacin. The PAE of gemifloxacin was significantly higher ($p<0.05$) than that of rifampicin (0.93 h), clarithromycin (1.9 h), azithromycin (0.9) and levofloxacin (2.59 h). Against erythromycin-susceptible *L. pneumophila* only gemifloxacin, moxifloxacin, ofloxacin and ciprofloxacin had a PAE over 3 h. For other erythromycin-resistant *Legionella* spp. gemifloxacin, grepafloxacin, levofloxacin, ofloxacin and rifampicin had PAEs in excess of 3 h, which was significantly longer ($p<0.05$) than the PAE of ciprofloxacin (2.13 h), moxifloxacin (2.02 h) and erythromycin (0.44 h). The half-life of gemifloxacin and the data from this study indicate a significant PAE to support a once-daily administration of gemifloxacin for the treatment of *Legionella* infections.

In recent years, *Legionella* has emerged as an important pathogen in community acquired pneumonia and nosocomial pneumonia, (Stout, *et al.*, *Diag. Microbial. Infect.*

Dis., 30: 37-43 (1998)) with many studies ranking it among the top three pathogens in community-acquired pneumonia both in the Western hemisphere and in Europe (Aubertin, *et al.*, *Infection*, 15: 328-331 (1987)). Erythromycin has traditionally the drug of choice with some gastrointestinal side effects (Tsai, *et al.*, *Ann. Inter. Med.*, 90: 509-517 (1979)). However, the newer macrolides and quinolones have improved *in vitro* activity (Dubois, *et al.*, *Diag. Microbiol. Infec. Dis.*, 12: 89-91S (1989); Dubois, *et al.*, 36th ICAAC, Abstract 1992 (1996); Dubois, *et al.*, Diag. Microbiol. Infec. Dis., 33: 261-265 (1999); Dubois, *et al.*, *Clin. Microbiol. Infect.*, 5: 205-212 (1999)). Susceptibility studies have found the novel fluoroquinolone gemifloxacin to be appreciably more potent than other fluoroquinolones against many Gram-positive organisms. In addition, it retains activity against a range of Gram-negative bacilli, including strains resistant to other antimicrobial agents, and has potent activity against various atypical respiratory pathogens.

This study compared the MIC and postantibiotic effect (PAE) of gemifloxacin, trovafloxacin, moxifloxacin, grepafloxacin, levofloxacin, ofloxacin, ciprofloxacin, azithromycin, clarithromycin, erythromycin and rifampicin against a range of *Legionella* species isolated from environmental sources or from patients with nosocomial or acquired respiratory tract infection.

A variety of *Legionella* isolates were cultured from respiratory tract and environmental sources. Identification of strains was by standard methods (Washington, et al., Manual of Clinical Microbiology, 6th ed., 533-544 (1995)).

MICs were determined by standard, two-fold agar dilution (National Committee for Clinical Laboratory Standards, 4th ed., Approved Standard M7-A4 (1997)) using buffered yeast extract (BYE) agar. A final inoculum of approximately 10^4 cfu was plated onto buffered yeast extract (BYE) agar containing doubling dilutions of antibiotic (0.004–256 µg/mL), and incubated at 35°C for 48 h. *Pseudomonas aeruginosa* ATCC 27853 and *L. pneumophila* ATCC 33152 were included as reference strains.

PAE was determined a broth technique in BYE, (Craig, *et al.*, Antibiotics in Laboratory Medicine, Williams & Wilkins, pp. 515-536 (1986)) in which each strain was exposed to antibiotic concentrations at 4 × MIC. Fresh inoculum (1 mL, final concentration 10^6–10^7 cfu/ml) was added to 9 mL prepared antibiotic-containing medium and to 9 mL drug-free control medium and incubated at 37°C for 1 h. Antibiotic was then removed by three consecutive centrifugations at 1200 × g for 10 minutes. Bacterial counts were performed on all cultures at time 0, before and after washing, and every hour until turbidity developed. These counts were plotted on a graph, and the duration of PAE was calculated

using the equation:

$$PAE = T - C$$

where T is the time for the count in the antibiotic-exposed culture to increase by 1 $\log_{10}$ above the count recorded immediately after drug removal, and C is the time required for the count in the control culture to increase by 1 $\log_{10}$ above the count observed immediately after the completion of the same procedure.

The activity of the panel of test antibiotics against *L. pneumophila* serogroups 1–9 is shown in Tables 38 and 39. The MIC range of gemifloxacin against these serogroups was 0.008–0.06 μg/mL; this activity was around six-fold higher than erythromycin and azithromycin (0.008–1.0 μg/mL). Gemifloxacin showed similar activity to grepafloxacin (MIC_{90} 0.16 μg/mL), levofloxacin (MIC_{90} 0.16 μg/mL), and moxifloxacin (MIC_{90} 0.16 μg/mL), but was superior to ciprofloxacin ((MIC_{90} 0.03 μg/mL), ofloxacin(MIC_{90} 0.03 μg/mL) and ofloxacin (MIC_{90} 0.03 μg/mL) and was inferior to trovafloxacin (MIC_{90} <0.004 μg/mL).

Overall, *L. pneumophila* serogroups 1–3 (MIC_{90} 0.016 μg/mL) and 7–9 (MIC_{90} 0.016 μg/mL) were more susceptible to gemifloxacin than serogroups 4–6 (MIC_{90} 0.03 μg/mL). Against the most prevalent strain, *L. pneumophila* serogroup 1, the activity of gemifloxacin (MIC_{90} 0.004 μg/mL) was superior to azithromycin, clarithromycin, erythromycin, ofloxacin and ciprofloxacin (MIC_{90} 0.03–1.0 μg/mL).

The activity of gemifloxacin, grepafloxacin, trovafloxacin, moxifloxacin, levofloxacin, offloxacin, ciprofloxacin and clarithromycin (MIC_{90} 0.06 μg/mL) was superior to azithromycin (MIC_{90} 0.25 μg/mL) and erythromycin (MIC_{90} 0.5 μg/mL) against *Legionella dumoffii* and *Legionella longbeacheae* (Table 40). Against *L. micdadei*, gemifloxacin, trovafloxacin, grepafloxacin, levofloxacin, ciprofloxacin, ofloxacin and moxifloxacin (MIC_{90} 0.03 μg/mL) were five-fold more active than erythromycin.

Mean values for the PAE of test antibiotics against erythromycin-susceptible and -resistant *Legionella* spp. are shown in Table 41. Only gemifloxacin, moxifloxacin and grepafloxacin displayed a significant ($p<0.05$) mean PAE over 3 h against erythromycin-resistant *L. pneumophila*, with clarithromycin, erythromycin and rifampicin demonstrating a PAE of under 2 h against these strains. Against other erythromycin-resistant *Legionella* isolates, a mean PAE over 3 h was observed with gemifloxacin, grepafloxacin, levofloxacin, ofloxacin and rifampicin. Erythromycin and clarithromycin demonstrated the shortest PAE (under 2 h).

A mean PAE over 3 h was recorded for gemifloxacin, moxifloxacin, ofloxacin and ciprofloxacin against erythromycin-susceptible *L. pneumophila*. Gemifloxacin and ofloxacin were the only quinolones showing a significant ($p<0.05$) mean PAE over 2 h against other erythromycin-susceptible *Legionella* spp.

As *L. pneumophila* is a facultative, intracellular bacterium which multiplies within phagocytic cells, both PAE and MIC activity may be expected to have a relationship with antimicrobial dosing regimens. While rifampicin is remarkably active against both extra- and intracellular *Legionella* spp., it too is reversibly inhibitory and its use as monotherapy is discouraged on the theoretical grounds of emergence of resistance (Edelstein, *et al.*, *Clin. Infec. Dis.*, 21 (supp. 3), 265-276 (1989)). Erythromycin, alone or in combination with agents such as rifampicin, is the usual treatment of choice for legionella pneumonia, though the newer macrolides, clarithromycin and azithromycin, are also effective.

Based on the MIC data from this study, gemifloxacin can be an effective antimicrobial agent against most *Legionella* spp. In particular, these results demonstrate that gemifloxacin is significantly ($p<0.001$) superior to erythromycin, the agent most commonly used in the treatment of legionellosis. The *in vitro* activity of gemifloxacin was also significantly ($p<0.05$) superior to azithromycin.

Against erythromycin-susceptible isolates of *L. pneumophila*, gemifloxacin demonstrated a mean PAE of 3.49 h which was over 1 h longer than that of trovafloxacin and levofloxacin, and over 2.5 h longer than erythromycin and clarithromycin. The mean PAE of gemifloxacin against other erythromycin-susceptible *Legionella* spp. (2.27 h) was also at least 1 h longer than that of trovafloxacin, moxifloxacin and clarithromycin.

The PAE of gemifloxacin was greater against erythromycin-resistant *L. pneumophila* isolates than susceptible strains (4.65 h *vs* 3.49 h). This effect was significantly ($p<0.05$) superior to that of trovafloxacin, levofloxacin, ciprofloxacin, azithromycin, clarithromycin, erythromycin and rifampicin. A difference in mean PAE was also noted among some quinolones tested such as gemifloxacin and trovafloxacin against erythromycin-resistant *Legionella* spp. other than *L. pneumophila*.

Table 38. Antimicrobial susceptibility of *L. pneumophila* serogroups 1–4

Antimicrobial agent	*L. pneumophila* serogroup 1 (n = 85)			*L. pneumophila* serogroup 2 (n = 17)			*L. pneumophila* serogroup 3 (n = 15)			*L. pneumophila* serogroup 4 (n = 26)		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.008–0.06	0.016	**0.016**	0.008–0.016	0.008	0.016	0.008–0.016	0.016	0.016	0.008–0.03	0.016	0.03
Trovafloxacin	≤ 0.004–0.016	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004
Moxifloxacin	≤ 0.004–0.03	0.016	0.016	≤ 0.004–0.016	0.008	0.008	≤ 0.004–0.016	0.008	0.016	≤ 0.004–0.016	0.016	0.016
Grepafloxacin	≤ 0.004–0.06	0.016	0.016	≤ 0.004–0.03	0.008	0.016	≤ 0.004–0.016	0.008	0.016	0.008–0.016	0.008	0.016
Levofloxacin	≤ 0.004–0.016	0.016	0.016	≤ 0.004–0.016	0.008	0.008	0.008–0.016	0.008	0.016	0.004–0.016	0.016	0.016
Ofloxacin	0.008–0.03	0.03	0.03	0.008–0.03	0.016	0.03	0.016–0.03	0.016	0.03	0.008–0.03	0.03	0.03
Ciprofloxacin	0.016–0.25	0.03	0.03	≤ 0.004–0.03	0.016	0.016	≤ 0.004–0.03	0.03	0.03	0.016–0.12	0.03	0.06

Antimicrobial agent	*L. pneumophila* serogroup 1 (n = 85)			*L. pneumophila* serogroup 2 (n = 17)			*L. pneumophila* serogroup 3 (n = 15)			*L. pneumophila* serogroup 4 (n = 26)		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Azithromycin	0.008–1.0	0.06	0.5	0.008–0.12	0.06	0.12	0.016–0.25	0.12	0.25	0.008–0.25	0.12	0.12
Clarithromycin	≤ 0.004–0.12	0.06	0.06	≤ 0.004–0.06	0.03	0.06	0.016–0.06	0.03	0.06	0.004–0.06	0.03	0.06
Erythromycin	0.03–1.0	0.25	1.0	0.008–0.5	0.25	0.25	0.06–0.5	0.25	0.5	0.016–0.5	0.5	0.5
Rifampicin	≤ 0.004–0.008	≤ 0.004	0.008	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004–0.008	≤ 0.004	≤ 0.004

Table 39. Antimicrobial susceptibility of *L. pneumophila* serogroups 5–12

Antimicrobial agent	*L. pneumophila* serogroup 5 (n = 15)			*L. pneumophila* serogroup 6 (n = 40)			*L. pneumophila* serogroup 7 (n = 2)			*L. pneumophila* serogroups 8, 9 and 12 (n = 4)		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%

Antimicrobial agent	*L. pneumophila* serogroup 5 (n = 15)			*L. pneumophila* serogroup 6 (n = 40)			*L. pneumophila* serogroup 7 (n = 2)			*L. pneumophila* serogroups 8, 9 and 12 (n = 4)		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.03–0.06	0.03	0.03	0.008–0.03	0.016	0.03	0.008–0.016	0.008	0.016	0.016	0.016	0.016
Trovafloxacin	≤ 0.004–0.008	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004
Moxifloxacin	≤ 0.004–0.03	0.016	0.016	≤ 0.004–0.016	0.008	0.016	≤ 0.004–0.016	≤ 0.004	0.016	0.016	0.016	0.016
Grepafloxacin	≤ 0.004–0.003	0.016	0.03	≤ 0.004–0.016	0.008	0.016	≤ 0.004–0.008	≤ 0.004	0.008	0.008	0.008	0.008
Levofloxacin	≤ 0.004–0.016	0.008	0.016	0.008–0.016	0.008	0.016	0.008–0.016	0.008	0.016	0.008–0.016	0.008	0.016
Ofloxacin	0.008–0.03	0.016	0.03	0.008–0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ciprofloxacin	0.016–0.06	0.03	0.03	≤ 0.004–0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Azithromycin	0.008–0.5	0.03	0.25	0.016–0.25	0.06	0.12	0.06	0.06	0.06	0.06	0.06	0.06

Antimicrobial agent	*L. pneumophila* serogroup 5 (n = 15)			*L. pneumophila* serogroup 6 (n = 40)			*L. pneumophila* serogroup 7 (n = 2)			*L. pneumophila* serogroups 8, 9 and 12 (n = 4)		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Clarithromycin	0.03–0.06	0.03	0.06	≤ 0.004–0.06	0.016	0.06	0.016–0.06	0.016	0.06	0.06	0.06	0.06
Erythromycin	0.06–1.0	0.25	0.5	0.008–0.25	0.12	0.25	0.12–0.5	0.12	0.5	0.25	0.25	0.25
Rifampicin	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004–0.008	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004

Table 40. Susceptibility of other *Legionella* spp.

Antimicrobial agent	*L. dumoffii* (n = 10)			*L. micdadei* (n = 10)			*L. longbeacheae* (n = 7)			Other *Legionella* spp. (n = 7)*		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.06	0.06	0.06	0.008–0.03	0.016	0.03	0.016–0.06	0.06	0.06	0.016–0.06	0.03	0.06

Antimicrobial agent	*L. dumoffii* (n = 10)			*L. micdadei* (n = 10)			*L. longbeacheae* (n = 7)			Other *Legionella* spp. (n = 7)*		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Trovafloxacin	≤ 0.004–0.008	0.008	0.008	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004	≤ 0.004
Moxifloxacin	0.008–0.03	0.03	0.03	0.008–0.03	0.016	0.03	0.008–0.03	0.016	0.03	0.008–0.03	0.008	0.03
Grepafloxacin	0.06	0.06	0.06	≤ 0.004–0.016	0.008	0.016	≤ 0.004–0.06	0.03	0.06	≤ 0.004–0.03	0.03	0.03
Levofloxacin	0.016	0.016	0.016	0.008–0.016	0.016	0.016	0.008–0.016	0.016	0.016	0.008–0.06	0.016	0.016
Ofloxacin	0.03	0.03	0.03	0.03	0.03	0.03	0.016–0.03	0.03	0.03	≤ 0.004–0.06	0.016	0.06
Ciprofloxacin	0.016–0.03	0.016	0.03	0.016–0.03	0.016	0.03	≤ 0.004–0.03	0.016	0.03	≤ 0.004–0.03	0.016	0.03
Azithromycin	0.12–0.25	0.12	0.25	0.016–0.25	0.25	0.25	0.016–0.25	0.12	0.25	0.016–0.5	0.12	0.5
Clarithromycin	0.03–0.06	0.03	0.06	0.03–0.12	0.06	0.06	0.008–0.06	0.06	0.06	≤ 0.004–0.12	0.03	0.12
Erythromycin	0.25–0.5	0.25	0.5	0.25–1.0	0.5	1.0	0.008–0.5	0.25	0.5	0.016–1	0.5	1.0

Antimicrobial agent	*L. dumoffii* (n = 10)			*L. micdadei* (n = 10)			*L. longbeacheae* (n = 7)			Other *Legionella* spp. (n = 7)*		
	MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)			MIC (μg/mL)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Rifampicin	≤ 0.004–0.03	0.008	0.016	0.008	0.008	0.008	≤ 0.004–0.06	≤ 0.004	0.06	≤ 0.004–0.008	≤ 0.004	0.008

*Includes one isolate of *L. bozemanii, L. feelei, L. jordanis, L. gormanii, L. oakridgensis, L. sainthelensi* and *L. wadsworthii*

Table 41. Mean PAE of test antibiotics against erythromycin-resistant and -susceptible *Legionella* strains

Antimicrobial agent (4 × MIC)	Mean PAE (h)*			
	Erythromycin-resistant strains		Erythromycin-susceptible strains	
	L. pneumophila (n = 7)	*Legionella* spp.† (n = 9)	*L. pneumophila* (n = 15)	*Legionella* spp.** (n = 13)
Gemifloxacin	4.65 ± 3	3.34 ± 2	3.49 ± 3	2.27 ± 2
Trovafloxacin	2.83 ± 2	2.25 ± 2	1.71 ± 1	1.22 ± 1
Moxifloxacin	3.38 ±2	2.02 ± 1	3.59 ± 3	1.18 ± 2
Grepafloxacin	4.18 ±3	3.67 ± 1	2.62 ± 3	1.67 ± 1
Levofloxacin	2.59 ± 2	3.24 ± 1	2.14 ± 2	1.35 ± 1
Ofloxacin	2.99 ± 1	4.13 ± 2	3.53 ± 3	3.04 ± 2
Ciprofloxacin	2.86 ± 2	2.13 ± 3	3.61 ± 2	1.86 ± 2
Azithromycin	2.16 ± 1	2.13 ± 1	2.91 ± 3	1.86 ± 2
Clarithromycin	1.90 ± 1	1.60 ± 2	0.72 ± 2	0.98 ± 2
Erythromycin	0.90 ± 1	0.44 ± 1	0.93 ± 1	2.06 ± 2
Rifampicin	0.93 ± 4	5.6 ± 3	2.86 ± 5	3.09 ± 4

*Means are given ± SD

†*L. micdadei* (n=1), *L. dumoffii* (n=3), *L. bozemanii* (n=1), *L. wadsworthii* (n=1), *L. jordanis* (n=1), *L. longbeacheae* (n=2)

***L. micdadei* (n=4), *L. dumoffii* (n=5), *L. bozemanii* (n=1), *L. gormanii* (n=1), *L. jordanis* (n=1), *L. longbeacheae* (n=1).

The findings of this study indicate that gemifloxacin has promise in the treatment of lower respiratory tract infections caused by *Legionella* spp. Considering the excellent *in vitro* activity and the long PAE of gemifloxacin against intracellular *L. pneumophila* recorded in this study, the agent appears to offer an excellent therapeutic option in the empirical treatment of patient with community-acquired pneumonia.

The invention provides a method for modulating metabolism of *Legionella* spp. pathogenic bacteria. Skilled artisans can readily choose *Legionella* spp. pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Legionella* spp. pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Legionella* spp. pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Legionella* spp. pathogenic bacteria is selected from the group consisting of: *Legionella pneumophilia, Legionella bozemanii, Legionella wadsworthii, Legionella jordanis, Legionella dumoffii, Legionella longbeacheae, Legionella micdadei,* Erithromycin-susceptible strains of *Legionella spp* and Erithromycin-resistant strains of *Legionella spp..* Other *Legionella* spp. pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Streptococcus pneumoniae.*

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Streptococcus pneumoniae* pathogens. An objective of these analyses was to determine the post antibiotic effect (PAE) of GEM in ciprofloxacin (CIP) and trovafloxacin (TRO) -susceptible and -resistant *S. pneumoniae.* CIP-resistant, clinical isolates of *S. pneumoniae* were collected from across Canada. Another objective of these analyses was to determine the PAE of gemifloxacin against penicillin-susceptible and penicillin-resistant *S. pneumoniae* and to determine if reduced susceptibility to

fluoroquinolones adversely affected the PAE of gemifloxacin.

Gemifloxacin (GEM) is active against penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* and is active against CIP- and TRO -resistant isolates. MICs were determined using a microbroth dilution technique described by the NCCLS. Topoisomerase IV (parC) and DNA gyrase (gyrA) mutations were confirmed by sequencing the QRDR region of each gene. Two fluoroquinolone-susceptible and 8 -resistant isolates (3 CIP-resistant, 5 CIP,TRO -resistant) were selected for study. The PAE was determined by exposing logarithmic phase organisms at 4 x or 10 x MIC for 2 h. Antimicrobial agents were removed using dilution into sterile media and the PAE was assessed using a viable colony counting technique. The MICs of CIP ranged from 0.5–64 μg/ml and the MICs of TRO ranged from 0.06–8.0 μg/ml. The MICs of GEM ranged from ≤0.03–0.5 μg/ml. The mean PAE of CIP in susceptible *S. pneumoniae* was 1.6 h at 4 x MIC and 2.5 h at 10 x MIC. In TRO-susceptible isolates, the mean PAE at 4 x MIC was 2.1 h and 3.2 h at 10 x MIC. The mean PAE of GEM was 2.7 h at 4 x MIC and 3.8 h at 10 x MIC. There was no significant difference in the duration of the GEM PAE between CIP or TRO -susceptible and -resistant strains ($p < 0.05$). In conclusion, GEM is highly active against CIP and TRO-susceptible and -resistant *S. pneumoniae* and produces a prolonged PAE in isolates displaying diminished susceptibility to other fluoroquinolones.

Gemifloxacin possesses potent activity against both penicillin-susceptible and -resistant *Streptococcus pneumoniae*. Several *in vitro* studies have also demonstrated that gemifloxacin is highly active against *S. pneumoniae* with reduced susceptibility to other fluoroquinolones.

The postantibiotic effect (PAE) is defined as the persistent suppression of bacterial growth following brief exposure to antimicrobial agents. Clinically, the PAE can have a major impact on the dose regimen and efficacy of the drug *in vivo*. Depending upon the rate of elimination and half-life of an antimicrobial agent, tissue and blood levels of an antimicrobial may fall below the MIC before the next scheduled dose. As a result, drug-pathogen combinations that do not exhibit a PAE may require more frequent antimicrobial administration. However, demonstration of a prolonged PAE ensures that bacterial re-growth will not occur even if tissue and serum levels of the antimicrobial fall below the MIC. Thus, during the PAE phase, there is no loss of efficacy.

Ten clinical isolates of *S. pneumoniae*, (2 ciprofloxacin-susceptible, 3 with reduced susceptibility to ciprofloxacin, and 5 trovafloxacin-resistant, "ciprofloxacin-resistant") were selected for study. All isolates were obtained from respiratory sites.

MICs were determined using a microbroth dilution method, as recommended by the NCCLS. Briefly, 18–20 h blood agar cultures were used to prepare suspensions equal to a 0.5 MacFarland in saline. Cation-supplemented Mueller-Hinton broth (MHB) was inoculated to a final concentration of 5 x 10^5 CFU/ml. Lysed horse blood was added to a final concentration of 5%. The susceptible, intermediate, and resistant breakpoints for determining penicillin susceptibility of *S. pneumoniae* were: susceptible ≤0.06 μg/ml, intermediate 0.12–1 μg/ml, and resistant ≥2.0 μg/ml. Microtiter plates were incubated at 35°C and the MIC was defined as the lowest concentration of antimicrobial that inhibited bacterial growth after 20–24 h of incubation (NCCLS guidelines are 20–24 h for *S. pneumoniae).*

One to two colonies were used to inoculate 50 ml of MHB (with 5% lysed horse blood) and the culture was incubated overnight at 35°C. The overnight culture was diluted 1:5 into fresh medium, and allowed to incubate at 35°C until logarithmic growth was achieved. 1 ml of the resulting mid-log phase culture was added to i) a growth control containing 9 ml of broth and ii) a test culture containing 8.9 ml of broth and 100 μl of the test antimicrobial stock solution. A final concentration of approximately 10^6 isolates was achieved and confirmed by viable colony counting. After 2 h of incubation at 35°C, the test antimicrobial was removed using a dilution technique. One hundred (100) μl of the growth control and test culture were transferred to 9.9 ml of fresh broth resulting in a 1:100 dilution. The cultures were re-incubated at 35°C and aliquots removed for viable colony counts until marked turbidity was observed. The PAE was calculated using the formula; PAE=T-C, where T represents the time required for the organisms in the test culture to increase one $\log_{10}$ above the viable colony count immediately after dilution and C represents the time required for the organisms in the growth control to increase one $\log_{10}$ above the viable colony count immediately after dilution.

MICs of ciprofloxacin, trovafloxacin, and gemifloxacin are displayed in Table 42. Both gemifloxacin and trovafloxacin have excellent activity against penicillin-susceptible and penicillin-resistant *S. pneumoniae*. Gemifloxacin is active against isolates with reduced susceptibility to ciprofloxacin and resistance to trovafloxacin.

All agents producea prolonged PAE against fully susceptible strains of *S. pneumoniae* at both 4 x and 10 x MIC (Figures 16 and 17). Both trovafloxacin and gemifloxacin produce significant PAEs against isolates of *S. pneumoniae* with reduced susceptibility to ciprofloxacin. For isolates resistant to trovafloxacin, gemifloxacin retains a significant PAE (Table 43).

Gemifloxacin has excellent activity against both penicillin-susceptible and penicillin-resistant *S. pneumoniae*. Gemifloxacin is highly active against fluoroquinolone-resistant *S.*

pneumoniae isolates. Gemifloxacin produces a prolonged PAE against both penicillin-susceptible and penicillin-resistant strains of *S. pneumoniae*. Resistance to other fluoroquinolones does not significantly alter the PAE of gemifloxacin. The excellent anti-pneumococcal activity of gemifloxacin coupled with its prolonged PAE indicate that gemifloxacin can be used as an agent to treat lower respiratory tract infections.

Table 42. MICs of ciprofloxacin, trovafloxacin, and gemifloxacin against 10 clinical isolates of *S. pneumoniae*

Isolate susceptibility to penicillin	MIC (μg/ml)		
	Ciprofloxacin	Trovafloxacin	Gemifloxacin
Susceptible	0.5	0.06	<0.03
Susceptible	1	0.12	<0.03
Intermediate	4	0.5	<0.03
Resistant	4	1	0.06
Resistant	8	1	0.06
Resistant	16	4	0.12
Resistant	16	8	0.12
Resistant	32	8	0.25
Resistant	32	8	0.25
Resistant	64	8	0.5
MIC_{90}	**32**	**8**	**0.25**

Table 43. Duration of the postantibiotic effect (PAE) of ciprofloxacin, trovafloxacin, and gemifloxacin against 10 clinical isolates of *S. pneumoniae*

Isolate susceptibility to penicillin	PAE (h)					
	Ciprofloxacin*		Trovafloxacin*		Gemifloxacin	
	4 x MIC	10 x MIC	4 x MIC	10 x MIC	4 x MIC	10 x MIC
Susceptible	1.6	2.7	1.9	3.2	2.1	3.4
Susceptible	1.6	2.3	2.2	3.4	2.8	3.9
Intermediate			2.4	3.5	3.1	4.3
Resistant			1.9	2.8	2.7	4.0
Resistant	Not susceptible to ciprofloxacin		2.0	2. 9	3.1	4.0

Resistant					2.4	3.3
Resistant			Not susceptible to		2.7	3.7
Resistant			trovafloxacin		2.7	4.1
Resistant					2.4	3.4
Resistant					2.6	3.7
Mean PAE	**1.6**	**2.5**	**2.1**	**3.2**	**2.7**	**3.8**

*PAE only determined in susceptible strains

The invention provides a method for modulating metabolism of *Streptococcus pneumoniae* pathogenic bacteria. Skilled artisans can readily choose *Streptococcus pneumoniae* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Streptococcus pneumoniae* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Streptococcus pneumoniae* pathogenic bacteria is *Streptococcus pneumoniae* . Other *Streptococcus pneumoniae* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Haemophilus influenzae* or pneumococci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Haemophilus influenzae* or pneumococci pathogens. An objective of these analyses was to determine the post-antibiotic effect (PAE) of gemifloxacin, a novel, broad-spectrum fluoroquinolone. The gemifloxacin PAE was compared with that of ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin and trovafloxacin against five quinolone-susceptible strains and one quinolone-resistant strain of pneumococci. The PAE of gemifloxacin was also compared with that of the same five

quinolones and ampicillin, amoxycillin, amoxycillin/clavulanate, cefixime, cefuroxime and azithromycin against four quinolone-susceptible strains and one rare quinolone-resistant strain of *Haemophilus influenzae*.

Gemifloxacin had the lowest broth dilution MIC against both quinolone-susceptible pneumococci (0.016–0.03 μg/ml versus 0.06–2 μg/ml for other quinolones) and the quinolone-resistant strain (0.25 μg/ml versus 4–32 μg/ml for other quinolones). For all six pneumococcal strains, MICs of non-quinolones were in the range of 0.008–2.0 μg/ml. Gemifloxacin and the other quinolones had significant and similar PAEs in all strains (gemifloxacin PAE 0.4–1.6 h at 10 x MIC). At 10 x MIC, the PAEs for the non-fluoroquinolones were as follows: amoxycillin 0.4–5.8 h, cefuroxime 0.8–2.9 h, azithromycin 1.3–2.9 h and clarithromycin 1.8–4.5 h. Exposure of the quinolone-resistant pneumococcal strain at 5 x MIC gave lower PAEs than at 10 x MIC for all agents except gemifloxacin (0.9 h) and clarithromycin (4.3 h), where PAEs at both exposures were similar. Gemifloxacin also had low macrobroth dilution MICs against quinolone-susceptible strains of *H. influenzae* (0.002–0.004 μg/ml compared with 0.004–0.03 μg/ml for other quinolones) and the lowest quinolone MIC (0.5 μg/ml) against the quinolone-resistant strain, which also produced β-lactamase (MICs of other quinolones 1–4 μg/ml). For all five *H. influenzae* strains, MICs of non-quinolones were in the range 0.016–>16 μg/ml. Gemifloxacin gave PAEs for all quinolone-susceptible strains of *H. influenzae* tested (0.3–2.3 h at 10 x MIC compared with 0–6.2 h for other quinolones). Azithromycin PAEs for the five strains were 3.7–7.3. β-Lactams gave no PAEs in four strains and PAEs of 0.2–1.7 in one β-lactamase-negative strain. At 10 x MIC, no quinolone PAEs were found in the quinolone-resistant strain.

The worldwide incidence of pneumococci resistant to penicillin G and other β-lactam and non-β-lactam compounds has increased alarmingly, particularly in South Africa, Spain, Central and Eastern Europe, and parts of Asia. Penicillin resistance in pneumococci has also increased in the US, with values of 6.6% reported in 1991–92, (Breiman, *et al.*, *JAMA*, 271: 1831-1835 (1994)) which in a recent survey (1994–1995) had risen to 23.6% (Doern, *et al.*, *Antimicrob. Agents. Chemother.*, 40: 1208-1213 (1996)). There is an urgent need for oral compounds for the out-patient treatment of otitis media and respiratory tract infections (RTI) caused by penicillin-intermediate and penicillin-resistant pneumococci. Available quinolones, such as ciprofloxacin and ofloxacin, yield moderate *in vitro* activity against pneumococci, with MICs clustering around the breakpoints (Jacobs, *et al.*, *Rev.*

Med. Microbiol., 6: 77-93 (1995)). Preliminary studies with gemifloxacin (SB-265805; LB 20304a), a new, broad-spectrum fluoronaphthyridone carboxylic acid with a novel pyrrolidone substituent, (Cormican, *et al.*, *Antimicrob. Agents Chemother.*, 41: 204-211 (1997); Hohl, *et al.*, *Clin. Microbiol. Infect.*, 4: 280-284 (1996); Oh, *et al.*, *Antimicrob. Agents. Chemother.*, 40: 1564-1568 (1996)), have shown it to be highly active against pneumococci.

Although development of an effective vaccine against *Haemophilus influenzae* type b has led to disappearance of this organism in many parts of the world, its place has been taken by untypeable *H. influenzae* strains. The latter organisms (followed by *Streptococcus pneumoniae* and *Moraxella catarrhalis*) are now considered to be the leading causes of acute exacerbations of chronic bronchitis and important causes of acute otitis media, sinusitis and community-acquired RTI. Preliminary studies show gemifloxacin to be highly active against *H. influenzae* and *M. catarrhalis* (Cormican, *et al.*, *Antimicrob. Agents Chemother.*, 41: 204-211 (1997); Hohl, *et al.*, *Clin. Microbiol. Infect.*, 4: 280-284 (1996); Oh, *et al.*, *Antimicrob. Agents. Chemother.*, 40: 1564-1568 (1996)).

The post-antibiotic effect (PAE) is defined as the length of time that bacterial growth is suppressed following brief exposure to an antimicrobial (Craig, *et al. In: Antibiotics in laboratory medicine*, Williams and Wilkins, pp. 296-329 (1996)). This pharmacodynamic parameter is helpful when choosing antimicrobial dosing regimens. This study examined the MICs and PAEs of gemifloxacin compared with other antimicrobials against strains of pneumococci and *H. influenzae*.

Gemifloxacin, ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin and trovafloxacin were tested against five quinolone-susceptible (ciprofloxacin MIC <2 μg/ml) strains and one resistant (ciprofloxacin MIC >8 μg/ml)) strain of pneumococci. These quinolones and ampicillin, amoxycillin, amoxycillin/clavulanate, cefixime, cefuroxime and azithromycin were tested against four quinolone-susceptible strains (ciprofloxacin MIC ≤0.016 μg/ml) and one rare quinolone-resistant strain (ciprofloxacin MIC 2 μg/ml) of *H. influenzae*. Gemifloxacin susceptibility powder was obtained from SmithKline Beecham Laboratories, Harlow, Essex, UK. Other drugs were obtained from their respective manufacturers.

Broth MICs for the pneumococcal strains tested for PAE were performed according to NCCLS recommendations (National Committee for Clinical Laboratory Standards, Approved Standard M7-A4, Villanova, PA, USA (1997) using cation-adjusted Mueller–Hinton broth (MHB) with 5% lyzed defibrinated horse blood. Standard quality control

strains were included in each run of broth dilution MICs. For *H. influenzae*, β-lactamase production was tested by the Cefinase disk method (BBL Microbiology Systems, Cockeysville, MD, USA). As previous time-kill studies had shown that MICs determined by the macrobroth method were 1–2 dilutions lower than those obtained by microbroth techniques, MICs were taken visually from macrobroth time-kill assays. Standard quality control strains were included in each run.

The PAE was determined by the viable plate count method using MHB supplemented with 5% lysed horse blood for pneumococci and freshly made HTM for *H. influenzae* (Craig, *et al. In: Antibiotics in laboratory medicine*, Williams and Wilkins, pp. 296-329 (1996)). Inocula (at a turbidity of #1 McFarland) were diluted to yield a suspension of approximately 5 x 10^6 CFU/ml. PAE was induced by exposure of all strains to 10 x MIC of test drug for 1 h. The quinolone-resistant pneumococcal strain was also exposed to quinolone concentrations of 5 x MIC. Growth controls comprising inoculum but no antimicrobial and a control containing bacteria and the antimicrobial at 0.01 x MIC (to confirm that the antimicrobial was no longer bacteriostatic after dilution) were also tested. Viability counts were determined before exposure of bacteria to the test drug, immediately after dilution (0 h) and then every 2 h until tube turbidity reached a #1 McFarland standard. Recovery plates were incubated for 24 h and colony counts performed on plates yielding 30–300 colonies (Craig, *et al. In: Antibiotics in laboratory medicine*, Williams and Wilkins, pp. 296-329 (1996)).

The PAE is defined as $T - C$, where T is the time required for viability counts of an antimicrobial-exposed culture to increase by 1 $\log_{10}$ above counts immediately after dilution and C is the corresponding time for growth control. For each experiment, viability counts ($\log_{10}$ CFU/ml) were plotted against time and the results were expressed as the mean of two separate assays (Craig, *et al. In: Antibiotics in laboratory medicine*, Williams and Wilkins, pp. 296-329 (1996)).

Broth microdilution MICs (μg/ml) for the quinolone-susceptible pneumococcal strains were: gemifloxacin 0.016–0.03, ciprofloxacin 0.5–1, levofloxacin 1–2, sparfloxacin 0.125–0.5, grepafloxacin 0.06–0.25, trovafloxacin, 0.06–0.125, amoxycillin 0.016–1, cefuroxime 0.25–2, azithromycin 0.008–0.125 and clarithromycin 0.008–0.03. Corresponding MICs for the quinolone-resistant strain were 0.25, 32, 32, 16, 8, 4 μg/ml, respectively for the quinolones and 0.008, 0.016, 0.125 and 0.03 μg/ml, respectively for the non-quinolones. PAEs obtained at 10 x MIC for quinolone-susceptible pneumococcal strains are shown in Table 44. At 5 x MIC, PAEs (h) for the quinolone-resistant strain were:

gemifloxacin, 0.9; ciprofloxacin, 3.7; levofloxacin, 1.3; sparfloxacin and grepafloxacin, 1.5; and trovafloxacin, 1.3.

Of the four quinolone-susceptible strains and one quinolone-resistant strain of *H. influenzae*, three (including the resistant strain) were β-lactamase positive and not tested for PAE with ampicillin or amoxycillin. Macrobroth dilution MIC ranges of quinolones (μg/ml) for the quinolone-susceptible strains were: gemifloxacin, 0.002–0.004; ciprofloxacin, 0.004–0.016; levofloxacin, 0.008–0.03; and sparfloxacin, grepafloxacin and trovafloxacin 0.004–0.008. Corresponding MICs for the quinolone-resistant strain were 0.5, 2, 2, 2, 1 and 4 μg/ml, respectively. For all five *H. influenzae* strains, MICs (μg/ml) of the non-quinolones were: ampicillin, amoxycillin, 0.25–>16; amoxycillin/clavulanate, 0.25–0.5; cefixime, 0.016–0.25; cefuroxime, 0.5–2; and azithromycin, 0.5–2. PAEs for the quinolone-susceptible strains at 10 x MIC are given in Table 45. β-Lactams gave no PAEs in three strains and PAEs of 0.2–1.7 h in one β-lactamase-negative strain. At 10 x MIC, no quinolone PAEs were found in the quinolone-resistant strain.

Gemifloxacin is the most potent quinolone tested by MIC against both quinolone-susceptible and quinolone-resistant pneumococci.

Gemifloxacin, together with the other quinolones, has significant PAEs against all pneumococcal strains tested, including the quinolone-resistant strain. The latter is an advantage with regard to dosage regimens and indicates that the use of gemifloxacin can help to slow the development of quinolone resistance in pneumococci.

Gemifloxacin is highly active against quinolone-susceptible strains of *H. influenzae*. Only gemifloxacin demonstrated MICs ≤0.5 μg/ml against the rare strain with raised quinolone MICs. Gemifloxacin has a significant PAE against quinolone-susceptible *H. influenzae* strains. Gemifloxacin has a wide spectrum of activity against respiratory tract pathogens, such as pneumococci (including quinolone-resistant strains), *H. influenzae*, *Legionella* spp., *Chlamydia* spp. and mycoplasmas. This compound represents an attractive alternative to other quinolone and non-quinolone agents for the empiric treatment of community-acquired RTI.

Table 44. PAEs (h) Obtained at 10 x MIC for Five Quinolone-susceptible Strains of Pneumococci

Antimicrobial	Pneumococcal strain				
	60	WRU 294	ATCC 49619	19	24

Antimicrobial	Pneumococcal strain				
	60	WRU 294	ATCC 49619	19	24
Gemifloxacin	1.3	0.6	1.6	0.5	0.4
Ciprofloxacin	1.5	0.5	1.5	1.0	0.8
Levofloxacin	1.7	0.9	2.3	1.1	1.5
Sparfloxacin	0.8	0.3	1.1	0.9	0.5
Grepafloxacin	0.4	0.3	0.9	0.4	0.3
Trovafloxacin	2.0	1.3	3.0	1.6	1.3
Amoxycillin	1.8	0.4	5.8	0.8	1.8
Cefuroxime	2.1	0.8	2.9	1.1	1.0
Azithromycin	1.3	1.4	2.9	NT*	NT
Clarithromycin	2.6	1.8	4.5	NT	NT

*NT = not tested

Table 45. PAEs (h) Obtained at 10 x MIC for Four Quinolone-susceptible Strains of *H. influenzae*

Antimicrobial	*H. influenzae* strain			
	HH3	HH4	HH20	HH45
Gemifloxacin	2.3	0.3	1.9	2.0
Ciprofloxacin	3.4	1.3	4.2	1.7
Levofloxacin	5.4	2.8	6.2	2.9
Sparfloxacin	1.5	0.6	3.0	2.9
Grepafloxacin	1.5	0	2.1	1.4
Trovafloxacin	2.3	0.8	2.8	2.6
Ampicillin	0.5	0	NT*	NT

Antimicrobial	*H. influenzae* strain			
	HH3	HH4	HH20	HH45
Amoxycillin	0.7	0	NT	NT
Amoxycillin/clavulanate	0.9	0	0	0
Cefixime	0.2	0	0	0
Cefuroxime	1.7	0	0.1	0
Azithromycin	4.1	3.7	7.0	7.3

*NT = not tested

The invention provides a method for modulating metabolism of *Haemophilus influenzae* or pneumococci pathogenic bacteria. Skilled artisans can readily choose *Haemophilus influenzae* or pneumococci pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Haemophilus influenzae* or pneumococci pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Haemophilus influenzae* or pneumococci pathogenic bacteria.

While a preferred object of the invention provides a method wherein said pathogenic bacteria is selected from the group consisting of: *Haemophilus influenzae* and pneumococci . Other *Haemophilus influenzae* or pneumococci pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against Gram positive bacterial pathogens, such as streptococci and staphylococci, or Enterobacteriaceae bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various Gram positive bacterial pathogens, such as streptococci and

staphylococci, or Enterobacteriaceae pathogens. An objective of these analyses was to determine the effect of gemifloxacin on fecal flora in healthy subjects compared with placebo following 7 days of administration.

Quinolones have a selective effect on the normal human intestinal microflora. Published data on different quinolone agents (ciprofloxacin, enoxacin, norfloxacin, ofloxacin, pefloxacin, lomefloxacin, levofloxacin, sparfloxacin, rufloxacin, sitafloxacin, gatifloxacin, trovafloxacin and moxifloxacin) show that Gram negative aerobic bacteria, especially *Enterobacteriaceae*, are strongly suppressed or eliminated during therapy. Gemifloxacin is a new fluoroquinolone that has been shown to possess a broad spectrum of antimicrobial activity against Gram positive and Gram negative microrganisms, including methicillin-susceptible and methicillin-resistant staphylococci, *Streptococcus pneumoniae*, *Haemophilus influenzae* and most members of the family *Enterobacteriaceae*.

The aim of the present study was to investigate the effect of gemifloxacin on the human intestinal microflora. Gemifloxacin was given in oral doses of 320 mg for 7 days to 10 healthy subjects and 5 subjects received a once-daily dose of matched placebo for 7 days. Fecal samples were collected prior to administration (days -8 and -6), during the administration period (days 2 and 4) and after withdrawal of administration (days 8, 11, 21, 28 and 56). In the aerobic intestinal microflora the numbers of enterobacteria were suppressed during the gemifloxacin administration and the numbers of enterococci and streptococci also decreased. No other aerobic microrganisms were affected. In the anaerobic microflora the numbers of anaerobic cocci and lactobacilli were suppressed during the gemifloxacin administration while no other changes occurred. The microflora was normalized 49 days after the administration of gemifloxacin had stopped. No selection or overgrowth of resistant bacterial strains or yeasts occurred. The ecological impact of gemifloxacin was favorable and shown to be selective and similar to that of gatifloxacin, trovafloxacin and moxifloxacin.

Quinolones are a group of antimicrobials that typically have potent activity against aerobic Gram negative organisms but are less active against Gram positive organisms. However, newer quinolones have enhanced activity against Gram positive organisms. Quinolones reduce the *Enterobacteriaceae* in feces but have little effect on anaerobic microflora and enterococci. Colonization with quinolone-resistant bacteria has generally not been demonstrated in clinical studies investigating the fecal microflora.

Gemifloxacin has high activity against Gram positive bacteria, including streptococci. A single center, randomized, double blind, placebo controlled, parallel group

study was performed. Subjects consisted of eight healthy male and seven healthy female volunteers (weight range 53.10–93.80kg, height range 1.60–1.84m and age range 24–44 years). Subjects were randomized to receive either 320 mg gemifloxacin or placebo in a 2:1 ratio. Ten subjects received a once daily oral dose of 320 mg gemifloxacin for 7 days and five subjects received a once daily oral dose of matched placebo for 7 days.

Fecal samples were taken 6 and 8 days prior to dosing and then on days 2, 4, 8, 11, 21, 28 and 56 days after the first dose of gemifloxacin or matched placebo. Fecal samples were collected on the target day within ±24 hours for samples up to Day 8 and ±2 days for subsequent samples was acceptable.

The microbiological analysis of the fecal specimens was performed as described by Heimdahl and Nord (Heimdahl, *et al.*, *Scand. J. Infect. Dis.*, 11: 233-242 (1979)). The specimens were suspended in pre-reduced peptone-yeast extract medium, diluted and inoculated on selective media. The aerobic agar plates were incubated for 24 h at 37°C and the anaerobic plates for 48 h at 37°C in anaerobic jars (GasPlak, BBL, USA). After incubation, different colony types were counted, isolated in pure culture and identified to genus level using morphological and biochemical tests and gas-liquid chromatography. The lower limit of detection was 10 (Arronsson, *et al.*, *Antimicrob. Chemother.*, 14: 85-89 (1984)) microrganisms per gram of feces. Additionally a microbiological assay was performed to determine fecal concentrations of gemifloxacin.

Pulse, blood pressure, 12-lead ECG readings, clinical laboratory parameters and adverse events were monitored in all subjects throughout the study. The changes in fecal microflora endpoints were compared over time between gemifloxacin and placebo. There was no formal statistical analysis performed.

The effect on the aerobic fecal microflora by the administration of gemifloxacin is shown in Figure 20. *Escherichia coli* strains were suppressed during the administration period and the numbers of enterococci and streptococci decreased. These aerobic bacteria were normalized 21 days after the last dose of gemifloxacin. No other aerobic microrganisms were affected by the gemifloxacin administration. No marked alterations were observed in the placebo group.

The effect on the anaerobic fecal microflora by the administration of gemifloxacin is presented in Figure 21. The numbers of anaerobic cocci and lactobacilli decreased during the administration of gemifloxacin while no other major changes occurred. The anaerobic microflora returned to normal 21 days after the last dose. No marked changes were noticed in the anaerobic microflora during the placebo treatment. No selection or

overgrowth of gemifloxacin resistant bacteria or yeasts occurred.

Gemifloxacin (320 mg) was well tolerated in healthy male and female subjects. There were no clinically relevant changes in vital signs, ECG or laboratory values. The number of enterobacteria, enterococci and streptococci were suppressed during the gemifloxacin administration. No other aerobic microrganisms were affected.

The number of anaerobic cocci and lactobacilli were suppressed during the gemifloxacin administration while no other changes occurred. All aerobic and anaerobic microrganisms were normalized 49 days after the administration of gemifloxacin had stopped. No selection or overgrowth of resistant bacterial strains or yeasts occurred. The ecological impact of gemifloxacin is favorable and has been shown to be selective and similar to that of gatifloxacin, trovafloxacin and moxifloxacin.

The invention provides a method for modulating metabolism of Gram positive bacterial pathogens, such as streptococci and staphylococci, and Enterobacteriaceae pathogenic bacteria. Skilled artisans can readily choose Gram positive bacterial pathogens, such as streptococci and staphylococci, or Enterobacteriaceae pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive bacterial pathogens, such as streptococci and staphylococci, or Enterobacteriaceae pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with Gram positive bacterial pathogens, such as streptococci and staphylococci, or Enterobacteriaceae pathogenic bacteria.

While a preferred object of the invention provides a method wherein said pathogenic bacteria is selected from the group consisting of: Gram positive bacterial pathogens, such as streptococci and staphylococci, and Enterobacteriaceae bacteria. Other Gram positive or Gram negative pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against Gram positive pneumococci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various Gram positive pneumococcal pathogens. An objective of these analyses was to determine the antimicrobial susceptibility and clonality of strains with elevated MICs to ofloxacin, ciprofloxacin and levofloxacin using BOX and PFGE fingerprinting.

A small number of multi-drug resistant pneumococcal clones have disseminated globally and constitute a considerable threat to the management of invasive pneumococcal disease in many countries. Although fluoroquinolones have not been widely advocated for the empiric management of respiratory tract infections, the recent development of agents of the same class with enhanced activities against Gram positive organisms, in particular *Streptococcus pneumoniae,* is likely to lead to greater use of this class of agent in the future. A total of 361 strains were tested against a panel of antibiotics including ofloxacin, ciprofloxacin, levofloxacin and gemifloxacin (SB-265805). Twenty strains were resistant (MIC ≥8 μg/ml) to the "older" quinolones but MICs remained low (<0.12 μg/ml) for gemifloxacin. DNA fingerprinting of these resistant strains revealed serotype 9V and 23F strains identical to the pandemic Spanish^{23F}-1 and France^{9V}-3 clones, originally considered sensitive to quinolones. Approximately one third of fully penicillin-resistant pneumococci in the USA belong to the Spanish^{23F}-1 clone. The data indicates that fluoroquinolone resistance may be emerging in pandemic clones of multi-resistant pneumococci and continued surveillance of pneumococcal resistance to fluoroquinolones is essential. The novel fluoroquinolone gemifloxacin retains activity against the pneumococcal strains resistant to penicillin and older fluoroquinolones.

Streptococcus pneumoniae continues to be a significant cause of morbidity and mortality in humans. *Streptococcus pneumoniae* is the leading cause of bacterial pneumonia, sinusitis and otitis media, and is an important cause of meningitis. The past decade has seen a dramatic increase in the incidence of pneumococcal strains which are resistant to penicillin G and other β-lactam and non-β-lactam antimicrobials. The problem has been exacerbated by the spread of a limited number of pneumococcal clones from country to country and from continent to continent. Although the recent development of new fluoroquinolones (FQs) with enhanced activity against *S. pneumoniae* is a potentially important advance in the management of pneumococcal disease, reports of the emergence of resistance to currently available FQs is increasing. In order to determine the factors contributing to FQ resistance, both ofloxacin- resistant (≥8 μg/ml) and ofloxacin-sensitive

(≤4 μg/ml) strains were investigated. In this study the clonality of strains were assessed and mutations in the *gyr*A, *gyr*B, *par*C and *par*E genes of DNA gyrase and topoisomerase IV were identified .

Strains were obtained from the 1997 Alexander Project and a Northern Ireland study. Serotyping was performed by Quellung reaction with sera from the Statens Serum Institut. The antimicrobial agents used were as follows: Gemifloxacin (SB-265805), batch #PNS-A-03C, potency 75.4% (SmithKline Beecham Pharmaceuticals, UK); ofloxacin, ciprofloxacin and levofloxacin E-test strips (AB Biodisk, Sweden); penicillin G, erythromycin, chloramphenicol, clindamycin, tetracycline, rifampin, cefotaxime and trimethoprim and sulphamethoxazole (Sigma, USA).

Susceptibility tests were carried out using E-tests and broth microdilution methods according to NCCLS guidelines. DNA typing by BOX-fingerprinting was performed as follows: Genomic DNA was isolated by the proteinase K-SDS-phenol/chloroform method. PCR was performed in 50 μl volumes using primer AR1 for 30 cycles at 90°C for 1 min, 52°C for 1 min and 72°C for 2 min. Products were visualized on 2% agarose gels after 2 h at 120 V. Pulsed-field gel electrophoresis *(PFGE)* was performed as previously described, (Fox, *et al., J. Infect. Dis.*, 175: 1396-1403 (1997) with DNA restriction using *Sma*I enzyme. *Gyr*A, *gyr*B, *par*C and *par*E genes were amplified by PCR and sequenced manually using Sequenase version 2 Sequencing Kit (US Biochemicals, Ohio, USA).

Table 46 shows data for serotyping, susceptibility testing, DNA typing and sequencing of quinolone resistance determining regions (QRDRs). Twenty strains with ofloxacin MIC ≥8 μg/ml were identified among the 361 strains tested. A number of low level ofloxacin-susceptible strains (≤4 μg/ml) were included for comparison. The majority (75%) of resistant strains belonged to serotypes 9V, 23F and 6B. DNA typing using BOX-fingerprinting and PFGE revealed a basically heterogenous population with 26 types identified for the 41 strains tested.

Four serotype 9V ofloxacin-resistant isolates were identified as being identical to the France^{9V}-3 clone, present in Spain and France. This has become widely disseminated throughout the world, and has been identified in Italy, Sweden, Germany, the USA, the UK and in South America and the Far East. In addition, two serotype 23F FQ resistant isolates were identified as members of the well documented Spanish^{23F}-1 clone. A recent study in the USA revealed that 40% of all highly penicillin-resistant isolates belonged to the Spanish^{23F} clone (Kilmarx, *et al., J. Infect. Dis.*, 177: 677-682 (1998). No quinolone-resistant strains in the study were identified as belonging to the Spanish^{6B}-2 clone. The

emergence of FQ resistance in international clones and their capacity for dissemination is, therefore, of potential concern.

The putative QRDR amino acid changes were identified for gyrA, gyrB, parC and parE of both ofloxacin-sensitive and ofloxacin-resistant strains. The most frequent gyrA substitution was S81F with S81Y and E85K present in three and two isolates respectively. One isolate showed substitutions at S114G and A17T. Five isolates showed changes in gyrB. ParC contained the most changes, with K137N the most frequent substitution encountered either alone or in combination with S79F or S79Y; two other mutations were also observed, D83N and D83G alone or in combination with S79F as the major substitutions. The predominant parE substitution was I460V. Amino acid substitutions I460V and K137N in parC are present in a large number of FQ sensitive strains and appear to play a lesser role in resistance. Substitution S79F in parC and S81F in gyrA appear to be associated with the highest average MIC.

Of concern is the presence of mutations in parC and parE of the quinolone-sensitive France^{9V}-3 control strain. The identification of putative mutations in the Spanish^{23F}-1 and Spanish^{6B}-2 control strains are in progress and could provide some insight in the emergence of FQ resistance into these international clones.

Gemifloxacin retains activity against pneumococcal strains resistant to penicillin and other fluoroquinolones. Quinolone-resistant strains of the international clones Spain^{23F}-1 and France^{9V}-3 were identified.

Table 46. Data for Quinolone-sensitive and -resistant Strains

Strain	Serotype	Fingerprint Box:PFGE	Other antimicrobials	MICs (μg/ml)				GyrA	GyrB	ParC	ParE
				OFL	LEV	CIP	GEM				
TL7/1993 (France^{9V}-3)	9V	A:A	PCot	3	0.5	1.5	<0.12	–	–	D83G	I460V
10	9V	E:E	PECtx	0.5	0.25	0.25	<0.015	–	–	K137N	I460V
703414	9N	F:H	PCTECdCot	0.5	0.19	0.19	<0.015	–	–	K137N	I460V
705234	9V	E:D	PCECdCot Ctx	1.5	0.25	0.38	<0.015	–	–	K137N	I46[illegible]
61	9V	A:A	PCot	1.5	0.75	0.75	<0.015	–	–	K137N	I460V
15	9V	A:A	PCotCtx	1.5	0.5	0.75	<0.015	–	–	K137N	I460V
305313	9V	D:C	TCot	2	0.5	0.75	0.12	–	–	S79Y K137N	A532V V535I + # other
734097	9V	A:A	PCot	2	0.5	0.75	<0.015	–	–	K137N	I460V
13	9V	A:D	PCotCtx	2	0.5	1	0.015	–	–	D83N K137N	I460V
603160	9V	B:B	PCot	2	3	2	0.03	–	–	D83N	I460V
205118	9V	A:A	PCot	8	2	4	0.015	–	E474K	S79Y K137N	I460V
27	9V	A:A	PCot	16	2	4	<0.015	–	E474K	S79F K137N	I460V
28	9V	A:A	PECotCtx	16	4	4	0.015	–	E474K	S79F K137N	I460V
62	9V	A:A	PECot	>32	3	>32	<0.015	–	–	S79F K137N	I46[illegible]
203120	9V	C:A	PCot	>32	>32	>32	0.06	E85K	–	S79F K137N	I460V
SP264	23F	F:F	PCTCot	4	0.75	3	0.015	–	TBC	TBC	TBC

Strain	Serotype	Fingerprint Box:PFGE	Other antimicrobials	MICs (µg/ml)				GyrA	GyrB	ParC	ParE
				OFL	LEV	CIP	GEM				
(Spain^{23F}-1)											
716272	23F	F:G	PCTCot	1	0.38	0.5	<0.015	–	–	K137N	I460V
717144	23F	G:G	PCTECot	1	0.38	0.5	<0.015	–	–	K137N	I460V
705309	23F	J:K	PCTECdCot Ctx	1	0.38	1	<0.015	–	–	K137N	–
735194	23F	F:F	PTCotCtx	2	0.5	1.5	<0.015	–	–	K137N	D435N I[illegible]V
403346	23F	F:G	PCTCotCtx	>32	8	16	0.015	S81F	–	S79F K137N	I460,
205229	23F	G:F	PCTCot	>32	8	16	0.03	S81F	–	S79F K137N	I460V
205324	23F	F:F	PCTCot	>32	6	32	0.06	S81F	–	S79F	I460V
503167	23F	F:F	PCTCot	32	12	8	<0.015	S81F	–	S79F K137N	I460V
403412	23F	G:F	PCECdCot	>32	8	16	0.06	–	–	K137N	I460V
622286	23F	H:I	TECd	>32	2	4	0.06	S81F	–	D83N	–
707172	23F	I:J	Rif	>32	8	12	0.06	S81F	–	S79F	I460V
GM17 (Spain^{6B}-2)	6B	K:L	PCTCot	1.5	0.38	0.38	<0.015	–	TBC	TBC	TBC
717145	6B	K:L	PCECdCot	1	0.25	0.25	<0.015	–	–	–	–
405183	6A	L:M	Cot	>32	>32	>32	0.0.15	S81Y	–	S79F	–
503244	6A	M:N	PTCot	>32	>32	>32	0.03	S81F	–	S79Y A189V	I460V
723084	6A	J:O	Cot	>32	6	>32	0.06	–	–	–	I46(
406261		X:Y		1.5	0.38	1.5	0.015	S114G A17T	–	S52G N91D	I493L
209165	34	N:P	Cot	2	0.75	4	0.06	–	–	–	–
782032	14	U:W	PTECd	2	0.5	1	0.015	–	E474N	K137N	–
791139	17	V:D	PCot	2	1	1.5	0.03	S81Y	E474N	K137N	I460V

Strain	Serotype	Fingerprint Box:PFGE	Other antimicrobials	MICs (µg/ml)				GyrA	GyrB	ParC	ParE
				OFL	LEV	CIP	GEM				
309350	20	Q:S	Sens	3	1	8	0.06	–	–	–	I460V
507103	3	R:T	Sens	3	0.75	1.5	<0.015	S81F	–	R95C	D435N
771036	3	T:V	Sens	3	0.5	2	0.03	–	–	–	I460V
214152	22	O:Q	Sens	>32	8	16	<0.12	S81Y	–	S79F	–
304232	14	P:R	TECd	>32	4	6	0.03	S81F	–	D83N K137N	I460V
509063	22	S:U	Sens	>32	6	6	0.06	S81F	–	S79F K137N	I460
403413		W:X		>32	16	16	0.015	E85K	–	S79Y K137N	I460V
502226		Y:Z		>32	8		<0.12	S81F	–	D83N	I460V
R6			Sens	1.5	0.38	0.38	<0.015				
ATCC 49619				1.0	0.38	0.25	<0.015				

(–) = no mutation
TBC = to be completed

The invention provides a method for modulating metabolism of Gram positive pneumococcal pathogenic bacteria. Skilled artisans can readily choose Gram positive pneumococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with Gram positive pneumococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said Gram positive pneumococcal pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae*. Other Gram positive pneumococca pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against gonococci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various gonococcal pathogens. An objective of these analyses was to determine the activity of gemifloxacin against 150 gonococci including one-third of strains with elevated ciprofloxacin MICs and to establish criteria for defining susceptibility of *N. gonorrhoeae* to the new drug. These analysis showed a high correlation between MICs and disk diffusion, as well as to E-test (AB BIODISK, Solna, Sweden), a simple and effective alternative to the more complicated agar dilution method. The E-test results were also compared to the reference National Committee for Clinical Laboratory Standards (NCCLS) agar dilution method, (National Committee for Clinical Laboratory Standards, Tentative guideline M23-T3, Wayne, PA USA (1998)) to validate the E-test for routine clinical laboratory use.

The activity of gemifloxacin was tested against 150 *Neisseria gonorrhoeae* strains, including 50 ciprofloxacin-resistant isolates using reference agar dilution, standardized disk diffusion, and E-test (AB BIODISK, Solna, Sweden) methods. Gemifloxacin is very potent against ciprofloxacin-susceptible strains (MIC_{90}, 0.008 μg/ml) but is 16-fold less active

against the ciprofloxacin-resistant gonococci. The rank order of fluoroquinolone activity against fluoroquinolone-resistant mutants was: gemifloxacin (MIC_{90}, 0.12 μg/ml) > trovafloxacin (0.25 μg/ml) > moxifloxacin = grepafloxacin (0.5 μg/ml) > ciprofloxacin (1 μg/ml). E-test and reference agar dilution MIC results indicate excellent correlation (r = 0.96), and >98% MICs are within ±1 $\log_2$ dilution (essential agreement). Agar dilution MICs were also compared to zone diameters obtained using gemifloxacin 5 μg disks; and complete intermethod categorical agreement (100%) was achieved applying breakpoints proposed as follows: ≤0.25 μg/ml (zone, ≥25 mm) for susceptible and ≥1 μg/ml (zone, ≤21 mm) for resistant. These criteria conform to those approved for trovafloxacin. Gatifloxacin MIC and disk diffusion test QC ranges were established for *N. gonorrhoeae* ATCC 49226. Data was collected from ≥7 laboratories, three GC agar medium lots for both agar MICs and disk methods, and two lots each of the 5 and 10 μg disks. The proposed MIC quality control (QC) range was 0.002–0.016 μg/ml and the calculated mm zone ranges (median ±0.5 x average range) for both disks were similar, but contained only 88.1 and 91.9% of results. To achieve the acceptable ≥95% of all study results within range, 43–54 mm limits were necessary. The excellent broad-spectrum activity and low adverse effects profile of gemifloxacin indicates a potential for treatment of quinolone-resistant gonorrhea.

Gonococcal infections continue to be a serious world-wide problem. Initially, penicillin and/or tetracycline were the first line agents against *Neisseria gonorrhoeae*, but in the last three decades there have been widespread and increasing infections with penicillinase-producing (PPNG) and tetracycline-resistant (TRNG) gonococci. Alternative therapies for uncomplicated gonorrhea have been recommended that include β-lactamase stable 'second- or third-generation' cephalosporins such as ceftriaxone, cefixime, cefpodoxime, cefotetan, cefotaxime, cefoxitin, ceftazidime, and cefuroxime. Another avenue of treatment has been the fluoroquinolone class of antimicrobial agents. Ciprofloxacin and ofloxacin proved to be very effective during the initial single-dose implementation. However, gonococci rapidly emerged with ciprofloxacin resistance, and the rate of occurrence of these strains world-wide appears to be increasing (Fox, *et al.*, *J. Infect. Dis.*, 175: 1396-1403 (1997); Kilmarx, *et al.*, *J. Infect. Dis.*, 177: 677-682 (1998); Tapsall, *et al.*, *Med. J. Aust.*, 156: 143 (1992)); Van de Laar, *et al.*, *Genitourinary Med.*, 73: 510-517 (1997). Moreover, the PPNG strains have demonstrated higher MICs for ceftriaxone and ciprofloxacin compared to the non-PPNG. A recent WHO report, (Anonymous, *Genitourinary Med.*, 73: 355-361 (1997)) on gonococcal surveillance between 1992–1994, documents that fluoroquinolone resistance had increased and become

widespread, in terms of number and extent of MICs for the resistant isolates. During the same time frame, a study in the United States showed a 20% increase in the gonococcal infections having reduced susceptibility to ciprofloxacin (Fox, *et al.*, *J. Infect. Dis.*, 175: 1396-1403 (1997)).

Elevation in ciprofloxacin MICs has been associated with mutations of the *gyr A* and *par C* genes in the bacterial chromosome (Deguchi, *et al.*, *Antimicrob. Agents Chemother.*, 40: 1020-1023 (1996)). Very high levels of ciprofloxacin resistance (8–64 μg/ml) follows double mutations in the *par C* gene. These multiple mutations can also produce organisms less susceptible to the newest advanced spectrum fluoroquinolones, because of their structural similarity.

Gemifloxacin has been shown to have superior *in vitro* activity compared with other currently available fluorinated quinolones against a wide variety of pathogens (Cormican, *et al.*, *Antimicrob. Agents Chemother.*, 41: 204-211 (1997); Hohl, *et al.*, *Clin. Microbiol. Infect.*, 4: 280-283 (1998); Marco, *et al.*, *J. Antimicrob. Chemother.*, 40: 605-607 (1997); Oh, *et al.*, *Antimicrob. Agents Chemother.*, 40: 1564-1568 (1996)). Gemifloxacin is also bactericidal for *Staphylococcus aureus* and *Escherichia coli* while showing bacteriostatic effects like that of ciprofloxacin versus *Pseudomonas aeruginosa*. Cormican and Jones found that gemifloxacin was the most active agent tested against Gram positive species, including fluoroquinolone- and glycopeptide-resistant strains (Cormican, *et al.*, *Antimicrob. Agents Chemother.*, 41: 204-211 (1997)). Gemifloxacin also had activity similar to ciprofloxacin against many Gram negative pathogens. Some earlier reports studied a limited number of gonococci (not including ciprofloxacin-resistant isolates), with gemifloxacin being very potent. If gemifloxacin is to be used as a treatment for gonorrhea, it is important to study a larger number of organisms, including fluoroquinolone-resistant strains, thus assessing the possibility of cross-resistance. Also quality control limits must be established for routine clinical laboratory testing or use in resistance surveillance studies.

SmithKline Beecham Pharmaceuticals (Philadelphia, PA) supplied the investigational drug, gemifloxacin. The other comparator drugs were obtained from their respective manufacturers. Gemifloxacin E-test strips were made by AB BIODISK. The gemifloxacin 5 and 10 μg disks were produced by Oxoid (Hampshire, UK); and BD Microbiological Systems (Cockeysville, MD) produced the control ciprofloxacin (5 μg) disks. Antimicrobial solutions for reference agar MIC tests were prepared according to manufacturer's instructions, and stored in aliquots at -70°C until used. Gemifloxacin

solution was made fresh each day of testing.

A total of 150 strains of *N. gonorrhoeae* were used in this investigation, including recent and reference stock clinical isolates. One hundred gonococcal strains were ciprofloxacin-susceptible (zone diameter at ≥41 mm; MIC at ≤0.06 μg/ml). These ciprofloxacin-susceptible strains were further categorized based on their susceptibility to penicillin: Nineteen penicillin-susceptible (MIC ≤0.06 μg/ml), 25 moderately-susceptible or intermediate (MIC 0.12–1 μg/ml), 29 strains resistant to penicillin (MIC ≥2 μg/ml) by non-β-lactamase mechanisms. An additional 27 strains were resistant to penicillin by virtue of β-lactamase production.

The other 50 *N. gonorrhoeae* strains categorized as ciprofloxacin-resistant demonstrated two levels of decreased susceptibility to that fluoroquinolone: 43 strains showing low-level resistance (MIC 0.12–0.25 μg/ml) and seven strains with high-level resistance (MIC 0.5–8 μg/ml). The ciprofloxacin-resistant strains were derived from diverse locations: 27 from Japan, 17 from the United States, and six from The Netherlands. The strains from Japan have previously been characterized for their *gyr A* and *par C* mutations by PCR analysis: 16 with a *gyr A* mutation and 11 with a *gyrA + par C* substitutions. The ciprofloxacin-susceptible isolates were a part of a laboratory collection (University of Iowa Hospitals and Clinics) and represented routine, recent domestic clinical strains.

The *N. gonorrhoeae* isolates were grown on chocolate agar plates and stored in lysed horse blood at -70°C until processed. Fresh overnight subcultures were used for susceptibility testing. For the 100 ciprofloxacin-susceptible isolates, one E-test strip containing gemifloxacin, and one 5 μg gemifloxacin diffusion disk were tested, as well as reference agar dilution MIC test with gemifloxacin and ciprofloxacin. The ciprofloxacin non-susceptible or -resistant isolates were tested against an E-test strip, a 5 μg disk and reference agar dilution test with gemifloxacin and other comparator drugs (ciprofloxacin, trovafloxacin, grepafloxacin, moxifloxacin, amoxicillin, penicillin, and azithromycin). All tests were performed by NCCLS [1997a and b] methods. The *N. gonorrhoeae* ATCC 49226 QC strain repeatedly produced a narrow range of values (MIC, 0.004 or 0.008 μg/ml); all within subsequently established ranges (see section below).

A seven laboratory study was designed to comply with the specification of NCCLS M23-A for the disk diffusion method using 1) seven qualifying sites; 2) two disk lots for each of two disk potencies (5 and 10 μg); 3) three GC agar lots; 4) 10 replicate tests over 10

days; and 5) a control fluoroquinolone (trovafloxacin) tested concurrently. This study design produced 60 replicate zone diameters for each disk potency in each participating laboratory or 840 zones overall, excluding the trovafloxacin control results. The agar dilution QC design used 1) seven laboratories; 2) 30 tests per laboratory (10 tests/day); and 3) three GC agar lots with three groups of two laboratories each testing one lot and a single reference site testing all three medium lots (90 MIC results). This protocol produced 270 MIC results. Control trovafloxacin results for the agar dilution test (range of results 0.008–0.016 μg/ml) and the disk diffusion test (range of results 42–53 mm) were all within NCCLS (1999) recommended ranges.

Gemifloxacin was very potent versus ciprofloxacin-susceptible strains (MIC_{90} 0.004–0.008 μg/ml), but was 16- to 32-fold less active against the ciprofloxacin-resistant organisms (MIC_{90} 0.125 μg/ml). The highest gemifloxacin MIC recorded was 1 μg/ml (0.5 μg/ml by E-test) for a strain having multiple mutations in *gyr A* and *par C*.

Among the ciprofloxacin-susceptible strains, the levels of penicillin resistance and penicillinase production generally did not influence the fluoroquinolone susceptibilities.

Among the ciprofloxacin-resistant strains, those with mutations in *gyr A* only had ciprofloxacin and gemifloxacin MIC ranges of 0.12–1 and 0.03–0.12 μg/ml, respectively. The gonococci having mutations in both *gyr A* and *par C* had eight-fold higher MIC ranges (0.25–8 and 0.06–8 μg/ml) for each fluoroquinolone.

In Table 47, MIC results from the E-test are compared to those obtained from the reference agar dilution method [NCCLS, 1997b]. A trend toward a slightly lower MIC for the E-test was noted when using the MIC_{50} and MIC_{90} values derived from the 150 tested strains (Table 47). A regression scattergram plot (not shown) prepared with these data demonstrates a correlation coefficient (r) of 0.96 and a regression equation of $y = 0.66 + 0.95x$. Essential agreement (±1 $\log_2$ dilution) between methods was 98.7%, but 76 (50.7%) E-test MIC values were two-fold lower than those determined by the reference agar dilution test.

A scattergram comparison was also made of disk diffusion tests (plotted on the x-axis) and the reference gemifloxacin MIC values (plotted on the y-axis)(not shown). The correlation was high ($r = 0.91$) and the regression equation was $y = 13.2 - 0.24x$. The susceptible MIC breakpoint of ≤0.25 μg/ml would correlate to a zone diameter of ≥25 mm (Table 49). The single isolate with a gemifloxacin MIC of 1 μg/ml could be considered either as resistant or as undetermined.

The results from seven laboratory investigations to establish gemifloxacin QC

ranges are found in Tables 50 and 51. Using QC *N. gonorrhoeae* ATCC 49226, the gemifloxacin MICs ranged from 0.002–0.016 μg/ml with 100 and 130 occurrences at 0.004 and 0.008, respectively. This broad modal value requires consideration of a 4 $\log_2$ dilution control range of 0.002– 0.016 μg/ml. The results for each disk drug content were not significantly different, and the 5 μg disk was selected for routine clinical use. For the 5 μg disk, the calculated range was 49 ± 4 mm or 45–53 mm. However, this range only contained 88.1% of generated results and therefore, was expanded to 43–54 mm (97.6% of results within range).

Gemifloxacin activity against *N. gonorrhoeae*, especially those strains resistant to ciprofloxacin is outstanding (MIC_{90}, 0.12 μg/ml), and superior to all other tested fluoroquinolones including trovafloxacin. This level of potency can permit gemifloxacin use for the therapy of gonococcal infections observed to be resistant to older fluoroquinolones by virtue of greater affinity for mutant DNA gyrase/topoisomerase targets.

Table 47. Activity of Gemifloxacin Against Two Groups of *N. gonorrhoeae* Strains by Reference Agar Dilution [NCCLS, 1997b] and E-test Methods

Ciprofloxacin susceptibility (No. isolates tested)	Test method	Gemifloxacin MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
Susceptible (100)	Agar dilution	≤0.002–0.03	0.004	0.008
	E-test	≤0.002–0.025	≤0.002	0.004
Resistant (50)	Agar dilution	0.03–1	0.06	0.12
	E-test	0.012–0.5	0.032	0.125

Table 48. Comparison of MIC Frequency Distributions of Fluoroquinolone (Ciprofloxacin)-resistant Isolates for Gemifloxacin and Six Comparator Drugs

Antimicrobial	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Gemifloxacin	0.03–1.0	0.06	0.12
Trovafloxacin	0.03–1.0	0.12	0.25
Grepafloxacin	0.12–1.0	0.25	0.5
Moxifloxacin	0.06–2.0	0.12	0.5
Penicillin	0.06–4.0	1.0	2.0
Amoxicillin	0.25–2.0	2.0	2.0
Azithromycin	0.06–0.25	0.06	0.12

Table 49. Proposed Susceptibility Interpretive Criteria for Gemifloxacin Tested by Agar

Dilution and Disk Diffusion Methods

Proposed MIC criteria (correlate zone diameter)		Error rates (%)[a]		
Susceptible	Resistant	Very major	Major	Minor
≤0.25 (≥25)[b]	≥1 (≤21)	0.0	0.0	0.0
≤0.25 (≥25)	NC[c]	0.0	–	–

[a]Very major = false-susceptible, major = false-resistant and minor = an intermediate result by one method and susceptible or resistant by the other

[b]MIC in μg/ml and zone diameter in mm

[c]NC = no criteria, strains with gemifloxacin MICs of ≥0.5 μg/ml should be sent to a reference laboratory for confirmation and clarifications of clinical outcomes

Table 50. Distribution of Quality Control (QC) *N. gonorrhoeae* ATCC 49226 MIC Results for Gemifloxacin from Seven Laboratories Establishing NCCLS [1999] Control Ranges (270 total tests)

Gemifloxacin	Occurrences by laboratory							
MIC (μg/ml)	A	B	C	D	E	F	G[a]	Total
0.002	10	0	0	0	0	0	0	10[b]
0.004	10	10	0	20	30	30	0	100[b]
0.008	10	20	0	10	0	0	90	130[b]
0.016	0	0	30	0	0	0	0	30[b]

[a]Three lots were tested by the organizing reference laboratory only. All other sites produced 30 replicate tests on one GC agar lot

[b]Proposed MIC QC range (0.002–0.016 μg/ml) includes all reported values

Table 51. Distribution Statistics from the Gemifloxacin 5 and 10 μg Disk Diffusion Quality Control (QC) Studies in Seven Laboratories [NCCLS, 1998]

Laboratory	5 μg disks (zone in mm)		10 μg disks (zone mm)	
	Median	Range	Median	Range
A	51	50–55	52	50–55
B	50	47–53	50	46–53
C	44	40–47	46	41–48
D	50	47–52	50	47–53
E	51	48–55	52	48–56
F	47	45–52	48	45–52
G	48	46–49	47	46–50
Total	49	40–55[a]	49	41–56[b]

[a]Proposed QC range = 43–54 mm that contains 97.6% of reported results (420 tests)

[b]Proposed QC range = 44–54 mm that contains 97.9% of reported results (420 tests)

The invention provides a method for modulating metabolism of gonococcal pathogenic bacteria. Skilled artisans can readily choose gonococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by gonococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with gonococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said gonococcal pathogenic bacteria is selected from the group consisting of: *Neisseria gonorrhoeae,* including Ciprofloxacin-resistant strains of *Neisseria gonorrhoeae.* Other gonococcal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against pneumococci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various pneumococcal pathogens. An objective of these analyses was to identify pneumococci which are resistant to fluoroquinolones (ofloxacin MIC≥8μg/ml), analyze strains from Northern Ireland where an association of ciprofloxacin and penicillin-resistance was previously reported, and evaluate the potency of gemifloxacin against various pneumococci strains.

The report of Chen and co-workers (Chen, *et al., New England Journal of Medicine*, 341: 233-239 (1999)) documents increasing resistance of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Pulsed-field gel electrophoresis identified many clones of pneumococci in that study, but did not find fluoroquinolone resistance in multi-resistant clones (eg. Spanish serotype 23F clone) (Munoz, *et al., J. Infect. Dis.*, 164: 302-306 (1991)) which now have a global distribution. The authors (Chen, *et al., New England Journal of Medicine*, 341: 233-239 (1999)) expressed concern that fluoroquinolone resistance may emerge in such a clone.

The Alexander study is a global surveillance program of respiratory pathogens

sponsored by SmithKline Beecham. Pneumococci were identified from this collection which are resistant to fluoroquinolones (ofloxacin MIC≥8μg/ml) and analyzed strains from Northern Ireland where an association of ciprofloxacin and penicillin-resistance was previously reported (Goldsmith, *et al.*, *J. Antimicrob. Chemother.*, 41: 420-421 (1998)). The 23F multi-resistant Spanish clone which currently contributes up to a third of highly resistant pneumococci in the United States was identified to have mutated to fluoroquinolone resistance in isolates from France and Spain. The serotype 9V multi-resistant global pneumococcal clone (Lefevre, *et al.*, *Eur. J. Clin. Micro. Infec. Dis.*, 14: 491-497 (1995)) was identified to be fluoroquinolone resistant in both Spain and Northern Ireland. Of particular interest is the recent observation that the type strain of the 9V clone (TL7) isolated in 1993 (Lefevre, *et al.*, *Eur. J. Clin. Micro. Infec. Dis.*, 14: 491-497 (1995)) carries a first step mutation to fluoroquinolone resistance in the *parC* gene (D83G), previously undetected since this mutation alone is insufficient to express the phenotype resistance (ofloxacin MIC 3μg/ml; ciprofloxacin MIC 1.5μg/ml). Gemifloxacin was found to be the most potent of the new fluoroquinolones tested, retained activity against these strains.

This data describes the emergence of fluoroquinolone resistance in multi-resistant international clones of pneumococci and indicate the need for continued surveillance of anitmicrobial resistance in the pneumococcus and the prudent use of fluoroquinolones for pneumococcal infections.

The invention provides a method for modulating metabolism of pneumococcal pathogenic bacteria. Skilled artisans can readily choose pneumococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: pneumococcal pathogenic bacteria. Other pneumococcal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as

using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against aerobic bacteria..

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various aerobic pathogens. An objective of these analyses was to determine the PAE for gemifloxacin and ciprofloxacin against 10 isolates representing a broad spectrum of Gram positive and Gram negative aerobic organisms.

The post-antibiotic effect (PAE) of gemifloxacin was compared with that of ciprofloxacin against 10 isolates comprising *Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Proteus vulgaris*. Isolates were incubated in the presence of 2 and 4 x MIC. After 1 h of exposure, the antimicrobial was removed by ultrafiltration and viable counts were made for 6 h. The PAE was defined as *T* - C, where *T* is the time for the antimicrobial-exposed culture to increase in viable count by 1 log10 and *C* is the time for the growth control to increase in viable count by 1 log10. A measurable PAE was observed with both compounds at 2 and 4 x MIC. The PAEs of gemifloxacin were comparable to those of ciprofloxacin, and for both compounds exposure to 2 x MIC resulted in shorter PAEs than exposure to 4 x MIC. The PAE of gemifloxacin at 4 x MIC was >6 h against *P. aeruginosa, P. vulgaris* and *H. influenzae*, and ranged from 0.1 to 2.5 h against the other isolates tested. These results indicate that gemifloxacin has a pronounced PAE against a broad spectrum of organisms, which, in combination with gemifloxacin's pharmacokinetic profile, indicates a less frequent dosing schedule for this compound in clinical use.

The post-antibiotic effect (PAE) is defined as the persistent suppression of bacterial growth after brief exposure of a bacterial culture to an antimicrobial. See, Craig WA, Gudmundsson S. The post antibiotic effect. *In: Antibiotics in laboratory medicine (4th edition)*. Ed. Lorian, V. Baltimore, MD, USA: The Williams and Wilkins Co., 1986: pp 296–329. The measurement of a PAE can be useful in the design of an appropriate dosing regimen for an antimicrobial. In theory, an antimicrobial with a long PAE would require a less frequent dosing regimen than one with a short PAE.

Gemifloxacin, batch # 03R1P2-1-1, potency 73.8%, was obtained from SmithKline Beecham Pharmaceuticals, Harlow, UK. Ciprofloxacin, lot G, potency 100%, was obtained as a USP reference standard (Rockville, MD, USA).

The following isolates were obtained from the SmithKline Beecham: Anti-

infectives Research Culture Collection: *Staphylococcus aureus* ATCC 29213, *Staphylococcus saprophyticus* 662, *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247, *Enterococcus faecalis* ATCC 29212, *Moraxella catarrhalis* MC2, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* KP2 and *Proteus vulgaris* ATCC 13315. Trypticase soy agar containing 5% sheep blood (BBL, Cockeysville, MD, USA) was used to subculture frozen isolate stocks and for *S. pneumoniae* colony counts. Mueller–Hinton II agar (BBL) was used for colony counts of the non-fastidious organisms. Chocolate II agar (BBL) was used for colony counts of the *H. influenzae* isolate. Cation-adjusted Mueller–Hinton broth (CAMHB) (BBL) was used to test the non-fastidious organisms. CAMHB supplemented with 5% lyzed horse blood (BBL) was used to test the *S. pneumoniae* isolate. *Haemophilus* Test Medium (BBL) was used to test the *H. influenzae* isolate.

Organisms were subcultured from a frozen stock (-80°C) on to a trypticase agar plate containing 5% sheep blood and incubated for 20–24 h at 35°C. A 5 ml tube of saline was inoculated with a sufficient number of colonies from the overnight culture to obtain a turbidity equivalent to a 0.5 McFarland standard. This inoculum was diluted 100-fold into 18 ml of the appropriate media (contained in a 50 ml flask) to produce a final test inoculum of approximately $1x10^6$ CFU/ml.

The MICs of gemifloxacin and ciprofloxacin were determined using the NCCLS recommended procedure for broth microdilution. (*See*, National Committee for Clinical Laboratory Standards. 1997. Approved Standard M2-A6. Performance standards for antimicrobial disk susceptibility tests (sixth edition). NCCLS, Wayne, PA, USA.)

Both compounds were tested in serial two-fold dilutions ranging in concentration from 0.0001 to 32 µg/ml. A positive growth control (antimicrobial free) was included on each microtiter plate. Following inoculation, plates were incubated at 35°C in air for 18–24 h. A 10 ml aliquot of the inoculum was plated on to trypticase soy agar containing 5% sheep blood to determine the purity of the
final test inoculum.

The PAE effect was determined using a filtration method as previously described. (*See*, Thornburn CE, Molesworth SJ, *et al*. Post antibiotic and post-b-lactamase inhibitor effects of amoxicillin plus clavulanate. *Antimicrob Agents Chemother* 1996; 40: 2796–2801.) Each test isolate was added to a 50 ml flask containing the antimicrobial (at 2 or 4 ´ MIC) in 20 ml of the appropriate broth. After addition of the isolate, a colony count was performed to determine thedensity (CFU/ml) of the starting inoculum. Flasks were

incubated on a shaker at 35°C. After 2 h hours of antimicrobial exposure a further colony count was performed. The contents of each flask were then filtered using a 0.2 micron filter to remove the antimicrobial. The filtrate was washed twice with 10 ml of pre-warmed broth and the filter was resuspended into 20 ml of pre-warmed media. A colony count was performed immediately after resuspension of the test isolate. Flasks were returned to the incubator and colony counts were performed at 1 h intervals for 6 h. Due to filtration problems associated with the media supplements used for *S. pneumoniae* and *H. influenzae*, a 1:100 dilution of the inoculum was made intopre-warmed broth to remove the antimicrobial. The control for determination of the PAE included incubation of the test isolate for 2 h in drug-free media followed by filtration and incubation of the isolate in drug-free media.

Five 10-fold dilutions were made for each isolate/antimicrobial and control at every time interval. Using a 10 µl disposable loop, 50 µl from each well were spread on to the appropriate agar media. The plates were incubated overnight and colony counts were made at the dilution that provided 30–300 colonies.

The MIC was determined as the lowest concentration of compound that inhibited visible growth of the isolate. A microtiter mirror reader was used to assist in determining the MIC endpoint. A semi-logarithmic graph with number of colonies on the y-axis and time on the x-axis was prepared for each isolate. The PAE effect was determined using the equation:

$$PAE = T - C$$

where T is the time required for the test isolate count to increase by 10-fold (1 x log10) above the count observed immediately after removal of the antimicrobial and C is the time required for the control to increase by 10-fold above the count observed immediately after removal of the antimicrobial.

The MICs of gemifloxacin and ciprofloxacin are shown in Table 52.

A summary of the PAE results is shown in Table 53.

In general, a shorter PAE was observed at 2 x MIC as opposed to 4 x MIC. At 4 ´ MIC, the gemifloxacin PAE was >6 h against *H. influenzae, P. aeruginosa* and *P. vulgaris*, and ranged from 0.1 to 2.5 h against the other isolates tested. The ciprofloxacin PAE values obtained at 4 ´ MIC ranged from 0.3 to 5.1 h, with the exception of *E. faecalis* ATCC 29212, where no effect was observed.

The PAE of gemifloxacin is comparable to that of ciprofloxacin against all isolates

tested. A shorter PAE is observed for both compounds at 2 x MIC compared with 4 x MIC. At 4 x MIC, a longer PAE for gemifloxacin (>6 h) than ciprofloxacin is observed against *H. influenzae, P. aeruginosa* and *P. vulgaris*. Overall, the results of this study indicate that gemifloxacin has a pronounced PAE against a broad spectrum of organisms.

Table 52. MICs of Gemifloxacin and Ciprofloxacin Against a Broad Spectrum of Bacterial isolates

Microorganism	MIC (ug/ml)	
	Gemifloxacin	Ciprofloxacin
Staphylococcus aureus ATCC 29213	0.016	0.25
Staphylococcus saprophyticus 662	0.016	0.25
Streptococcus pneumoniae ATCC 49619	0.016	0.5
Haemophilus influenza ATCC 49247	0.004	0.008
Enterococcus faecalis ATCC 29212	0.03	0.25
Moraxella catarrhalis MC2	0.004	0.008
Escherichia coli ATCC 25922	0.008	0.008
Pseudomonas aeruginosa ATCC 27853	0.5	0.5
Klebsiella pneumoniae KP2	0.5	0.5
Proteus vulgaris ATCC 13315	0.06	0.008

Table 53. PAE of Gemifloxacin and Ciprofloxacin at 2 and 4 x MIC

Microorganism	PAE(h)			
	Gemifloxacin		Ciprofloxacin	
	2xMIC	4xMIC	2xMIC	4xMIC
Staphylococcus aureus ATCC 29213	1.1	1.0	1.3	1.5
Staphylococcus saprophyticus 662	1.9	2.5	2.4	3.9
Streptococcus pneumoniae ATCC 49619	0.7	1.5	0.9	1.5
Haemophilus influenza ATCC 49247	2.4	>6	0.6	2.4
Enterococcus faecalis ATCC 29212	0.1	0.6	0	0.3
Moraxella catarrhalis MC2	0.6	0.5	1.1	3.7
Escherichia coli ATCC 25922	1.1	1.9	1.2	3.7
Pseudomonas aeruginosa ATCC 27853	2.4	6.6	4.5	5.1
Klebsiella pneumoniae KP2	0.2	0.1	0.2	0.5
Proteus vulgaris ATCC 13315	3.9	>6	1.3	3.8

The invention provides a method for modulating metabolism of aerobic pathogenic bacteria. Skilled artisans can readily choose aerobic pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial

infection by aerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with aerobic pathogenic bacteria.

While a preferred object of the invention provides a method wherein said aerobic pathogenic bacteria is selected from the group consisting of: *Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Proteus vulgaris*. Other aerobic pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against pneumococci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various pneumococcal pathogens. An objective of these analyses was to determine the *in vitro* antipneumococcal activity of gemifloxacin (SB-265805)

Gemifloxacin was compared to other quinolones against *S. pneumoniae* with specific resistant mutations in their *parC* and *gyrA* genes, the two principal mechanisms of quinolone resistance in pneumococci (Varon, *et al., Antimicrob. Agents Chemother.*, 43: 302-306 (1999)).

The worldwide increase in beta-lactam- and macrolide-resistant *S. pneumoniae* has become major clinical problem. Newer quinolones with good anti-Gram positive activity remain effective against these organisms and can be a therapeutic alternative. However, the widespread use of quinolones in the community might select for quinolone-resistant *S. pneumoniae* and limit the use of these compounds.

Provided herein are data from a study investigating the *in vitro* antipneumococcal activity of gemifloxacin against quinolone-susceptible and quinolone-resistant pneumococci (Kelly, *et al.*, 38th ICAAC, Abstract F-087 (1998)).

Gemifloxacin was compared to other quinolones against *S. pneumoniae* with specific resistant mutations in their *parC* and *gyrA* genes, the two principal mechanisms of quinolone resistance in pneumococci (Varon, *et al., Antimicrob. Agents Chemother.*, 43: 302-306 (1999)). The newly described efflux-mediated quinolone resistance (*pmrA* mutants) (Gill, *et al., Antimicrob. Agents Chemother.*, 43: 187-189 (1999)) was not tested.

S. pneumoniae ATCC 49619 was used as the quality control. Three isogenic

isolates of *S. pneumoniae* were studied: WB4, a ciprofloxacin-susceptible, but penicillin- and erythromycin-resistant strain; WB4CR, a ciprofloxacin-resistant, laboratory mutant of the strain WB4; and WB4TR, a trovafloxacin-resistant, laboratory mutant of the strain WB4. Both mutants were generated by cyclic exposure to either of the drugs. The parent (WB4) was ciprofloxacin(CIPRO)-susceptible, but penicillin- and erythromycin-resistant. The two derivatives were selected either for CIPRO-resistance (WB4CR) or trovafloxacin (TROVA)-resistance (WB4TR) by cyclic exposures to these drugs. The CIPRO-susceptible ATCC and WB4 strains carried no parC or gyrA mutations. The CIPRO-resistant mutant WB4CR carried one mutation in parC (79S Y) and the TROVA-resistant mutant carried one mutation in parC (79S F) and one mutation in gyrA (81S F). MICs (in mg/l) are listed in Table 55. Mutations in parC and/or gyrA increased the MICs of all the quinolones. However, gemifloxacin and clinafloxacin were the least affected.

Antibiotics used in the study comprised: Gemifloxacin; ciprofloxacin and moxifloxacin; levofloxacin; sparfloxacin; clinafloxacin; grepafloxacin; trovafloxacin.

Minimun Inhibitory Concentrations (MICs) were determined by the broth dilution method in Brain-Heart infusion (BHI, Difco) with an inoculum of 10^6 cfu/ml.

PCR Procedures and DNA Sequencing of QRDR genes used the primers and amplification conditions published by Pan *et al.* (Pan, *et al.*, *Antimicrob. Agents Chemother.*, 40: 2321-2326 (1996)) were used for PCR amplification of *parC* and *gyrA* fragments corresponding to the QRDRs. The DNA sequence of PCR-amplified products were run on an automatic sequencer.

Gemifloxacin and clinafloxacin had the most potent activity against *S. pneumoniae* among the tested quinolones. In contrast to the other drugs, gemifloxacin and clinafloxacin retained MICs in the therapeutic range even against the *parC/gyrA* double mutant quinolone-resistant pneumococci. These results warrant further studies to determine the activity of such drugs against *parC/gyrA* double mutants *in vivo*.

Table 54

Mutations in the parC and gyrA Genes		
	Mutation	
Strain (phenotype)	parC	gyrA
WB4 (parent)[a]	none	none
WB4CR(cipro-R)[b]	79Ser Tyr	none
WB4TR(trova-R)[c]	79Ser Phe	81Ser Phe

[a] **ciprofloxacin-susceptible strain.**
[b] **ciprofloxacin-resistant, laboratory mutant of strain WB4**
[c] **trovafloxacin-resistant, laboratory mutant of strain WB4.**

Table 55

Minimum Inhibitory Concentrations				
	MIC (mg/L)			
Drug	ATCC49619	WB4	par-C-mutant WB4CR	parC/gyrA-mutant WB4TR
Gemifloxacin	0.03	0.03	0.25	0.5
Ciprofloxacin	0.25	0.25	8	>32
Levofloxacin	0.5	0.25	8	>32
Sparfloxacin	0.25	0.25	2	>32
Clinafloxacin	0.06	0.06	0.12	0.5
Grepafloxacin	0.12	0.12	2	>32
Trovafloxacin	0.06	0.06	0.25	4
Moxifloxacin	0.25	0.12	2	8
Comment: Mutations in parC and/or gyrA increased the MICs of all the quinolones. However, gemifloxacin and clinafloxacin were the least affected.				

The invention provides a method for modulating metabolism of pneumococcal pathogenic bacteria. Skilled artisans can readily choose pneumococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: *S. pneumoniae.* Other pneumococcal pathogenic bacteria may also be included in the methods. The skilled

artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against Gram positive cocci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various Gram positive cocci pathogens. An objective of these analyses was to determine the spectrum and potency of gemifloxacin against comparison drugs. Gemifloxacin was compared to ampicillin, oxacillin, penicillin, ciprofloxacin, trovafloxacin, doxycycline, clindamycin, erythromycin and vancomycin.

Previous studies found greatest potency of gemifloxacin against Gram positive species, and this additional investigation sampled 6,790 of these organisms from more than 50 medical centers. Gemifloxacin was tested against trovafloxacin and four other comparison drugs to best define spectrum/potency. Reference broth microdilution method with recommended medium supplements was used throughout. The collection included 4962 staphylococci, 600 fastidious streptococci, 1182 enterococci and 46 strains of other species. Selected results (No. of strains tested; MIC_{90} for gemifloxacin/trovafloxacin; % ≤1 μg/ml for gemifloxacin/trovafloxacin) were: *Staphylococcus aureus* (3672; 2/2; 86/85), *S. epidermidis* (404; 1/>4; 92/71), *Enterococcus faecalis* (630; 4/>4; 76/66), *E. faecium* (216; >4/>4; 15/11), *Streptococcus pneumoniae* (300; 0.06/0.25; 100/97), β-hemolytic streptococci (150; 0.06/0.25; 100/100) and viridans group streptococci (150; 0.12/0.25; 99/97). Rates of resistance among monitored species were 31% for oxacillin in *S. aureus*, 35% for penicillin in pneumococci, and 11% vancomycin in enterococci, respectively. Gemifloxacin appears equal or superior to trovafloxacin in its overall Gram positive activity pending a choice of the susceptible breakpoint concentration. Continued *in vitro*, pharmacodynamic and clinical investigations appear warranted.

Initial evaluations of gemifloxacin spectrum indicate very potent activity against Gram positive cocci including many strains observed to be resistant to ciprofloxacin or ofloxacin. The gemifloxacin activity against Gram negative bacilli was most similar to that of trovafloxacin or gatifloxacin among the newer fluorinated quinolones but more modest potencies versus strict anaerobes were reported. These features conform to the structure–activity relationships described by Domagala in 1994, (Domagala, *et al., J. Antimicrob. Chemother.*, 33: 685-706 (1994)) and preliminary safety information suggest acceptable toleration for gemifloxacin (Kim, *et al., J. Appl. Pharmacol.*, 3: 322-326 (1995)).

To supplement existing *in vitro* information on gemifloxacin, an investigation was initiated to study a larger population of routine clinical isolates of Gram positive cocci and bacilli. These strains from diverse geographic locations in Europe, North America, and South America were processed by reference broth microdilution methods (*National Committee for Clinical Laboratory Standards*, Approved standard M7-A4, Wayne, PA USA (1997)).

The test organisms for these experimenst were Gram positive species and included routine clinical isolates, the majority of which were isolated in 1998–99. The general categorizations of genus and species were: enterococci (1182 strains; seven species and 310 strains that were not identified to species level), *Staphylococcus aureus* (3672 strains), coagulase-negative *Staphylococcus* spp. (1290 strains; 13 species with 775 strains that were not identified to species level), streptococci (600 strains), Coryneforme organisms (27 strains; four species), and *Bacillus* species (19 strains). All strains were from clinically significant infections, most from blood cultures, respiratory tract infections, and cutaneous/wound infections reported from more than 50 medical centers and referral laboratories.

The organisms were tested by the reference broth microdilution method with results interpreted by the current guidelines (*National Committee for Clinical Laboratory Standards*, Ninth Infomational Supplement M100-S9, Wayne, PA USA (1999)). Susceptibility interpretation and breakpoints for gemifloxacin and trovafloxacin were assigned at ≤1 μg/ml for comparison purposes only, pending approval by the National Committee for Clinical Laboratory Standards (NCCLS).

Nine comparison drugs including, ampicillin, oxacillin, penicillin, ciprofloxacin, trovafloxacin, doxycycline, clindamycin, erythromycin and vancomycin were tested. All compounds were obtained from their manufacturers and were utilized in the reference susceptibility method as specified by the drug provider or the NCCLS.

Susceptibility results are presented in Table 56. Gemifloxacin was the most active fluoroquinolone tested against the enterococci (≥4-fold more potent), inhibiting 76% of *E. faecalis*, 15% of *E. faecium* and 68% of other *Enterococcus* spp. isolates. For all enterococci (1182 strains), gemifloxacin was active against 63% of strains at ≤1 μg/ml (76% at ≤2 μg/ml) compared with trovafloxacin which inhibited 7% and 17% fewer strains at ≤1 and ≤2 μg/ml, respectively. This population of enterococci had the following resistance demographics: 10% resistance to vancomycin, 21% resistance to ampicillin, 28% gentamicin high-level resistance (>500 μg/ml) and 40% streptomycin high-level resistance

(>1000 μg/ml). Vancomycin remained the single best drug overall against all three tabulated Enterococcus groups.

The *S. aureus* strains showed 31% resistance to oxacillin and the rank order of spectrums of activity for the tested compounds was (MIC_{50} [μg/ml]/% susceptible): vancomycin (1/100) > doxycycline (≤0.5/93) > gemifloxacin (≤0.03/86) = trovafloxacin (≤0.03/85) > clindamycin (0.25/72) > ciprofloxacin (0.5/69) = oxacillin (1/69) > erythromycin (0.5/52). Gemifloxacin and trovafloxacin were the most potent fluoroquinolones tested and were ≥16-fold more active than ciprofloxacin.

The 1290 coagulase-negative staphylococci were also very susceptible to gemifloxacin (MIC_{90} 1 or 2 μg/ml) with 16–21% more strains inhibited at ≤1 μg/ml compared with trovafloxacin (MIC_{90}, >4 μg/ml).

A total of 600 streptococci were tested against gemifloxacin in this experiment, two fluoroquinolones and penicillin. The penicillin non-susceptible rate among *S. pneumoniae* was 42% (15% high-level at ≥2 μg/ml). Gemifloxacin was two- or four-fold more active than trovafloxacin and inhibited 99–100% of all streptococci at ≤1 μg/ml.

The fluoroquinolones are comparable to the tetracyclines and vancomycin against the *Bacillus* spp., but only 41– 59% of *Corynebacterium* spp. isolates are susceptible.

Against this contemporary sample of Gram positive organisms gemifloxacin is the most potent fluoroquinolone tested. The gemifloxacin spectrum and potency against enterococci and staphylococci are equal or superior to that of trovafloxacin, an agent recognized as being among the most active of the fluoroquinolone class against these pathogens.

Table 56. Comparative Antimicrobial Activity of Gemifloxacin and Nine Selected Compounds Tested Against 6,790 Strains of Gram positive Bacteria

Microrganism (No. of strains)	Antimicrobial	MIC (μg/ml)		% susceptible (breakpoint MIC)
		MIC_{50}	MIC_{90}	
Enterococcus faecalis (630)	Gemifloxacin	0.06	4	76 (≤1)
	Ciprofloxacin	2	>2	45 (≤1)
	Trovafloxacin	0.25	>4	66 (≤1)
	Ampicillin	1	2	98 (≤8)
	Doxycycline	>4	>4	50 (≤4)
	Erythromycin	>8	>8	8 (≤0.5)
	Vancomycin	1	2	98 (≤4)
Enterococcus faecium (216)	Gemifloxacin	>4	>4	15 (≤1)

Microrganism (No. of strains)	Antimicrobial	MIC (μg/ml)		% susceptible (breakpoint MIC)
		MIC_{50}	MIC_{90}	
	Ciprofloxacin	>2	>2	5 (≤1)
	Trovafloxacin	>4	>4	11 (≤1)
	Ampicillin	>16	>16	13 (≤8)
	Doxycycline	4	>4	61 (≤4)
	Erythromycin	>8	>8	3 (≤0.5)
	Vancomycin	1	>16	57 (≤4)
Enterococcus spp. (336)	Gemifloxacin	0.12	4	68 (≤1)
	Ciprofloxacin	2	>2	40 (≤1)
	Trovafloxacin	0.5	>4	62 (≤1)
	Ampicillin	1	>16	89 (≤8)
	Doxycycline	>4	>4	47 (≤4)
	Erythromycin	>8	>8	9 (≤0.5)
	Vancomycin	1	2	98 (≤4)
Staphylococcus aureus (3,672)	Gemifloxacin	≤0.03	2	86 (≤1)
	Ciprofloxacin	0.5	>2	69 (≤1)
	Trovafloxacin	≤0.03	2	85 (≤1)
	Oxacillin	1	>8	69 (≤2)
	Doxycycline	≤0.5	2	93 (≤4)
	Erythromycin	0.5	>8	52 (≤0.5)
	Clindamycin	0.25	>8	72 (≤0.5)
	Vancomycin	1	1	100 (≤4)
Staphylococcus epidermidis (404)	Gemifloxacin	≤0.03	1	92 (≤1)
	Ciprofloxacin	0.5	>2	55 (≤1)
	Trovafloxacin	0.06	>4	71 (≤1)
	Oxacillin	8	>8	21 (≤0.25)
	Doxycycline	≤0.5	>4	85 (≤4)
	Erythromycin	>8	>8	31 (≤0.5)
	Clindamycin	0.25	>8	58 (≤0.5)
	Vancomycin	2	2	100 (≤4)
Coagulase-negative staphylococci (886)	Gemifloxacin	≤0.03	2	88 (≤1)
	Ciprofloxacin	0.5	>2	56 (≤1)
	Trovafloxacin	0.06	>4	72 (≤1)
	Oxacillin	8	>8	23 (≤0.25)
	Doxycycline	≤0.5	>4	89 (≤4)
	Erythromycin	>8	>8	32 (≤0.5)
	Clindamycin	0.25	>8	58 (≤0.5)
	Vancomycin	2	2	100 (≤4)
Streptococcus pneumoniae (300)	Gemofloxacin	0.03	0.06	100 (≤1)
	Ciprofloxacin	1	2	66 (≤1)
	Trovafloxacin	0.12	0.25	97 (≤1)
	Penicillin	0.06	2	58 (≤0.06)

Microrganism (No. of strains)	Antimicrobial	MIC (μg/ml)		% susceptible (breakpoint MIC)
		MIC_{50}	MIC_{90}	
Streptococcus β-hemolytic (150)				
	Gemifloxacin	0.03	0.06	100 (≤1)
	Ciprofloxacin	0.5	1	97 (≤1)
	Trovafloxacin	0.12	0.25	100 (≤1)
	Penicillin	0.12	0.25	85 (≤0.12)
Streptococcus viridans group (150)	Gemifloxacin	0.06	0.12	99 (≤1)
	Ciprofloxacin	2	4	37 (≤1)
	Trovafloxacin	0.12	0.25	97 (≤1)
	Penicillin	0.12	>2	62 (≤0.12)
Bacillus spp. (19)	Gemifloxacin	≤0.03	0.06	95 (≤1)
	Ciprofloxacin	0.12	0.5	95 (≤1)
	Trovafloxacin	≤0.03	0.12	95 (≤1)
	Ampicillin	8	>16	58 (≤8)
	Doxycycline	≤0.5	≤0.5	100 (≤4)
	Erythromycin	0.25	1	89 (≤0.5)
	Vancomycin	0.5	2	100 (≤4)
Corynebacterium spp. (27)	Gemifloxacin	0.5	>4	59 (≤1)
	Ciprofloxacin	>2	>2	41 (≤1)
	Trovafloxacin	1	>4	52 (≤1)
	Ampicillin	4	>16	81 (≤8)
	Doxycycline	≤0.5	>4	89 (≤4)
	Erythromycin	>8	>8	26 (≤0.5)
	Vancomycin	0.5	1	100 (≤4)

The invention provides a method for modulating metabolism of Gram positive coccal pathogenic bacteria. Skilled artisans can readily choose Gram positive coccal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by Gram positive coccal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with Gram positive coccal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said Gram

positive coccal pathogenic bacteria is selected from the group consisting of: *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, Coagulase-negative staphylococci, *Streptococcus pneumoniae*, *Streptococcus* β-hemolytic, *Streptococcus* viridans group, *Bacillus* spp., and *Corynebacterium* spp. Other Gram positive coccal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Streptococcus pneumoniae* .

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Streptococcus pneumoniae* pathogens. An objective of these analyses was to determine the mechanism of action of gemifloxacin in S. *pneumoniae*.

Gemifloxacin was compared to ciprofloxacin, norfloxacin pefloxacin, levofloxacin, trovafloxacin, sparfloxacin and gatifloxacin.

Quinolone targets in *Streptococcus pneumoniae* include Topo IV and Gyrase. Ciprofloxacin, norfloxacin pefloxacin, levofloxacin and trovafloxacin target Topo IV while sparfloxacin and gatifloxacin target Gyrase.

Gemifloxacin is highly potent against Gram positive bacteria such as S. aureus and S. *pneumoniae* and common respiratory tract pathogens (e.g., M. catarrhalis and H. influenza, with MICs<0.06 micrograms/ml). Gemifloxacin is also active against pen-R and -S strains of S. *pneumoniae* (MICs < 0.125 micrograms/ml) and shows activity against ciprofloxacin-resistant S. *pneumoniae* (MICs < 0.5 micrograms/ml).

Two approaches were used to investigate the mechanism of action of gemifloxacin in S. *pneumoniae*: (i) to study susceptibilities of strains with characterized QRDR mutations and (ii) to select gemifloxacin-resistant strains and determine QRDR sequences. The MICs of gemifloxacin are lower than those of ciprofloxacin and sparfloxacin (from two-timees to 32 times lower than the MIC from sparfloxacin and from eight-times to 128-times lower than the MIC from ciprofloxacin).

Mutations in both parC and gyrA are required to significantly reduce susceptibility to gemifloxacin. First-step gemifloxacin mutants (MIC 0.25 micrograms/ml) harbor gyrA mutations and show cross-resistance to sparfloxacin. ParC changes are acquired on further selection (MIC 0.5-1.0 micrograms/ml). Gemifloxacin has in vivo preference for gyrase and activity against strains with topoisomerase mutations.

The invention provides a method for modulating metabolism of *Streptococcus pneumoniae* pathogenic bacteria. Skilled artisans can readily choose *Streptococcus pneumoniae* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Streptococcus pneumoniae* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Streptococcus pneumoniae* pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae* . Other *Streptococcus pneumoniae* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae*.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* pathogens. An objective of these analyses was to determine the bactericidal activity and mechanisms of action of the fluoroquinolone gemifloxacin against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Gemifloxacin was compared to Trovafloxacin and Sitafloxacin. Gemifloxacin was found to be highly bactericidal against these bacteria producing a biphasic dose response curve typical of the fluoroquinolones. This novel fluoroquinolone was more bactericidal than all other fluoroquinolones so far tested against *S. aureus* and was more bactericidal than most other fluoroquinolones against *E. coli* or *S. pneumoniae*. The data shows gemifloxacin to be an improved member of the fluoroquinolone class of antibacterials.

The bactericidal activity of numerous fluoroquinolones has been assessed in a series of studies (Morrissey, *et al.*, *J. Med. Microbiol.*, 43: 4-8 (1995)). This bactericidal activity has been characterized into four mechanisms of action (A, B, C and B_1) based on experiments investigating kill in phosphate-buffered saline (PBS), i.e. against non-

multiplying bacteria, or kill in the presence of chloramphenicol (to prevent protein synthesis). Mechanism A, requires bacteria to be multiplying and to be actively undergoing protein and RNA synthesis (Lewin, *et al., Eur., J. Clin. Microbiol. Infect. Dis.*, 10: 240-248 (1991)). This is the basic mechanism of action shared by all quinolones and is the sole mechanism of action of older quinolones such as nalidixic acid. Mechanism B, does not require multiplying bacteria or protein and RNA synthesis (Lewin, *et al., Eur., J. Clin. Microbiol. Infect. Dis.*, 10: 240-248 (1991)). Mechanism B is shown by many modern fluoroquinolones against *E. coli* (Morrissey, *et al., J. Med. Microbiol.*, 43: 4-8 (1995)). However, this does not guarantee that this mechanism ofaction will be present against other bacteria. For example, ciprofloxacin does not possess mechanism B against *S. aureus* (Lewin, *et al., J. Antimicrob. Chemother.*, 22 (Suppl. C): 1-8 (1988)) whereas levofloxacin does (Lewin, *et al., J. Med. Microbiol.*, 30: 227-231 (1989)). Against *S. pneumoniae*, only sitafloxacin has been found to possess this additional mechanism (Morrissey, *et al., J. Med. Microbiol.*, 43: 4-8 (1995)). Mechanism C, on the other hand, does not require multiplying bacteria but does require active protein and RNA synthesis (Morrissey, *et al., J. Med. Microbiol.*, 43: 4-8 (1995)). This mechanism has only been identified with enoxacin (Lewin, *et al., Eur. J. Clin. Microbiol. Infect. Dis.*, 8: 731-733 (1989)) and norfloxacin (Ratcliffe, *et al., J. Pharm. Pharmacol.*, 37 (Suppl): 92P (1987)). Mechanism B_1 is the most recently discovered mechanism of action. This mechanism does not require protein or RNA synthesis, but is lost against non-dividing bacteria. Mechanism B_1 has been identified with clinafloxacin against *E. coli* or staphylococci (Lewin, *et al., J. Med. Microbiol.*, 33: 67-70 (1990)) and sitafloxacin or trovafloxacin against *Enterococcus faecalis* (Morrissey, *et al., J. Med. Microbiol.*, 43: 4-8 (1995); Morrissey, *et al., J. Antimicrob. Chemother.*, 38: 1061-1066 (1996)).

This study was carried out to assess the bactericidal activity of gemifloxacin (SB-265805), a new (C-7 3-aminomethyl-4-methyloxime substituted 1,8-napthyridine) fluoroquinolone. Preliminary studies suggest that gemifloxacin has enhanced antibacterial activity, especially against Gram positive bacteria (Paek, *et al.*, 38th ICAAC, Abstract F-92, p. 255 (1998)).

Gemifloxacin was supplied by SmithKline Beecham Pharmaceuticals R & D (New Frontiers Science Park (South), Third Avenue, Harlow, Essex). Stock solutions of 1 mg/ml were prepared in sterile distilled water. Chloramphenicol (Sigma-Aldrich, Poole, Dorset) was firstly dissolved in methanol and then dissolved in sterile distilled water. Both antibacterials were prepared fresh on each day of experimentation.

The following laboratory strains were used in this study:

1. *Escherichia coli* KL16
2. *Staphylococcus aureus* E3T
3. *Streptococcus pneumoniae* C3LN4

These strains were chosen because of their previous use to determine the bactericidal mechanisms of action of other quinolones. The bacteria were stored at –70 °C and sub-cultured onto nutrient broth No.2 (Unipath Ltd., Basingstoke, Hants) solidified with 1.5% (w/v) agar bacteriological (Unipath Ltd) prior to use. For *S. pneumoniae*, agar was supplemented with 5% (v/v) laked horse blood (Unipath Ltd.).

The bactericidal activity of gemifloxacin was investigated using the method of Morrissey and Smith (Morrissey, *et al.*, *J. Med. Microbiol.*, 43: 4-8 (1995)). Briefly, a range of concentrations between 0.005 and 10 mg/L were prepared in nutrient broth No.2. Bacteria were inoculated to an initial inoculum size of about 10^7 cfu/L and incubated for 3 h at 37 °C. When *S. pneumoniae* was used, the medium was supplemented with laked horse blood to 7% (v/v). The presence of additional mechanisms B, B_1 or C was assessed by the addition of 20 mg/L chloramphenicol (2.5 mg/L for *S. pneumoniae*) to prevent protein synthesis or by replacing nutrient broth with phosphate-buffered saline to prevent bacterial multiplication. To prevent autolysis of *S. pneumoniae*, horse serum (Unipath) at 7% (v/v) was added when this organism was studied in phosphate-buffered saline. After incubation 1 mL samples were taken, centrifuged and resuspended in an equal volume of sterile nutrient broth no.2. This washing step was repeated twice in total to prevent drug carry-over. Viable counts of these samples were made on solid agar by spiral plating and the plates incubated for 48 h at 35 °C. Percent survival was calculated and plotted against drug concentration tested.

Gemifloxacin produces a biphasic dose response against *E. coli* KL16, *S. aureus* E3T or *Str. pneumoniae* C3LN4, producing an optimum bactericidal concentration for gemifloxacin against all 3 bacteria (Figures 24, 25, and 26). This phenomenon is typical of the fluoroquinolone antibacterials (Lewin, *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.*, 10: 240-248 (1991)). The optimum bactericidal concentration for gemifloxacin against *E. coli* KL16, *S. aureus* E3T and *S. pneumoniae* C3LN4 is 1.0, 0.5 and 0.5 mg/L, respectively.

The addition of a bacteriostatic concentration of chloramphenicol reduced the bactericidal activity of gemifloxacin against *E. coli* (Figure 24). However, significant bactericidal activity still occurred in the presence of this protein synthesis inhibitor. On the other hand, when the activity of gemifloxacin was tested in phosphate-buffered saline

considerably less bactericidal activity was observed against *E. coli* (Figure 24). It would appear therefore that gemifloxacin can kill bacteria devoid of protein synthesis but is less able to kill fully non-multiplying bacteria incubated in phosphate-buffered saline. Therefore, gemifloxacin possesses bactericidal mechanisms A and B_1 against *E. coli*.

Against *S. aureus* slightly stronger bactericidal activity occurred in nutrient broth than that seen against *E. coli* (Figure 25). Reduced kill was produced against the staphylococcus when chloramphenicol was added or when experiments were carried out in phosphate-buffered saline (Figure 25). Nevertheless, good bactericidal activity still occurred against *S. aureus* E3T under these conditions i.e. against staphylococci unable to undergo protein synthesis or against staphylococci unable to multiply. It is evident therefore that gemifloxacin possesses bactericidal mechanisms A and B against *S. aureus*.

It can be seen from Figure 26, as with the other two bacterial species tested, gemifloxacin was bactericidal against *S. pneumoniae* in nutrient broth (supplemented with laked horse blood). This bactericidal activity was lower than that observed for gemifloxacin against either *E. coli* or *S. aureus*. Furthermore, little or no bactericidal activity occurred using gemifloxacin when chloramphenicol was added or when experiments were carried out in phosphate-buffered saline (supplemented with horse serum). Therefore against the pneumococcus, gemifloxacin did not possess any bactericidal mechanisms of action in addition to mechanism A.

The results of this study show that gemifloxacin is bactericidal against *E. coli* KL16, *S. aureus* E3T and *S. pneumoniae* C3LN4. Gemifloxacin possesses additional bactericidal mechanisms of action B_1 and B against *E. coli* KL16 and *S. aureus* E3T, respectively. However, no such additional mechanisms are present against *S. pneumoniae* C3LN4.

For comparative purposes, the results of this study have been presented with the equivalent results obtained previously obtained with trovafloxacin and sitafloxacin (Table 57). It can be seen that gemifloxacin has a comparable optimum bactericidal concentration to sitafloxacin and a lower OBC than trovafloxacin against *E. coli*. Gemifloxacin also has the lowest OBC against *S. pneumoniae*. Most significantly, however, gemifloxacin has a considerably lower OBC than trovafloxacin or sitafloxacin against *S. aureus*. In addition, the bactericidal activity of gemifloxacin at the optimum bactericidal concentration against *S. aureus* in nutrient broth was also greater than that found with the other fluoroquinolones (Table 57). These results indicate that gemifloxacin is the most potent fluoroquinolone so far tested against *S. aureus* E3T using this system.

Interestingly, however, the bactericidal advantage of gemifloxacin against *S. aureus* is not retained when chloramphenicol is added or when experiments were carried out in phosphate-buffered saline (Table 57). In other words, bactericidal mechanism B with gemifloxacin is not as potent as that seen with trovafloxacin or sitafloxacin

As seen with trovafloxacin (Table 57), gemifloxacin does not possess bactericidal activity in the presence of chloramphenicol or in phosphate-buffered saline against *S. pneumoniae*. In fact, sitafloxacin is the only fluoroquinolone known to possess bactericidal activity against *S. pneumoniae* C3LN4 under these experimental conditions.

Gemifloxacin shows reduced bactericidal activity when bacteria are unable to multiply due to weak additional mecanisms B or B_1 compared to trovafloxacin or sitafloxacin. Nevertheless, against multiplying bacteria gemifloxacin shows very strong bactericidal activity. The clinical significance of these differences will become clear after further evaluation of gemifloxacin.

Table 57. Comparable bactericidal activity of gemifloxacin

Organism	Quinolone	OBC (mg/L)	Percent survival			Reference
			NB	NB & Cm	PBS	
E. coli	**Gemifloxacin**	**1.0**	**0.004**	**2.5**	**12.3**	**This study**
KL16	Trovafloxacin	**1.5**	0.009	1.9	1.4	8
	Sitafloxacin	0.9	0.0005	0.028	0.07	1
S. aureus	**Gemifloxacin**	**0.5**	**0.0017**	**3.00**	**1.84**	**This study**
E3T	Trovafloxacin	3.0	0.005	0.16	0.44	8
	Sitafloxacin	3.0	0.004	0.09	0.18	**1**
S. pneumoniae	**Gemifloxacin**	**0.5**	**0.21**	**16.2**	**25.7**	**This study**
C3LN4	Trovafloxacin	1.5	0.24	24.8	57.5	8
	Sitafloxacin	0.9	0.10	0.14	0.41	1

OBC = optimum bactericidal concentration, NB = nutrient broth,

Cm = chloramphenicol, PBS = phosphate-buffered saline.

The invention provides a method for modulating metabolism of *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria. Skilled artisans can readily choose *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus pneumoniae* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said pathogenic bacteria is selected from the group consisting of: *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. Other pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *enterococci*.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *enterococci* pathogens. An objective of these analyses was to determine the in vitro activity of gemifloxacin against 100 clinical isolates collected at the University Hospital in 1998 and 1999.

Gemifloxacin was compared to gemifloxacin, trovafloxacin, grepafloxacin, levofloxacin, ofloxacin, ciprofloxacin, nalidixic acid, penicillin, ampicillin, gentamicin and trimethoprim/sulfamethoxazole

The increased resistance of *enterococci* to many antimicrobials, including glycopeptides, has highlighted the need for new agents. Among the fluoroquinolones, several newer compounds have enhanced activities against Gram positive bacteria. The purpose of this study was to examine the in vitro activity of gemifloxacin (SB-265805) against 100 clinical isolates collected at the University Hospital in 1998 and 1999. The

isolates comprised 50 *Enterococcus faecalis* and 50 *Enterococcus faecium* strains, seven of which were glycopeptide resistant (VRE). The MICs for gemifloxacin and 10 other antimicrobials were determined using a broth microdilution method according to NCCLS methodology.

Table 58

Antimicrobial	Enterococcus faecalis		Enterococcus faecium	
	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Gemifloxacin	0.06	0.25	0.06	1
Nalidixic acid	>64	>64	>64	>64
Ofloxacin	2	8	4	8
Ciprofloxacin	1	8	1	4
Levofloxacin	1	4	2	2
Trovafloxacin	0.25	0.5	0.5	1
Grepafloxacin	0.25	0.5	0.5	4
Penicillin	2	4	4	>16
Ampicillin	2	2	2	8
Gentamicin	16	>64	8	16
Trimethoprim/ sulfamethoxazole	≤0.06/1.14	≥64/1216	0.5/9.5	4/76

Gemifloxacin was more active against *enterococci* than the other quinolones tested. MICs were, in general, 1–2 dilutions lower than those of other quinolones with activity against Gram positive bacteria. There was a 3–4 dilution difference between the MIC_{50} and MIC_{90} for both gemifloxacin and grepafloxacin resulting from a tendency to show a bimodal distribution: MIC_{50}/ MIC_{90} values for VRE strains were 0.5/16 μg/ml for grepafloxacin and 0.06/8 μg/ml for gemifloxacin. Of 17 strains with MICs ≥2 for grepafloxacin, MIC_{50}/ MIC_{90} values for grepafloxacin and gemifloxacin were 4/16 μg/ml and 0.5/2 μg/ml, respectively, meaning a three dilution difference in favour of gemifloxacin.

Antimicrobial resistance is an increasing clinical problem, particularly among Gram positive organisms. Available quinolones, such as ofloxacin and ciprofloxacin, yield moderate activities against Gram positive organisms, with MIC values clustering around the

breakpoints. Newer quinolones with broader spectrums of activity against Gram positive cocci include levofloxacin, grepafloxacin and trovafloxacin. Gemifloxacin (SB-265805) is a new fluoronaphthyridone carboxylic acid with a novel pyrrolidine substituent. Preliminary studies have shown that gemifloxacin is highly active against Gram positive bacteria, including strains that are resistant to other classes of agents.

In this study, the in vitro antimicrobial activity of gemifloxacin was examined against strains of *Enterococcus faecalis* and *Enterococcus faeci*um and compared with that of other quinolones and β-lactams.

A total of 100 *enterococci* were isolated from clinical specimens from both hospitalized patients and out-patients. The isolates comprised 50 *Enterococcus faecalis* and 50 *Enterococcus faecium* strains. Seven *E. faecium* isolates were vancomycin resistant (VRE) and were isolated from stool specimens from patients screened for strains resistant to vancomycin.

MICs were determined using the microbroth dilution method according to the NCCLS (*National Committee for Clinical Laboratory Standards*, Ninth Informational Supplement, Table 2H, Vol. 19, No.: 1, Villanova, PA USA (199)). The antimicrobials tested were: gemifloxacin, trovafloxacin, grepafloxacin, levofloxacin, ofloxacin, ciprofloxacin, nalidixic acid, penicillin, ampicillin, amoxycillin/clavulanic acid, clarithromycin, azithromycin, cefuroxime, gentamicin and trimethoprim/sulfamethoxazole. The microtiter trays were manufactured by Sensititre®(AccuMed International Ltd, UK).

The appropriate quality control organisms were included in each run. Results were only accepted if the quality control ranges recommended by the NCCLS were within range. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were included as control organisms.

The *in vitro* antimicrobial activities of gemifloxacin and other antimicrobials against *Enterococcus spp.* are shown in Table 59. Table 60 shows MIC_{50}/MIC_{90} values for gemifloxacin and other quinolones against vancomycin-susceptible and VRE strains.

The current study indicates that gemifloxacin had the most potent in vitro activity against *enterococci* among the quinolones tested. At concentrations of 0.5 μg/ml and 1 μg/ml, gemifloxacin and both trovafloxacin and grepafloxacin inhibited 90% of all E. faecalis isolates compared with only 6% and 78%, respectively, for ciprofloxacin and 6% and 88%, respectively, for levofloxacin. Against *E. faecium*, inhibition rates of gemifloxacin at 0.5 μg/ml and at 1 μg/ml were higher than those of all other quinolones

tested. At a concentration of 1 μg/ml 94% of all *E. faecium* isolates were inhibited by gemifloxacin compared with 62% for ciprofloxacin, 46% for levofloxacin and 66% for grepafloxacin.

For both *E. faecalis* and *E. faecium*, the MIC_{50} for gemifloxacin was 0.06 μg/ml; this was 2–3 dilutions lower than the MIC_{50} of the comparable fluoroquinolones trovafloxacin (0.25 μg/ml) and grepafloxacin (0.5 μg/ml). MIC_{90}s of gemifloxacin were 1–2 dilutions lower than those of trovafloxacin and grepafloxacin for both *enterococcal* species.

There was a 3–4 dilutions difference between the MIC_{50} and the MIC_{90} for both gemifloxacin and grepafloxacin, resulting from a tendency to show a bimodal distribution. This was only partly explained by the VRE, for which MIC_{50}/MIC_{90} values were 0.5/16 μg/ml for grepafloxacin and 0.06/8 μg/ml for gemifloxacin.

Of 17 strains with MICs ≥2 for grepafloxacin, MIC_{50}/MIC_{90} values for grepafloxacin and gemifloxacin were 4/16 μg/ml and 0.5/2 μg/ml, respectively, meaning a three dilution difference in favour of gemifloxacin.

Table 59. *In Vitro* Antimicrobial Activity of Gemifloxacin Compared With Other Antimicrobials Against *Enterococcus* spp.

Micro-rganism	Antimicrobial agent	Cumulative % inhibited at MIC			MIC (μg/ml)			% susce-ptible*
		0.5 μg/ml	1 μg/ml	2 μg/ml	50%	90%	Range	
Enterococcus faecalis (n = 50)	Gemifloxacin	90	90	92	0.06	0.25	0.008–8	–
	Ofloxacin	0	2	72	2	8	1–>64	–
	Ciprofloxacin	6	78	88	1	8	0.5–>16	78
	Levofloxacin	6	88	88	1	4	0.5–>16	88
	Trovafloxacin	90	90	90	0.25	0.5	0.12–16	–
	Grepafloxacin	90	90	90	0.25	0.5	0.12–>16	–
	Penicillin	0	0	66	2	4	2–8	100
	Ampicillin	0	40	100	2	2	1–2	100

Micro-rganism	Antimicrobial agent	Cumulative % inhibited at MIC			MIC (μg/ml)			% susce-ptible*
		0.5 μg/ml	1 μg/ml	2 μg/ml	50%	90%	Range	
Enterococcus faecium (n = 50)	Gemifloxacin	84	94	98	0.06	1	0.008–2	–
	Ofloxacin	4	12	24	4	8	0.5–32	–
	Ciprofloxacin	20	62	86	1	4	0.12–>16	62
	Levofloxacin	18	46	90	2	2	0.25–16	90
	Trovafloxacin	66	90	98	0.5	1	0.03–8	–
	Grepafloxacin	64	66	80	0.5	4	0.12–16	–
	Penicillin	20	22	46	4	>16	0.03–>16	80
	Ampicillin	22	36	78	2	8	≤0.06–64	90

*Percentage susceptibility results relate to the NCCLS (1999) breakpoints for levofloxacin (≤2 μg/ml), ciprofloxacin (≤1 μg/ml), penicillin (≤8 μg/ml) and ampicillin (≤8 μg/ml).

Table 60. MIC_{50}/MIC_{90} Values for Gemifloxacin and Other Antimicrobials Against Vancomycin-susceptible and VRE Strains of *E. faecium*

Antimicrobial	Vancomycin susceptible (n = 43)		VRE (n = 7)	
	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Gemifloxacin	0.06	1	0.06	8
Ofloxacin	4	8	4	32
Ciprofloxacin	1	4	1	16
Levofloxacin	2	2	2	16
Trovafloxacin	0.5	1	1	8
Grepafloxacin	0.5	4	0.5	16
Penicillin	2	8	4	≥16
Ampicillin	2	4	8	32

The invention provides a method for modulating metabolism of *enterococcal* pathogenic bacteria. Skilled artisans can readily choose *enterococcal* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *enterococcal* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *enterococcal* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *enterococcal* pathogenic bacteria is selected from the group consisting of: *Enterococcus faecalis* and *Enterococcus faecium.* Other *enterococcal* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against streptococci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various streptococcal pathogens. An objective of these analyses was to determine the *in vitro* antimicrobial activity of gemifloxacin as compared with that of other quinolones, macrolides and β-lactams against streptococci..

Although early quinolones were developed primarily for the treatment of Gram negative infections, several newer compounds with improved activity against *streptococci* and other Gram positive pathogens are currently under investigation and are becoming available. The *in vitro* activity of gemifloxacin (SB-265805) was evaluated against 150 recent clinical isolates of streptococci, collected at the University Hospital Antwerp in 1998–99, including 50 *Streptococcus pyogenes*, 50 *Streptococcus agalactiae* and 50 viridans streptococci strains. MICs were determined by the broth microdilution method according to NCCLS methodology and compared with those of 14 other antimicrobials.

Among all the quinolones tested, gemifloxacin showed the most potent activity: both the MIC_{50} and the MIC_{90} were generally 1–2 dilutions lower than those of other quinolones with activity against Gram positive microrganisms. Four strains of *S. agalactiae* intermediately susceptible or resistant to ofloxacin had gemifloxacin MICs of 0.03–0.06 μg/ml. Three *S. pyogenes* strains intermediately susceptible to ofloxacin, two of which were

resistant to grepafloxacin, had gemifloxacin MICs of 0.03–0.06 μg/ml. These results indicate the potential activity of gemifloxacin against strains of streptococci that are becoming resistant to other quinolones.

Antimicrobial resistance is an increasing clinical problem, particularly among Gram positive organisms. vailable quinolones, such as ofloxacin and ciprofloxacin, yield moderate activities against Gram positive organisms, with MICs clustering around the breakpoints. A number of newer quinolones, such as trovafloxacin and grepafloxacin, with enhanced activity against Gram positive bacteria have become available. Gemifloxacin (SB-265805) is a naphthyridone molecule in the fluoroquinolone class of antimicrobials. It has broad-spectrum antimicrobial activity against both Gram positive and Gram negative pathogens, including strains resistant to other classes of agents.

A total of 150 streptococci were isolated from clinical specimens from both hospitalized patients and out-patients: 50 *Streptococcus agalactiae*, 50 *Streptococcus pyogenes* and 50 viridans streptococci.

MICs were determined using the microbroth dilution method according to the NCCLS (*National Committee for Clinical Laboratory Standards*, Ninth Informational Supplement, Table 2H, Vol., 19, Villanova, PA USA (1999)). The antimicrobials tested were: gemifloxacin, trovafloxacin, grepafloxacin, levofloxacin, ofloxacin, ciprofloxacin, nalidixic acid, penicillin, ampicillin, amoxycillin/clavulanic acid, clarithromycin, azithromycin, cefuroxime, gentamicin and trimethoprim/sulfamethoxazole. The microtiter trays were manufactured by Sensititre® (AccuMed International Ltd, UK).

The appropriate quality control organisms were included in each run. Results were only accepted if the quality control ranges recommended by the NCCLS were within range. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were included as control organisms.

The *in vitro* antimicrobial activities of gemifloxacin and other antimicrobials against *Streptococcus* spp. are shown in Table 61.

Gemifloxacin inhibited 98–100% of all streptococci at 0.5 μg/ml compared with 16–76% for ciprofloxacin, 94–98% for grepafloxacin and 98–100% for trovafloxacin.

Among all the quinolones tested, gemifloxacin showed the most potent activity. Both MIC_{50} and MIC_{90}, varying from 0.015 to 0.06 μg/ml, were 1–4 dilutions lower than those of the comparable fluoroquinolones: trovafloxacin (0.12–0.25 μg/ml) and grepafloxacin (0.12–0.5 μg/ml).

Gemifloxacin MICs in four *S. agalactiae* strains intermediately susceptible (MIC 4 μg/ml) or resistant (MIC 8 μg/ml) to ofloxacin were in the range 0.03–0.06 μg/ml. Three *S. pyogenes* strains intermediately susceptible to ofloxacin, two of which were resistant to grepafloxacin (MIC 2 μg/ml), had MICs for gemifloxacin of 0.03–0.06 μg/ml.

Gemifloxacin is a new fluoroquinolone showing high activity against streptococci, including potential activity against streptococci that are becoming resistant to other quinolones.

The invention provides a method for modulating metabolism of streptococcal pathogenic bacteria. Skilled artisans can readily choose streptococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Table 61. ***In Vitro*** **Antimicrobial Activity of Gemifloxacin Compared With Nine Other Antimicrobials Against** ***Streptococcus*** **spp.**

Microrganism	Antimicrobial agent	Cumulative % inhibited at MIC			MIC (µg/ml)			% susceptible*
		0.5 µg/ml	1 µg/ml	2 µg/ml	50%	90%	Range	
Streptococcus agalactiae (n = 50)	Gemifloxacin	100	100	100	0.06	0.06	0.008–0.12	–
	Ofloxacin	0	24	92	2	2	1–8	92
	Ciprofloxacin	34	64	98	1	1	0.5–4	–
	Levofloxacin	38	100	100	1	1	0.25–1	100
	Trovafloxacin	100	100	100	0.25	0.25	0.06–0.5	100
	Grepafloxacin	98	100	100	0.25	0.5	0.12–1	98
	Penicillin	90	90	100	0.03	0.06	≤0.015–2	90
	Ampicillin	90	96	100	0.12	0.25	≤0.06–2	88
	Clarithromycin	80	82	90	0.03	2	≤0.015–>16	80
	Azithromycin	78	78	78	≤0.06	>64	≤0.06–>64	78
Streptococcus pyogenes (n = 50)	Gemifloxacin	100	100	100	0.015	0.03	0.008–0.06	–
	Ofloxacin	4	72	94	1	2	0.5–4	94
	Ciprofloxacin	76	96	96	0.5	1	0.25–4	–

Microrganism	Antimicrobial agent	Cumulative % inhibited at MIC			MIC (µg/ml)			% susceptible*
		0.5 µg/ml	1 µg/ml	2 µg/ml	50%	90%	Range	
	Levofloxacin	90	96	100	0.5	0.5	0.25–2	100
	Trovafloxacin	98	100	100	0.12	0.12	0.03–1	100
	Grepafloxacin	94	96	100	0.25	0.5	0.06–2	94
	Penicillin	100	100	100	≤0.015	≤0.015	≤0.015	100
	Ampicillin	100	100	100	≤0.06	≤0.06	≤0.06	100
	Clarithromycin	94	94	94	0.03	0.03	≤0.015–16	94
	Azithromycin	94	94	94	≤0.06	0.12	≤0.06–64	94
Viridans group (n = 50)	Gemifloxacin	98	98	100	0.03	0.06	0.004–2	–
	Ofloxacin	2	22	76	2	4	0.5–16	–
	Ciprofloxacin	16	52	76	1	4	0.25–16	–
	Levofloxacin	40	90	98	1	1	0.25–4	98
	Trovafloxacin	98	98	100	0.12	0.25	≤0.015–2	98
	Grepafloxacin	98	98	98	0.12	0.5	0.06–4	98
	Penicillin	62	74	84	0.25	4	≤0.015–>16	46
	Ampicillin	60	64	74	0.5	8	≤0.06–>64	46

Microrganism	Antimicrobial agent	Cumulative % inhibited at MIC			MIC (μg/ml)			% susceptible*
		0.5 μg/ml	1 μg/ml	2 μg/ml	50%	90%	Range	
	Clarithromycin	48	58	76	1	>16	≤0.015–>16	42
	Azithromycin	48	54	64	1	>64	≤0.06–>64	48

*Percentage susceptibility results relate to the NCCLS (1999) breakpoints for grepafloxacin (≤0.5 μg/ml), trovafloxacin (≤1μg/ml), levofloxacin (≤2 μg/ml), ofloxacin (≤2 μg/ml for β-hemolytic streptococci), clarithromycin (≤0.25 μg/ml), azithromycin (≤0.5 μg/ml), penicillin (≤0.12 μg/ml) and ampicillin (≤0.25 μg/ml)

Also provided by the invention is a method of treating or preventing a bacterial infection by streptococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with streptococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said streptococcal pathogenic bacteria is selected from the group consisting of: *Streptococcus pyogenes*, *Streptococcus agalactiae* and viridans streptococci. Other streptococcal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Acinetobacter* spp.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Acinetobacter* spp. pathogens. An objective of these analyses was to determine the MIC range, MIC_{50} and MIC_{90} values of gemifloxacin and the comparator quinolones against the 5 species of *Acinetobacter* and the killing kinetics for the seven antibiotics tested at both the OBC and 4xMIC.

The in-vitro bacteriostatic activity of gemifloxacin was compared with 11 antibacterial agents: sparfloxacin, grepafloxacin, ciprofloxacin, moxifloxacin, trovafloxacin, levofloxacin, ofloxacin, gatifloxacin, imipenem, cefuroxime and azithromycin by MIC against 100 clinical isolates of *Acinetobacter* species (47 *A.baumannii*, *18 A.anitratus*, 18 *A.lwoffii*, 13 *A. calcoaceticus*, 4 *Acinetobacter spp.*). Gemifloxacin ($MIC_{50/90}$ 0.06/16) was over eight-fold more potent than ciprofloxacin ($MIC_{50/90}$ 0.5/>128) and two-eight fold more potent than grepafloxacin, moxifloxacin, levofloxacin, ofloxacin and gatifloxacin, and within one dilution of trovafloxacin and sparfloxacin. Cross-resistance was seen only within the group of quinolones and did not extend to the non-quinolone antibiotics. The bactericidal activity of gemifloxacin and six comparator quinolones was investigated by dose-response and time-kill against *A. baumannii* ATCC 19606 at their optimum bactericidal concentration (OBC) and four-times MIC. At the OBC there was no significant difference between the quinolones but at four-times MIC gemifloxacin shows an advantage, reducing the viable count by almost 2 $\log_{10}$ in 30 minutes compared with a 1 $\log_{10}$ reduction seen with the other drugs. This enhanced

killing extended over 24 hours reducing cell numbers by over 4 $\log_{10}$. These data indicate that gemifloxacin can be of therapeutic value against *Acinetobacter* infections.

Bacteria from the genus *Acinetobacter* and, in particular *Acinetobacter baumannii*, are increasingly isolated from hospitalised patients where they have been responsible for bacteraemia, secondary meningitis, urinary tract infections and pneumonia especially in the immunocompromised (Towner, *et al.*, *Journal of Medical Microbiology*, 46: 721-746 (1997)). These organisms have become resistant to almost all the currently available antibiotics including aminoglycosides and the older fluoroquinolones and broad-spectrum β-lactams; they are now almost all resistant to cephalosporins and some are resistant to the carbapenems including imipenem (Paton, *et al.*, *International Journal of Antimicrobial Agents*, 2: 81-88 (1993); Lyytikainen, *et al.*, *Journal of Hospital Infection*, 31: 41-54 (1995)). The inability to control these bacteria has allowed them to colonise those niches left vacant when more susceptible microbes are eradicated. Resistance to ciprofloxacin has been found in *Acinetobacter* spp within the hospital environment (Villers, *et al.*, *Annals of Internal Medicine*, 129: 182-189 (1998); Rodriguez-Bano, *Reviews in Medical Microbiology*, 10: 67-77 (1999)) though it has been found to be more pronounced in areas where fluoroquinolone use is high, and in particular where there is a history of prior use (Muder, *et al.*, *Antimicrobial Agents and Chemotherapy*, 35: 256-258 (1991)). The relative delay in the emergence of fluoroquinolone resistance indicates that newer, more potent quinolones could prove extremely useful in the control of *Acinetobacter* spp. (Pieroni, *et al.*, *Journal of Antimicrobial Chemotherapy*, 39: 419-422 (1997); Pan, *et al.*, *Antimicrobial Agents and Chemotherapy*, 42: 2810-2816 (1998)). Gemifloxacin is a new generation fluoroquinolone that has demonstrated a good spectrum of activity against both Gram-positive and Gram-negative organisms (Cormican, *et al.*, *Antimicrobial Agents and Chemotherapy*, 41: 204-211 (1997); Kim, *et al.*, *Program and Abstracts of the Sixth International Symposium on New Quinolones*, p.170 (1998); Paek, *et al.*, *Program and Abstracts of the Sixth International Symposium onNew Quinolones*, Denver, p. 170 (1998); Kelly, *et al.*, *Program and Abstracts of the Sixth International Symposium on New Quinolones*, Denver, p. 170 (1998)). In this study the *in vitro* activity of gemifloxacin was compared with eight comparator fluoroquinolones as well as the carbapenem imipenem, the cephalosporin cefuroxime and the macrolide azithromycin against five representative species of *Acinetobacter* (*A. baumannii, A. calcoaceticus, A. lwoffii, Acinetobacter* spp (which could not be speciated) and *A. anitratus*). The clinical action of fluoroquinolones is bactericidal but,

unusually, they exhibit a biphasic dose response (Crumplin, *et al.*, *Antimicrobial Agents and Chemotherapy*, 8: 251-261 (1975)). This is measurable *in vitro* where it can be demonstrated that the lethality of the drug increases proportionally with its concentration until an optimum bactericidal concentration (OBC) is reached. Above this point, the bactericidal activity steadily decreases with further increases in concentration. The newer fluoroquinolones have been tested predominantly against Gram-positive species and the biphasic response has rarely been demonstrated (Lewin, *et al.*, *Journal of Antimicrobial Chemotherapy*, 30: 625-632 (1992); Morrissey, *et al.*, *Journal of Medical Microbiology*, 43: 4-8 (1995)). Perhaps for this reason, concentration-dependent dose response studies have not been performed on Gram-negative bacteria such as *Acinetobacter* spp. So this study also compared the *in vitro* fixed-time killing activity of increasing concentrations of gemifloxacin on *A. baumannii* ATCC 19606 against the comparator quinolones trovafloxacin, moxifloxacin, levofloxacin, ciprofloxacin, grepafloxacin and sparfloxacin and then to examine the time-dependent kill at both their respective OBCs and four-times their MIC.

The following antibacterial agents were used: gemifloxacin, ciprofloxacin, trovafloxacin, grepafloxacin, azithromycin, imipenem, sparfloxacin, cefuroxime, moxifloxacin, levofloxacin, ofloxacin and gatifloxacin. The standard laboratory strains *Escherichia coli* ATCC 10418, *Pseudomonas aeruginosa* ATCC 10662, *Staphylococcus aureus* ATCC 6571 and *Acinetobacter baumannii* ATCC 19606 were used as controls for the determination of MIC and the *A. baumannii* strain was used for both the dose-response and kill-curves. The 100 *Acinetobacters* (47 *A. baumannii*, 18 *A. anitratus*, 18 *A. lwoffii*, 13 *A. calcoaceticus*, 4 *Acinetobacter spp.)*, were bloodstream isolates from the University of Iowa College of Medicine.

The agar dilution method was used to determine the Minimum Inhibitory Concentrations (MIC) on Iso-sensitest agar (Oxoid) following the British Society for Antimicrobial Chemotherapy (British Society for Antimicrobial Chemotherapy, *Journal of Antimicrobial Chemotherapy*, 27 (Suppl. D) (1991)) (BSAC) guidelines for susceptibility testing.

To determine the OBC, nutrient broth (Nutrient Broth No. 2, Oxoid, UK) was inoculated with *A. baumannii* ATCC 19606 and incubated overnight at 37°C. Doubling dilutions of antibiotics ranging from 0.03-256 mg/l in nutrient broth were inoculated with 0.2ml of the overnight culture (final volume 10ml) and incubated for a further 3 hours at 37°C. The

viable count of the resulting cultures was determined by serial dilution and plating onto nutrient agar (Oxoid, UK) (Lewin, *et al.*, *Journal of Medical Microbiology*, 29: 139-144 (1989)). The results were plotted on a graph to determine the optimum bactericidal concentration.

The killing kinetics were performed at both the OBC and four-times the MIC. Briefly 5ml sterile double strength nutrient broth was dispensed into 20ml universals, antibiotic and sterile distilled water were added to give a final volume of 9.8mls and the universals were pre-incubated at 37°C for 15 minutes. A log-phase culture (0.2ml) was used to start the experiment and an initial count was determined by serial dilution and plating onto nutrient agar. Samples were taken every 30 minutes for three hours and these were serially diluted and plated onto nutrient agar. A final count was taken after 24 hours. All plates were incubated overnight in air at 37°C (Lewin, *et al.*, *Journal of Medical Microbiology*, 29: 139-144 (1989)).

The MIC range, MIC_{50} and MIC_{90} values of gemifloxacin and the comparator quinolones against the 5 species of *Acinetobacter* are shown in Table 62. Gemifloxacin was over eight-fold more potent than ciprofloxacin ($MIC_{50/90}$ 0.06/16 mg/l versus 0.5/>128 mg/l, respectively) and 2-8 fold more potent than grepafloxacin, gatifloxacin, moxifloxacin, levofloxacin, ofloxacin and grepafloxacin and within one dilution of the comparator quinolones sparfloxacin and trovafloxacin. These values were irrespective of the susceptibility of the organism to the non-quinolone antibiotics. Resistance among the *Acinetobacters* as a whole against cefuroxime and azithromycin was 99% & 94% respectively, with *A. lwoffii* and *A. spp* recording low MIC ranges, although still having an MIC_{50} and MIC_{90} above the breakpoints. None of the *Acinetobacters* had an MIC against imipenem above the breakpoint of 8mg/l (British Society for Antimicrobial Chemotherapy, *Journal of Antimicrobial Chemotherapy*, 27 (Suppl. D) (1991), and at the species level recorded MIC_{50}s of 0.12mg/l and MIC_{90}s of 0.25 or 0.5 mg/l, thus exhibiting 100% susceptibility.

Figure 27 illustrates the biphasic dose-response of *A. baumannii* ATCC 19606 against gemifloxacin. The lowest point of the graph corresponds to its OBC. The concentrations of antibiotics used in the kill-curve experiments are shown in Table 63. Figures 28-34 show the killing kinetics for the seven antibiotics at both the OBC and 4xMIC. At their OBCs there was no significant difference in bactericidal activity between gemifloxacin and the other quinolones, with regrowth prevented for 24 h and a reduction in viable cells of over 5 $\log_{10}$. However, gemifloxacin at four times the MIC (0.5μg/ml), a concentration that is achievable *in vivo*, shows an advantage over the other quinolones, reducing the viable count by almost 2 $\log_{10}$

after only 30 minutes and reducing the viable count by over 4 $\log_{10}$ after 24 h. In contrast the other quinolones only managed to reduce the viable cell count by 1 $\log_{10}$ in the first 30 minutes at four-times the MIC. Levofloxacin (Figure 31) reduced the viable count by 3 $\log_{10}$ in the first 30 minutes, however after this time-point the rate of kill decreases, killing only 0.5 $\log_{10}$ in the following 150 minutes. Within this time-period, gemifloxacin killed over 1.5 $\log_{10}$ (Figure 28). This enhanced killing shown by gemifloxacin was markedly better than that seen with the Gram negative quinolones ciprofloxacin, trovafloxacin and sparfloxacin (Figures 29, 32, and 34).

In this study, gemifloxacin inhibited 72% of the isolates if the breakpoint of 0.5μg/ml set by Jevons, *et al.* (Jevons, *et al.*, *Programme and Abstracts of the 21st International Congress of Chemotherapy*, Birmingham, p. 141 (1999)) was used. The rank order of inhibitory activity of the quinolones against *A. baumannii* was similar to those reported by Cormican & Jones (Cormican, *et al.*, *Antimicrobial Agents and Chemotherapy*, 41: 204-211 (1997)) with trovafloxacin > gemifloxacin > levofloxacin > ciprofloxacin = ofloxacin, except that they report sparfloxacin as having equal potency to gemifloxacin but in this study sparfloxacin was one dilution more potent than gemifloxacin. MIC_{50} & MIC_{90} values for gemifloxacin, trovafloxacin, grepafloxacin, sparfloxacin, ciprofloxacin and ofloxacin against *A. calcoaceticus* shown here are in agreement with Paek *et al.*[11] but are slightly different from those recorded by Oh (Oh, *et al.*, *Antimicrobial Agents and Chemotherapy*, 40: 1564-1568 (1996)) who report MIC_{90}s that are one dilution higher. Pascual (Pascual, *et al.*, *Journal of Antimicrobial Chemotherapy*, 40: 140-142 (1997)) records ciprofloxacin, trovafloxacin and sparfloxacin resistance levels of 93.3%, 56.7% & 66.7% respectively (breakpoints: ciprofloxacin ≥2mg/l, sparfloxacin & trovafloxacin ≥4mg/l) against multidrug resistant *A. baumannii* compared to this study which records 34% for ciprofloxacin, 27% for trovafloxacin and 25% for sparfloxacin, using the same breakpoints. As a group, the level of resistance exhibited by the *Acinetobacters* was 26% for ciprofloxacin, 19% for trovafloxacin and 23% for sparfloxacin. Gemifloxacin showed good activity against *A. lwoffii* and *A. anitratus*, with MIC_{50}s eight-fold below the breakpoint. Against *A. lwoffii* gemifloxacin was more potent than sparfloxacin (MIC_{90}:1 & 4 mg/l respectively). The small sample of *A.spp* were sensitive to all the antibiotics except for cefuroxime, with sparfloxacin the most potent quinolone and all four isolates recording an MIC of 0.008 mg/l. A decrease in sensitivity to one quinolone was mirrored by a decrease to them all. This however was not mirrored by a similar decrease towards the non-quinolones, as all the strains were sensitive to imipenem and most were resistant to cefuroxime

and azithromycin. Previous studies by Fass (Fass, *et al.*, *Antimicrobial Agents and Chemotherapy*, 37: 2080-2086 (1993)) with *Acinetobacter* and azithromycin have shown that a concentration of 4 mg/l is sufficient to inhibit most isolates of this species. The MIC_{50}s for azithromycin in this study were below 4mg/l except for *A. baumannii* which recorded an MIC_{50} of 4mg/l. However this is still above the breakpoint set by the BSAC (British Society for Antimicrobial Chemotherapy, *Journal of Antimicrobial Chemotherapy*, 27 (Suppl. D)(1991)) of 0.5mg/l therefore are recorded as being resistant. The high MIC_{50}s recorded by the *Acinetobacters* against cefuroxime (64 mg/l) is in agreement with a survey by Traub and Spohr (Traub, *et al.*, *Antimicrobial Agents and Chemotherapy*, 33: 1617-1619 (1989)) who recorded similarly high MICs against the cephalosporins, with little species difference. The 100% susceptibility to imipenem reported here is in agreement with Traub & Spohr (Traub, *et al.*, *Antimicrobial Agents and Chemotherapy*, 33: 1617-1619 (1989)) and Pieroni (Pieroni, *et al.*, *Journal of Antimicrobial Chemotherapy*, 39: 419-422 (1997)).

In the dose-response assays all the quinolones exhibited the typical biphasic dose response. The OBC's were found to be within one dilution of each other which indicates that the killing mechanism for each drug is similar even though their respective MIC's vary greatly. At the OBC, killing is at its optimum but this concentration is not necessarily found *in vivo,* therefore four-times the MIC is a better indication of how the drug can act *in vivo*. In this study at four-times the MIC, gemifloxacin exhibits superior killing-kinetics compared to ciprofloxacin. Further to this, the gemifloxacin killing-kinetics at 4xMIC was almost equal to that of its OBC even though it is one-eighth the concentration (0.5 & 4 mg/l respectively). Only levofloxacin showed equal killing, although at a concentration four times that of gemifloxacin (2mg/l compared with 0.5 mg/l).

The results of this study demonstrating good *in vitro* bactericidal and bacteriostatic activity of gemifloxacin, indicate that this drug can be effective in the treatment of *Acinetobacter* infections.

Table 62. Agar dilution MICs for 5 species of *Acinetobacter*

Microorganism	Antimicrobial	MIC mg/l

Microorganism	Antimicrobial	MIC mg/l		
		Range	MIC_{50}	MIC_{90}
Acinetobacter baumannii (n = 47)	Gemifloxacin	0.015 - >128	0.06	16
	Ciprofloxacin	0.06 - >128	0.5	>128
	Levofloxacin	0.06 – 64	0.25	16
	Gatifloxacin	0.03 – 64	0.25	8
	Sparfloxacin	0.008 – 32	0.03	8
	Grepafloxacin	0.015 – 64	0.06	32
	Moxifloxacin	0.015 – 64	0.12	16
	Trovafloxacin	0.015 – 32	0.03	16
	Ofloxacin	0.012 – 128	0.5	32
	Imipenem	0.008 – 1	0.12	0.25
	Cefuroxime	8 – 128	64	128
	Azithromycin	0.12 – 128	4	32
Acinetobacter anitratus (n =18)	Gemifloxacin	0.03 – 64	0.06	32
	Ciprofloxacin	0.12 – 128	0.5	128
	Levofloxacin	0.12 – 16	0.25	8
	Gatifloxacin	0.03 – 32	0.12	16
	Sparfloxacin	0.015 – 16	0.03	16
	Grepafloxacin	0.015 – 64	0.06	32
	Moxifloxacin	0.06 – 32	0.12	32
	Trovafloxacin	0.015 – 16	0.03	8
	Ofloxacin	0.25 – 16	0.5	16
	Imipenem	0.008 – 2	0.12	0.25
	Cefuroxime	32 – 256	64	256
	Azithromycin	1.0 – 64	2	64
Acinetobacter calcoaceticus (n = 13)	Gemifloxacin	0.015 – 64	0.06	0.12
	Ciprofloxacin	0.06 - >128	0.5	1
	Levofloxacin	0.06 – 64	0.25	0.5
	Gatifloxacin	0.03 – 128	0.12	0.25
	Sparfloxacin	0.008 – 32	0.03	0.06
	Grepafloxacin	0.015 – 32	0.06	0.12
	Moxifloxacin	0.03 – 64	0.12	0.12
	Trovafloxacin	0.008 – 32	0.03	0.06
	Ofloxacin	0.12 - >128	0.5	1
	Imipenem	0.008 – 1	0.12	0.25
	Cefuroxime	16 – 128	64	128
	Azithromycin	0.5 – 64	2	4

Microorganism	Antimicrobial	MIC mg/l		
Acinetobacter lwoffii (n = 18)	Gemifloxacin	0.015 – 32	0.06	1
	Ciprofloxacin	0.015 - >128	0.25	8
	Levofloxacin	0.06 – 16	0.25	4
	Gatifloxacin	0.03 – 8	0.12	4
	Sparfloxacin	0.008 – 32	0.06	4
	Grepafloxacin	0.015 – 64	0.06	2
	Moxifloxacin	0.015 – 16	0.06	2
	Trovafloxacin	0.008 – 8	0.03	0.5
	Ofloxacin	0.12 – 32	0.5	8
	Imipenem	0.008 – 2	0.12	0.5
	Cefuroxime	0.25 – 128	64	128
	Azithromycin	0.12 – 32	1	32
Acinetobacter spp (n = 4)	Gemifloxacin	0.008 - 0.03		
	Ciprofloxacin	0.03 - 0.06		
	Levofloxacin	0.008 - 0.12		
	Gatifloxacin	0.03 - 0.06		
	Sparfloxacin	0.008 - 0.008		
	Grepafloxacin	0.015 - 0.03		
	Moxifloxacin	0.008 - 0.06		
	Trovafloxacin	0.008 - 0.015		
	Ofloxacin	0.03 - 0.25		
	Imipenem	0.008 - 0.12		
	Cefuroxime	2 - 8.0		
	Azithromycin	0.12 - 0.5		

Table 63. MIC and OBC values for the antibiotics used against *A. baumannii* ATCC 19606.

Drug	OBC (mg/l)	MIC (mg/l)	4xMIC (mg/l)
Gemifloxacin	4	0.125	0.5
Trovafloxacin	4	0.06	0.25
Moxifloxacin	4	0.25	1

Drug	OBC (mg/l)	MIC (mg/l)	4xMIC (mg/l)
Levofloxacin	4	0.5	2
Ciprofloxacin	4	0.5	2
Grepafloxacin	8	0.125	0.5
Sparfloxacin	8	0.125	0.5

The invention provides a method for modulating metabolism of *Acinetobacter* spp. pathogenic bacteria. Skilled artisans can readily choose *Acinetobacter* spp. pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention can be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Acinetobacter* spp. pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Acinetobacter* spp. pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Acinetobacter* spp. pathogenic bacteria is selected from the group consisting of: *Acinetobacter baumannii, Acinetobacter calcoaceticus, Acinetobacter lwoffii,* and *Acinetobacter anitratus.* Other *Acinetobacter* spp. pathogenic bacteria can also be included in the methods. The skilled artisan can identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Chlamydia pneumoniae.*

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Chlamydia pneumoniae* pathogens. An objective of these analyses was to determine the in vitro activity of gemifloxacin and newer fluoroquinolones against *Chlamydia pneumoniae* by minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).

Gemifloxacin was compared to Erythromycin,Doxycycline, Azithromycin, Clarithromycin, Ciprofloxacin, Ofloxacin, Levofloxacin, Sparfloxacin, Grepafloxacin, Trovafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin and Sulfamethoxazole.

Quinolones are currently being used as empiric therapy for treatment of community-acquired pneumonia and other respiratory infection as they cover a broad range of conventional bacterial and "atypical" pathogens, including *Chlamydia pneumoniae*. *C. pneumoniae* has been associated with 10% to 20% of community-acquired pneumonia in adults and recently has been implicated as being associated with several nonrespiratory conditions, including atherosclerosis. The newer "third generation" quinolones have enhanced activities against Gram-positive bacteria, including *Streptococcus pneumoniae*, and prolonged serum half-lives, permitting once a day dosing. Although gemifloxacin and the other new quinolones have good activity against *C. pneumoniae* in vitro, practically all published treatment studies have relied on serologic diagnosis, thus microbiologic efficacy has not been assessed. Anecdotal experience indicates that in vitro activity can not always correlate with efficacy in vivo.

Chlamydia pneumoniae, was first described as a respiratory tract pathogen by Grayston and colleagues in 1986 (Grayston, *et al.*, *J. Infect. Dis.*, 161: 618-625 (1990)). Mode of transmission remains uncertain but can be via infected respiratory secretions. The proportion of community-acquired pneumonias associated with *C. pneumoniae* infection has ranged from 6-22%, varying with geographic location, age group examined and diagnostic methods used (Hammerschlag, *Infect. Dis. Clin. Pract.*, 8: 232-240 (1999)). Most of these studies were based on serology alone. *C. pneumoniae* has also been associated with other respiratory infections including acute exacerbations of chronic bronchitis, otitis media, sinusitis and reactive airway disease (Grayston, et al., J. Infect. Dis., 168: 1231-1235 (1993); Emre, *et al.*, *Arch. Pediatr. Adolesc. Med.*, 148: 727-732 (1994); Block, *et al.*, *Pediatr. Infect. Dis. Journal*, 16: 855-862 (1997)). Persistent nasopharyngeal infection with *C. pneumoniae* following acute respiratory infection has been documented in adults for periods of up to several years (Hammerschlag, *et al.*, *Clin. Infec. Dis.*, 14: 178-182 (1992); Dean, *et al.*, *Proceedings of the Ninth International Symposium on Human Chlamydial Infection*, San Francisco: USCF, pp. 219-223 (1998)). Chronic infection with *C. pneumoniae* has also been implicated in the pathogenesis of atheroscelrosis (Weiss, *et al.*, *Bull. Instr. Pasteur*, 95: 107-113 (1997)). The lack of standardized serological and nonculture methods for the detection of the organism has had a significant impact on efforts to study treatment of *C. pneumoniae* infections. Most treatment

studies have relied entirely on serology for diagnosis, thus microbiological efficacy has not been assessed.

C. pneumoniae is susceptible in vitro to agents that affect protein or DNA synthesis; macrolides, tetracyclines and fluoroquinolones (Table 64) (Hammerschlag, *Antimicrob. Agents Chemother.*, 38: 1873-1878 (1994)). Extrapolating from studies with *C. trachomatis,* DNA gyrase is probably the primary target for action of quinolones against *C. pneumoniae* (Dessus-Babus, *et al., Antimicrob. Agents Chemother.*, 42: 2474-2481 (1998)). The older quinolones were less active in vitro than macrolides and tetracyclines with MICs and MBCs ranging from 1 to $\geq$ 16 micrograms/ml (9). The newer quinolone agents, including levofloxacin, trovafloxacin and moxifloxacin are slightly more active than ofloxacin, with MICs 0.25 - 1 g/ml (Hammerschlag, Antimicrob. Agents Chemother., 38: 1873-1878 (1994); Hammerschlag, *Antimicrob. Agents Chemother.*, 36: 682-683 (1992); Roblin, *et al., Antimicrob. Agents Chemother.*, 41: 2033-2034 (1998); Roblin, *et al., Antimicrob. Agents Chemother.*, 42: 951-952 (1998); Hammerschlag, *Antimicrob. Agents Chemother.*, 36: 1573-1574 (1992); Woodcock, *et al., Antimicrob. Agents Chemother.*, 41: 101-106 (1997); Ridgeway, *et al., J. Antimicrob. Chemother.*, 14: 471-477 (1997); Miyashita, *et al., Antimicrob. Agents Chemother.*, 41: 471-477 (1997); Kimura, *et al., Antimicrob. Agents Chemother.*, 37: 801-803 (1993)). Sparfloxacin, grepafloxacin, gatifloxacin and gemifloxacin are the most active compounds with MICs 0.03 - 0.5 micrograms/ml (Hammerschlag, *Antimicrob. Agents Chemother.*, 38: 1873-1878 (1994); Miyashita, *et al., Antimicrob. Agents Chemother.*, 41: 1331-1334 (1997); Wise, *et al., Antimicrob. Agents Chemother.*, 37: 497-504 (1993); Roblin, *et al., Antimicrob. Agents Chemother.*, 38: 1402-1403 (1994); Roblin, *et al., Int. J. Antimicrob Agents*, 12: 181-184 (1999); Donati, *et al., J. Antimicrob. Chemother.*, 43: 825-827 (1999); Roblin, *et al., J. Antimicrob Chemother.*, in press (1999); Ridgeway, *et al., 38th ICAAC*, Abstract F-097, (1998); Roblin, *et al., Antimicrob. Agents Chemother.*, in press (1999)). Within an individual study the susceptibility of different isolates of *C. pneumoniae* to a particular quinolones is very uniform, usually differing by only 1 to 2 dilutions. However, the susceptibilities reported can vary as much as 10 -fold from study to study. The methods used for in vitro susceptibility testing of *C. pneumoniae* have largely been adapted from those used for *C. trachomatis*. The methods are not standardized and the results can be influenced by a number of variables including the tissue culture system, inoculum size, time of addition of antibiotic, the number and which isolates are used for testing. Many in vitro studies reported in

the literature have used less than 5 isolates of *C. pneumoniae*, many have used only one, frequently TW 183, IOL 207 or laboratory isolates that have been passaged extensively. There are a limited number of studies that have tested large numbers of clinical isolates. Table 65 summarizes the results of individual studies of the activity of the newer fluoroquinolones.

Data are limited on the activity of gemifloxacin against *C. pneumoniae*. The two studies available illustrate the problem described above. Ridgway (Ridgeway, *et al.*, *38th ICAAC*, Abstract F-097 (1998)) tested five isolates of *C. pneumoniae* against gemifloxacin; TW 183, IOL 207, 2043(ATCC VR 1355), TW27.9 and CWL-029 (ATCC VR 1310). The MIC range was 0.06 - 0.12 micrograms/ml. Subsequently, Roblin (Roblin, *et al.*, *Antimicrob. Agents Chemother.*, in press (1999)) tested 20 isolates of *C. pneumoniae*, including 15 recent clinical isolates from patients enrolled in a US multicenter pneumonia treatment study against gemifloxacin and other antibiotics. The MIC_{90} and MBC_{90} of gemifloxacin was 0.[illegible]g/ml, which was 2 to 4-fold less active than the values reported by Ridgeway (Ridgeway, *et al.*, *38th ICAAC*, Abstract F-097 (1998)). This discrepancy can be within the standard error of the test, secondary to the methods used or related to variation in susceptibilities of different isolates. Ridgway et al used McCoy cells, which are 10 to 100-fold less susceptible to *C. pneumoniae* infection than HEp-2 cells, potentially leading to lower endpoints. Both studies tested TW 183, but as shown in Table 65, there was no overlap in the MICs of this isolate to gemifloxacin

Although the newer "third generation" quinolones are 2 to 10-fold more active than the older agents, they are still less active in vitro than macrolides and tetracyclines. Can one extrapolate from the results of in vitro susceptibility testing to efficacy of these new quinolones for treatment of infection in vivo? Although clarithromycin is 10-100-fold more active than erythromycin in vitro, it was not more effective than erythromycin in eradicating *C. pneumoniae* from the nasopharynx of children with community-acquired pneumonia (Block, *et al.*, *Pediatr. Infect. Dis. J.*, 14: 471-477 (1995)). The eradication rate was 79% for clarithromycin and 86% for erythromycin. Considering the enhanced pharmacokinetics and tissue penetration of the newer quinolones, one would expect these agents to be as effective as macrolides in treatment of *C. pneumoniae* infection. However, practically every study evaluating quinolones for treatment of *C. pneumoniae* infection published to date have used serology alone for diagnosis, which is essentially a clinical endpoint.

In 1990, Lipsky et al. (Lipsky, *et al.*, *Am. J. Med.*, 89: 722-724 (1990)) described four patients with bronchitis and pneumonia, treated with a 10-day course of ofloxacin, who were

retrospectively identified as having serologic evidence of acute *C. pneumoniae* infection (4-fold rise in IgG/IgM, single IgM ≥ 16 or IgG ≥ 512). All reportedly demonstrated marked clinical improvement. Based on the MICs of 3 laboratory strains to ofloxacin (1-2 □g/ml), the authors concluded that ofloxacin was effective in these patients as the MICs were less than the achievable serum levels. Plouffe et al (Plouffe, *et al.*, *Antimicrob. Agents Chemother.*, 40: 1175-1179 (1996)) found a clinical response rate of 83% in those patients with community-acquired pneumonia with serologic evidence of *C. pneumoniae* infection, who were treated with ofloxacin compared to 75% of those who received standard therapy (a beta-lactam antibiotic plus a macrolide or tetracycline). Similarly, File et al (File, *et al.*, *Antimicrob. Agents Chemother.*, 41: 1965-1972 (1997)) reported a clinical cure rate of 98% among patients who were treated with levofloxacin compared to 93% of those treated with ceftriaxone and/or cefuroxime axetil. In addition, either erythromycin or doxycycline could be added at the discretion of the investigator, and the response rate of those with serologic evidence of *C. pneumoniae* infection did not differ between those patients who had erythromycin or doxycycline added to their treatment regimen. There was also no difference in the response rate among those patients who had definite infection, i.e. a 4-fold rise in MIF IgG or IgM, compared to those who had probable infection, i.e. a single IgG ≥ 512 or IgM ≥ 32.

Several recent studies have taken this one step further, indicating that if a patient who had serologic evidence of infection improved clinically, the organism was presumed to be eradicated even though cultures were not done (Leophonte, *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.*, 17: 434-440 (1998); Deabate, *et al.*, *Chest*, 114: 120-130 (1998)). One study compared trovafloxacin to amoxicillin for the treatment of acute exacerbations of chronic bronchitis. The "eradication" rate in those patients with serologic evidence of *C. pneumoniae* who were treated with amoxicillin was also 100%. The apparent success of regimens using antibiotics which have poor or no activity against *C. pneumoniae* in vitro, such as amoxicillin and cephalosporins, raises a number of questions about the specificity of the serologic criteria used to diagnose *C. pneumoniae* infection.

There is one anecdotal report of treatment with a quinolone where cultures were done. Roblin et al (Roblin, *et al.*, *Antimicrob. Agents Chemother.*, 38: 1402-1403 (1994)) treated three patients with culture documented *C. pneumoniae* infection (bronchitis and pneumonia) with grepafloxacin, two of three patients remained culture-positive and symptomatic despite 2 weeks of treatment with the drug. Two of these culture positive patients did not meet the

serologic criteria for acute *C. pneumoniae* infection. Preliminary results of two multicenter pneumonia treatment studies have found that levofloxacin and moxifloxacin had 70-80% efficacy in eradication of *C. pneumoniae* from the nasopharynx, which was very similar to previously reported experience with erythromycin, clarithromycin and azithromycin (Block, *et al., Pediat. Infect. Dis. J.*, 14: 471-477 (1995); Roblin, *et al., Antimicrob. Agents Chemother.*, 42: 194-196 (1998)). The MICs and MBCs of isolates of *C. pneumoniae* obtained from patients after therapy did not change from those of the isolates obtained pretreatment, indicating that persistence was not due to the development of resistance. These studies illustrate several important issues dealing with the treatment of *C. pneumoniae* infections. In vitro activity does not always predict microbiologic efficacy. Although the development of antibiotic resistance has not as yet been described for *C. pneumoniae*, a four-fold increase in MICs to azithromycin was reported in isolates of *C. pneumoniae* obtained after therapy with the drug in two patients (Block, *et al., Pediat. Infect. Dis. J.*, 14: 471-477 (1995)). Dessus-Babus et al (Dessus-Babus, *et al., Antimicrob., Agents Chemother.*, 42: 2474-2481 (1998)) were able to induce resistance to ofloxacin and sparfloxacin in *C. trachomatis* after serial passage of the organism in subinhibitory concentrations of the drugs. Unless cultures are done and microbiologic efficacy is assessed, they can never be able to be surveyed for, or document the emergence of resistance. The use of quinolones for empiric treatment of community acquired pneumonia is becoming a popular option as these drugs have activity against conventional and "atypical" pathogens (Bartlett, *et al., Clin. Infec. Dis.*, 26: 811-838 (1998)). These issues need to be taken into account when evaluating the efficacy of quinolones for the treatment of *C. pneumoniae* infection.

Table 64

Comparative in vitro activities of various antibiotics against *C. pneumoniae*

Drug	MIC Range (□g/ml)
Erythromycin	0.008-0.25
Doxycycline	0.016-0.25

Azithromycin	0.05-0.25
Clarithromycin	0.004-0.03
Ciprofloxacin	1-4
Ofloxacin	0.5-2
Levofloxacin	0.25-1
Sparfloxacin	0.031-0.125
Grepafloxacin	0.125-0.5
Trovafloxacin	0.5-1
Moxifloxacin	0.125-1
Gatifloxacin	0.125-0.25
Gemifloxacin	0.06-0.25
Sulfamethoxazole	>500

Table 65 In vitro Activity of Gemifloxacin and Newer Fluoroquinolones Against *C. pneumoniae*: Comparison of Published Studies

			MIC*(ug/ml)			MBC(ug/ml)	
Drug	(Reference)	No. of isolates tested	Range	50%	90%	Range	90%
Gemifloxacin	(24)	5	0.06-0.125	-	-	-	-
	(25)	20	0.125-0.25	0.25	0.25	0.125-0.25	0.25
Levofloxacin	(14)	11	0.25-0.5	0.5	0.5	0.25-0.5	0.5
	(17)	25	0.25-1	0.25	0.5	0.25-1	0.5
	(21)	14	0.5-1	0.5	1.0	0.5-1	1.0
Sparfloxacin	(11)	12	0.06-0.5	0.25	0.25	0.06-0.25	0.25

	(17)	25	0.03-0.25	0.06	0.06	0.03-0.125	0.06
Grepafloxacin	(18)	1	0.06	-	-	0.03	-
	(19)	1	0.06	-	-	0.03	-
	(21)	12	0.25-0.5	0.25	0.5	0.25-0.5	0.5
	(16)	5	0.06-0.125	-	-	0.06-0.25	-
	(21)	14	0.03-0.5	0.125	0.5	0.03-0.5	0.5
Trovafloxacin	(12)	12	0.5-1	1.0	1.0	0.5-1	1.0
Gatifloxacin	(18)	25	0.06-0.125	0.06	0.125	0.06-0.125	0.125
	(23)	20	0.125-0.25	0.25	0.25	0.125-0.25	0.25
Moxifloxacin	(15)	1	0.06	-	-	0.06	-
	(13)	10	0.5-1	1.0	1.0	1.0	1.0
	(22)	3	0.06-0.125	-	-	0.06-0.125	-
	(21)	14	0.125-1	0.5	0.5	0.125-1	0.5

[a] MIC (or MBC) for 50% and 90% of strains, respectively.

The invention provides a method for modulating metabolism of *Chlamydia pneumoniae* pathogenic bacteria. Skilled artisans can readily choose *Chlamydia pneumoniae* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention can be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Chlamydia pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Chlamydia pneumoniae* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Chlamydia*

pneumoniae pathogenic bacteria is selected from the group consisting of: *Chlamydia pneumoniae*. Other pathogenic bacteria can also be included in the methods. The skilled artisan can identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against streptococci.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various streptococci pathogens. An objective of these analyses was to determine the in vitro activity of gemifloxacin (SB-265805) against 50 clinical isolates each of *Streptococcus pyogenes, Streptococcus agalactiae* and viridans streptococci by the microdilution method and compared the activity with that of nalidixic acid, ofloxacin, ciprofloxacin, levofloxacin, trovafloxacin and grepafloxacin, penicillin, ampicillin, gentamicin and trimethoprim / sulfamethoxazole.

Gemifloxacin was significantly more active than the other quinolones tested. Gemifloxacin was as active as penicillin against *S. agalactiae* and more active than penicillin against viridans streptococci. Against *S. pyogenes* the MIC50 was equal to that of penicillin, the MIC90 value was one dilution higher. Gemifloxacin was also active against isolates of *S. agalactiae* and *S. pyogenes* with reduced susceptibility for ofloxacin and grepafloxacin.

Microbial antibiotic resistance is an ever-increasing clinical problem, presently particularly important among Gram-positive organisms.

Whereas in the seventies 70% of bacteraemias in neutropenic patients were caused by Gram-negative organisms, in the eighties viridans streptococci became a major cause of bacteraemia in these patients (Oppenheim, *et al., Journal of Antimicrobial Chemotherapy*, 41 (Suppl. D): 7-11 (1998)); during the nineties these became increasingly penicillin-resistant (Cormican, *et al., Drugs*, 51 (Suppl. 1): 6-12 (1996)).

From the results of a large scale surveillance program, covering the USA, Canada and Latin America published by Pfaller et al. (Phaller, *et al., Diagn. Microbiology and Infectious Diseases*, 33: 283-297 (1999)) it appears that streptococcal isolates represent 9.8% of all bloodstream infections. Whereas *Streptococcus pneumoniae* was most frequently isolated (50.7%), β-hemolytic streptococci represented 32.8% and viridans streptococci 16.5% of the isolates.

The first fluoroquinolones developed had virtually no activity against gram positive

microbes. Gradually new derivatives were developed with improved activity against these organisms while retaining a broad-spectrum against gram negatives (Johnson, *et al.*, *Diagn. Microbiology and Infectious Disease*, 33: 85-91 (1999)). This is the case for clinafloxacin, grepafloxacin, moxifloxacin, sparfloxacin and trovafloxacin.

Gemifloxacin (SB-265805) is a novel fluoronaphthyridone (Kim, et al., 35th *ICAAC*, Abstract F204 (1995)) with wide spectrum of activity against both gram negative and gram positive aerobes, including *Staphylococcus aureus* and penicillin-resistant *S. pneumoniae* and enterococci (Oh, *et al.*, *Antimicrobial Agents and Chemother.*, 40: 1564-1568 (1996); Cormican, *et al.*, *Antimicrobial Agents and Chemother.*, 41: 204-211 (1997); Hohl, *et al.*, *Clinical Microbiology and Infection*, 4: 280-283 (1998)) and anaerobes (Mavco, *et al.*, *Journal of Antimicrobial Chemother.*, 40: 605-607 (1997)).

In the present study the activity of gemifloxacin, nalidixic acid, ofloxacin, ciprofloxacin, levofloxacin, trovafloxacin, grepafloxacin and trimethoprim / sulfamethoxazole were compared against 50 recent clinical isolates each of *S. pyogenes*, *S. agalactiae* and viridans streptococci.

Bacterial isolates were from the Microbiology Laboratory, University Hospital, Antwerp, both from hospitalized and outpatients: 50 isolates each of *S. agalactiae*, *S. pyogenes* and viridans streptococci. The organisms were kept frozen at -20°C until tested. For testing they were sub-cultured overnight on 5% horseblood agar. Suspensions were made in Mueller-Hinton broth at a density corresponding to McFarland 0.5. MIC values were determined by the microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) (*National Committee for Clinical Laboratory Standards*, Ninth Informational Supplement, Table 2H, Vol 19, No: 1 (1999)) , using microtiter trays (Sensititre®, AccuMed International Ltd., UK).

The following antibiotics were tested: gemifloxacin, trovafloxacin, grepafloxacin, levofloxacin, ofloxacin, ciprofloxacin, nalidixic acid, penicillin, ampicillin, gentamicin and trimethoprim/sulfamethoxazole. Control organisms *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* 29212 were included in each run. Results were accepted only when the results of the control strains were within the ranges recommended by the NCCLS (*National Committee for Clinical Laboratory Standards*, Ninth Informational Supplement, Table 2H, Vol 19, No: 1 (1999)).

The results in Table 66 illustrate the high activity of gemifloxacin for the aerobic gram positive organisms tested: 90% of the *S. agalactiae*, *S. pyogenes* and viridans streptococci isolates being inhibited by 0.06 μg/ml, 0.03 μg/ml and 0.06 μg/ml respectively. Compared with the other fluoroquinolones tested, gemifloxacin is significantly more active, with the exception of trovafloxacin against *S. pyogenes* where the difference is only a factor of 2. Against *S. agalactiae* and viridans streptococci the MIC90 of trovafloxacin is 4 fold higher than that of gemifloxacin. Four *S. agalactiae* isolates intermediately susceptible or resistant to ofloxacin (breakpoint ≤ 2 μg/ml) had low MIC values of 0.03 - 0.06 μg/ml for gemifloxacin. Three *S. pyogenes* isolates intermediately susceptible to ofloxacin, two of which were resistant to grepafloxacin (breakpoint ≤ 0.5 μg/ml) also had low MIC values of 0.03 - 0.06 μg/ml for gemifloxacin.

Gemifloxacin is as active against *S. agalactiae* as penicillin and 6 fold more active than penicillin against viridans streptococci. Only *S. pyogenes* is considerably more sensitive to penicillin compared with gemifloxacin, but this results from the exquisite sensitivity of *S. pyogenes* to penicillin.

The susceptibility results of the viridans streptococci for the quinolones other than gemifloxacin are in line with those of Pfaller and Jones (Pfaller, *et al.*, *Diagn. Microbiology and Infectious Diseases*, 29: 199-201 (1997)) and with those of Johnson et al. (Johnson, *et al.*, *Diagn. Microbiology and Infectious Disease*, 33: 85-91 (1999)), particularly for gemifloxacin against β-haemolytic streptococci. But the MIC results of Johnson et al. (Johnson, *et al.*, *Diagn. Microbiology and Infectious Disease*, 33: 85-91 (1999)) for penicillin against β-haemolytic streptococci are significantly higher (MIC90 = 0.25). Their β-haemolytic streptococci could have also contained species other than *S. pyogenes*. The MIC values for viridans streptococci are not significantly different in the two studies.

Carratala et al. (Carratala, *et al.*, *Clinical Infectious Diseases*, 20: 1169-1173 (1995)) in 1995 already called attention to the appearance of penicillin resistant viridans streptococci, confirmed by Doern et al. (Doern, *et al.*, *Antimicrobial Agents and Chemother.*, 40: 891-894 (1996)) and Tuhoy and Washington (Tuohy, *et al.*, *Diagn. Microbioligy and Infectious Diseases*, 29: 277-280 (1997)). Alcaide et al. (Alcaide, *et al.*, *Antimicrobial Agents and Chemotherapy*, 39: 2243-2247 (1995)) registered 33.6% penicillin resistance (MIC=0.25 - 8 μg/ml) among 410 isolates of viridans streptococci with some cross resistance with other β-lactams.

Gemifloxacin is very promising for clinical use, provided it has favorable pharmacokinetics and low toxicity. It has a high activity against gram positive (this study) and gram negative organisms (Oh, et al., Antimicrobial Agents and Chemotherapy, 40: 1564-1568 (1996); Cormican, *et al., Antimicrobial Agents and Chemotherapy*, 41: 204-211 (1997); Hohl, *et al., Clinical Microbiology and Infection*, 4: 280-283 (1998)) and anaerobes (*National Committee for Clinical Laboratory Standards*, Ninth Informational Supplement, Table 2H, Vol. 19, No.:1 (1999)). On the basis of preliminary observations in the present study it is also active against streptococci resistant against other quinolones.

Table 66 : *In vitro* antimicrobial activity of gemifloxacin compared with nine other antibiotics against *Streptococcus* spp.

Microorganism (No. of isolates)	Antimicrobial agent	Cumulative % inhibited at MIC			MIC (µg/ml)			% susceptible*
		0.5	1	2	50%	90%	range	
S. agalactiae (n=50)	Gemifloxacin	100	100	100	0.06	0.06	0.008 - 0.12	-
	Ofloxacin	0	24	92	2	2	1 - 8	92
	Ciprofloxacin	34	64	98	1	1	0.5 - 4	-
	Levofloxacin	38	100	100	1	1	0.25 - 1	100
	Trovafloxacin	100	100	100	0.25	0.25	0.06 - 0.5	100
	Grepafloxacin	98	100	100	0.25	0.5	0.12 - 1	98
	Penicillin	90	90	100	0.03	0.06	≤0.015 - 2	90
	Ampicillin	90	96	100	0.12	0.25	≤0.06 - 2	88
	Clarithromycin	80	82	90	0.03	2	≤0.015 - >16	80
	Azithromycin	78	78	78	≤0.06	>64	≤0.06 - >64	78
S. pyogenes (n=50)	Gemifloxacin	100	100	100	0.015	0.03	0.008 - 0.06	-
	Ofloxacin	4	72	94	1	2	0.5 - 4	94
	Ciprofloxacin	76	96	96	0.5	1	0.25 - 4	-
	Levofloxacin	90	96	100	0.5	0.5	0.25 - 2	100
	Trovafloxacin	98	100	100	0.12	0.12	0.03 - 1	100
	Grepafloxacin	94	96	100	0.25	0.5	0.06 - 2	94
	Penicillin	100	100	100	≤0.015	≤0.015	≤0.015	100
	Ampicillin	100	100	100	≤0.06	≤0.06	≤0.06	100
	Clarithromycin	94	94	94	0.03	0.03	≤0.015 - 16	94
	Azithromycin	94	94	94	≤0.06	0.12	≤0.06 - 64	94
Viridans group (n=50)	Gemifloxacin	98	98	100	0.03	0.06	0.004 - 2	-
	Ofloxacin	2	22	76	2	4	0.5 - 16	-
	Ciprofloxacin	16	52	76	1	4	0.25 - 16	-
	Levofloxacin	40	90	98	1	1	0.25 - 4	98

Microorganism (No. of isolates)	Antimicrobial agent	Cumulative % inhibited at MIC			MIC (μg/ml)			% susceptible*
		0.5	1	2	50%	90%	range	
	Trovafloxacin	98	98	100	0.12	0.25	≤0.015 - 2	98
	Grepafloxacin	98	98	98	0.12	0.5	0.06 - 4	98
	Penicillin	62	74	84	0.25	4	≤0.015 - >16	46
	Ampicillin	60	64	74	0.5	8	≤0.06 - >64	46
	Clarithromycin	48	58	76	1	>16	≤0.015 - >16	42
	Azithromycin	48	54	64	1	>64	≤0.06 - >64	48

* Percent susceptibility results relate to the NCCLS (1999) breakpoints for grepafloxacin (≤0.5μg/ml), trovafloxacin (≤1μg/ml), levofloxacin (≤2μg/ml), ofloxacin (≤2μg/ml for β hemolytic streptococci), clarithromycin (≤0.25μg/ml) azithromycin (≤0.5μg/ml), penicillin (≤0.12μg/ml) and ampicillin (≤0.25μg/ml).

The invention provides a method for modulating metabolism of streptococcal pathogenic bacteria. Skilled artisans can readily choose streptococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by streptococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with streptococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said streptococcal pathogenic bacteria is selected from the group consisting of: *Streptococcus pyogenes, Streptococcus agalactiae* and viridans streptococci. Other streptococcal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Bordetella* spp..

This invention was based, in part, on analyses evaluating the comparative activity of

gemifloxacin against various *Bordetella* spp. pathogens. An objective of these analyses was to determine MICs of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* to various antimicrobial agents, including gemifloxacin, a new quinolone antimicrobial agent.

Erythromycin is the mainstay of antibiotic therapy for pertussis, since it decreases contagiousness and ameliorates symptoms, especially in younger, more severely affected infants (Bass, *et al.*, *Ped. Infect. Dis.*, 5: 154-157 (1986)). Many *in vitro* studies have shown activity of other antibiotics to Bordetellae, specifically newer macrolides, ampicillin, trimethoprim-sulfamethoxazole, quinolones and third generation cephalosporins (Appleman, *et al.*, *Diagn. Microbiol. Infect., Dis.*, 8: 131-133 (1987); Bannatyne *et al.*, *Antimicrobial Agents Chemother.*, 21: 666-667 (1982); Hoppe, *et al.*, *Antimicrobl. Agents Chemother.*, 34: 2287-2288 (1990); Hoppe, *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.*, 8: 653-654 (1989); Hoppe, *et al.*, *Antimicrob. Agents Chemother.*, 42: 1868 (1998); Kurzinski, *et al. Antimicrob. Agents Chemother.*, 32: 137-140 (1988); Mortensen, *et al.*, *Antimicrob. Agents Chemother.*, 33: 771-772 (1989)). Antimicrobial susceptibility testing of clinical isolates of *B. pertussis* is not routinely performed due to the lack of standardization of laboratory methods, the slowly growing and fastidious nature of the organism, and until recently universal susceptibility to erythromycin. In 1995, Lewis et al (Lewis, *et al.*, *Ped. Infect. Dis. J.*, 14: 338-391 (1995)) described the first erythromycin resistant (minimum inhibitory concentrations [MIC] by E test >256 micrograms/ml) *B. pertussis* isolate in a 2 month old infant with clinical failure of erythromycin therapy. Thus alternative therapeutic agents are needed in case *Bordetella pertussis* resistance becomes common or for patients unable to tolerate conventional therapy. Although information exists about *in vitro* susceptibility data there is little *in vivo* efficacy data. Antibiotic efficacy of alternative agents in pertussis is predicted by *in vitro* susceptibility and achievable concentrations of these agents in respiratory secretions. Quinolones have been shown to achieve high concentrations in respiratory secretions (Gerding, *et al.*, *Rev. Infect. Dia.*, 11 (Suppl. 5): S1046-1047 (1989)).

The following bacterial isolates were tested :102 isolates of *B. pertussis,* including 99 clinical isolates from patients seen at St. Christopher's Hospital for Children in Philadelphia, PA collected between 1987 and 1997, strains #8467 and #9340 from the American Type Culture Collection (ATCC) and one erythromycin resistant strain originally from Yuma, AZ (Lewis, *et al.*, *Ped. Infect. Dis. J.*, 14: 338-391 (1995)) obtained from the Centers for Disease Control and Prevention; nine *B. parapertussis* isolates including seven clinical isolates from St.

Christopher's Hospital and ATCC strains #15311 and #15989; nine *B. bronchiseptica* isolates including 5 clinical isolates from St. Christopher's Hospital and ATCC strains, #785, #31124, #780, and #9395.

The assay conditions used were those proposed by Hoppe et al in 1988 and further developed by Mortensen et al. in 1998 (Hoppe, *et al., Antimicrob. Agents Chemother.*, 42: 1868 (1998); Mortensen, *et al., 38th ICAAC*, Abstract D-039 (1998)). In brief, the broth microdilution procedure was performed using Mueller-Hinton broth with 5% lysed horse blood. The inoculum used was a suspension of organisms equivalent to a final density of $5x10^5$ CFU/ml for all isolates and was confirmed with plate counts. The concentrations of the antimicrobials tested were as follows: ampicillin, azithromycin, erythromycin, ciprofloxacin, trimethoprim/sulfamethoxazole (SXT) (0.015 micrograms/ml to 32 micrograms/ml) and gemifloxacin (SB 265805) (0.008 micrograms/ml to 16 micrograms/ml). *S. pneumoniae* #49619 from ATCC was used for quality control. The microtiter plates were incubated in ambient air for 48 hours at 37°C. The MIC was defined as the lowest concentration of antimicrobial at which growth was inhibited.

Erythromycin, azithromycin, ciprofloxacin and gemifloxacin were consistently active against the *B. pertussis* isolates tested (Tables 67 and 68). The agents tested had variable activity against other species of *Bordetella* (Table 67). The azithromycin/erythromycin resistant isolate did not demonstrate cross resistance to any of the other agents tested: gemifloxacin, $\leq$ 0.008 micrograms/ml; ciprofloxacin, $\leq$ 0.015 micrograms/ml; ampicillin, 2.0 micrograms/ml; and SXT, 4.0 micrograms/ml.

Results of this *in vitro* testing of *B. pertussis* and *B. parapertussis* for ampicillin, azithromycin, erythromycin, ciprofloxacin and SXT were comparable to previous reports (Bannatyne, et al., Antimicrobial Agents Chemother., 21: 666-667 (1982); Hoppe, *et al., Antimicrobl. Agents Chemother.*, 34: 2287-2288 (1990); Hoppe, *et al., Eur. J. Clin. Microbiol. Infect. Dis.*, 8: 653-654 (1989); Hoppe, *et al., Antimicrob. Agents Chemother.*, 42: 1868 (1998); Kurzinski, *et al., Antimicrob. Agents Chomother.*, 32: 137-140 (1988)). *B. bronchiseptica* isolates were less susceptible in vitro than *B. pertussis* and *B. parapertussis.* MICs in the present study were higher than previously reported by Mortensen et al, (Mortensen. *et al., Antimicrob. Agents Chomther.*, 33: 771-772 (1989)) which could be indicative of the different methods used.

In vitro data for gemifloxacin has not been previously reported for *B. pertussis* and *B.*

parapertussis, but is comparable with the activity of other quinolones as reported in this study and others (Appleman, *et al.*, *Diagn. Microbiol. Infec. Dis.*, 8: 131-133 (1987); Hoppe, *et al.*, *Antimicrob. Agents Chemother.*, 34: 2287-2288 (1990); Hoppe, *et al.*, *Antimicrob. Agents Chemother.*, 42: 1868 (1998)). The potent *in vitro* activity of gemifloxacin for *B. pertussis* coupled with the high achievable concentrations of quinolones in respiratory secretions (Gerding, *et al.*, *Rev. Infect. Dia.*, 11 (Suppl. 5): S1046-1057 (1989)) can predict clinical efficacy and make this an attractive alternative agent for the treatment of pertussis.

Table 67 - The in vitro activity of various antimicrobial agents against isolates of *Bordetella* ssp.

	Number of isolates at each MIC (μg/ml)														
Organisms (n)	≤0.008	≤0.015	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	>32
***B. pertussis*(102)**															
Erythromycin		94		7											1*
Azithromycin		94		7											1*
Ciprofloxacin		88		9	5										
Gemifloxacin(97)**	80		4	10	3										
Ampicillin							4	15	78	5					
SXT***								1	1	8	71	10	10	1	
***B. parapertussis* (9)**															
Erythromycin						5		4							
Azithromycin					6	3									
Ciprofloxacin				4	4	1									
Gemifloxacin				2	6	1									
Ampicillin												2	4	3	
SXT***							1	2	6						
***B. bronchoseptica*(9)**															
Erythromycin												3	3	1	2
Azithromycin									1	2	2	1			3
Ciprofloxacin							2		3	4					
Gemifloxacin									6	3					

Ampicillin	3	3	3
SXT***	1	5	3

*Erythromycin resistant strain from Yuma, AZ
** Five strains of *B. pertussis* were not viable for testing against gemifloxacin
***SXT, trimethoprim/sulfamethoxazole (expressed as the MIC of trimethoprim)

Table 68 - MIC_{90} and Range (mg/ml) for 102 *B. pertussis* isolates

Antimicrobial	MIC_{50}*	MIC_{90}*	MIC range
Erythromycin	≤0.015	≤0.015	≤0.015 - >32
Azithromycin	≤0.015	≤0.015	≤0.015 - >32
Ciprofloxacin	≤0.015	0.03	≤0.015 - 0.06
Gemifloxacin**	≤0.008	0.03	≤0.008 - 0.06
Ampicillin	1.0	1.0	0.25 - 2.0
SXT***	4.0	4.0	0.5 - 32

*the MIC at which 50% and 90% of strains are inhibited, respectively.

** five strains of *B. pertussis* were not viable for testing against gemifloxacin
***SXT, trimethoprim/sulfamethoxazole (expressed as the MIC of trimethoprim)

The invention provides a method for modulating metabolism of *Bordetella* spp. pathogenic bacteria. Skilled artisans can readily choose *Bordetella* spp. pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Bordetella* spp. pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Bordetella* spp. pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Bordetella* spp. pathogenic bacteria is selected from the group consisting of: *Bordetella* spp.. Other *Bordetella* spp. pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Mycoplasma*

This invention was based, in part, on analyses evaluating the comparative activity of

gemifloxacin against various *Mycoplasma* pathogens. An objective of these analyses was to determine the *in vitro* activity of the quinolone gemifloxacin, in comparison to other new quinolones and macrolides was determined using low-passaged clinical isolates and type strains of *Mycoplasma* species commonly found in the respiratory and urogenital tract of humans. Organisms used in the analyses included *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma penetrans* and *Ureaplasma urealyticum*. Minimal inhibitory concentrations (MICs) were determined using a microbroth dilution method.

Comparator drugs included levofloxacin, trovafloxacin, grepafloxacin, azithromycin, clarithromycin, tetracycline and clindamycin. Analysis was performed on 200 Mycoplasma and 100 Ureaplasma isolates from different geographical regions. The overall range for gemifloxacin against different Mycoplasma species was ≤ 0.008 to 0.125 μg/ml. For Ureaplasma strains, the MIC range was ≤0.008 to 0.5 μg/ml. Depending on the species tested, gemifloxacin showed variable results when compared with the macrolides. However, gemifloxacin was as active as or more active than tetracycline, clindamycin and the other quinolones in the assays performed in this study.

Mycoplasma pneumoniae has long been recognized as a common pathogen, especially in younger age groups, causing as much as 20% of pneumonia in the general population and up to 50% in military settings (Cassell, *et al.*, *The Mycoplasma*, Academic Press, Inc., New York, NY, pp. 65-106 (1985)). Most respiratory infections due to *M. pneumoniae* are self-limiting, but in some instances life-threatening disease can occur (Cassell, *et al.*, *The Mycoplasma*, Academic Press, Inc., New York, NY, pp. 65-106 (1985)). Although naturally occurring strains of *M. pneumoniae* resistant to erythromycin have not been described to date, the effectiveness of this drug can be limited in some cases because of dose-related nausea, vomiting, or diarrhea justifying the need for alternative treatment modalities (Chow, *et al.*, *Antimicrobial Therapy*, Raven Press, New York, NY, pp. 209-221 (1988)). Knowledge of antibiotic susceptibilities of *M. hominis* and *U. urealyticum* is of increasing importance because of the recognition of these organisms as opportunistic pathogens in newborn infants (Cassell, *et al.*, *Pediatric Infect. Disease Journal*, 7: 535-541 (1988); Cassell, *et al.*, *Advanced in Experimental Medicine and Biology*, 24: 93-115 (1987); Cassell, *et al.*, *Lancet*, ii: 240-245 (1988); Waites, *et al.*, *Pediatrics*, 83: 84-89 (1989); Waites, *et al.*, *Lancet*, I: 17-21 (1988)) and other immunosuppressed persons Taylor-Robinson, *et al.*, *Pediatric Infectious Disease Journal*, 5

(Suppl.): 236-238 (1986)) and as primary pathogens in sexually transmitted diseases such as nongonococcal urethritis (Cassell, *et al.*, *Advances in Experimental Medicine and Biology*, 24: 93-115 (1987)) and pelvic inflammatory disease (Cassell, *et al.*, *Advances in Experimental Medicine and Biology*, 24: 93-115 (1987)). Resistance to traditional drugs such as tetracycline, which has been widely used in treating mycoplasmal infections and other sexually transmitted diseases is recognized in both *U. urealyticum* and *M. hominis* (Cummings, *et al.*, *Antimicrobial Agents and Chemother.*, 34: 2297-2299 (1990); Evans, *et al.*, *Journal of Antimicrobial Chemotherapy*, 4: 57-83 (1978); Roberts, *et al.*, *Pediatric Infectious Disease Journal*, 5 (Suppl): 338-S340 (1986); Taylor-Robinson, *et al.*, *Pediatric Infectious Disease Journal*, 5 (Suppl): 335-S340)).

Gemifloxacin has been shown to be highly active against the common respiratory tract pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* (Moore, *et al.*, *38th ICAAC*, Abstract F-098, p. 257 (1998); Kim, *et al.*, *38th ICAAC*, Abstract F-093, p. 256 (1998)) and the atypical organisms *Legionella pneumophila* (Critchley, *et al.*, *38th ICAAC*, Abstract F-100, p. 257 (1998); Dubois, *et al.*, *38th ICAAC*, Abstract F-105, p. 258 (1998)) and *Chlamydia pneumoniae* (Kim, *et al.*, *38th ICAAC*, Abstract F-093, p. 256 (1998); Ridgeway, *et al.*, *38th ICAAC*, Abstract F-097, p. 256 (1998)). Gemifloxacin also showed good antimicrobial activity against different *Mycoplasma* species when a limited number of samples were analysed (Hannan, *et al.*, *38th ICAAC*, Abstract F-101, p. 257 (1998)). To confirm the activity of gemifloxacin against different *Mycoplasma* species, the MIC was determined for a large number of clinical isolates and type strains. In this study, the *in vitro* effectiveness of gemifloxacin was compared with that of other new quinolones, macrolides, tetracycline and clindamycin.

Three hundred isolates of mycoplasmas, including low-passaged clinical isolates and type strains, were tested for antimicrobial susceptibility. Most of these isolates had been passaged only a few times in artificial media. *M. pneumoniae* isolates included 130 strains collected from six different countries (representing isolates collected over a 10-year period). All the isolates originated from the respiratory tract of individuals with proven respiratory disease. The isolates were identified by polymerase chain reaction as *M. pneumoniae* and were shown not to be cross-reactive with *M. genitalium* or any other *Mycoplasma* species known to occur in humans in either the respiratory or genital tract: ATCC, $n = 2$; Denmark, $n = 6$; Australia, $n = 5$; Japan, $n = 4$; England, $n = 9$; China, $n = 11$; and US, $n = 93$.

M. hominis isolates (n = 50) included seven type strains and 43 low-passaged clinical isolates. The origins of the clinical isolates are shown in Table 69. *M. fermentans* isolates (n = 18) included one type strain, two clinical isolates (from endometrial biopsy and urethra) and 15 well-characterised strains derived from cell culture, the respiratory tract, bone marrow or synovium *U. urealyticum* isolates (n = 100) included 14 serotypes and 86 clinical isolates (Table 70). *M. genitalium* strains consisted of two isolates and two isolates of *M. penetrans* were tested.

The following antimicrobials were used: gemifloxacin, trovafloxacin, levofloxacin, grepafloxacin, clarithromycin, azithromycin, tetracycline and clindamycin. Powders were dissolved according to the manufacturers' recommendations. Stock solutions of each drug containing 2048 µg/mL were prepared fresh for each assay.

A microbroth dilution method (Cassell, et al., Clinical and Pathogenic Microbiology, Mosby, St. Louis, MO, pp. 491-502 (1994)) was used to determine MICs. Serial two-fold dilutions of antimicrobials in mycoplasma media (10B for *U. urealyticum* and SP4 for all other *Mycoplasma* species) were carried out to give a range of concentrations from ≤ 0.008 to 256 µg/mL for each drug tested. An inoculum of 10^4–10^5 organisms, as measured by colour-changing units (CCU)/mL, was used. Plates were incubated aerobically at 37°C. All plates were examined daily until a colour change was detected in the drug-free (growth) control. Due to the rapid growth of *U. urealyticum*, the first reading occurred at 16 hours of incubation followed by multiple readings per day. The MIC for each drug was determined for all isolates. The initial MIC was defined as the lowest concentration of antimicrobial in which the metabolism of the organism was inhibited, as evidenced by lack of a colour change in the medium at the time the drug-free control first showed a colour change (time points varied for each species). Any mycoplasmal isolate in which resistance was suspected from the test was re-tested on another day to ensure reproducibility of results. Detailed descriptions of this method have been previously reported (Cassell, et al., Clinical and Pathogenic Microbiology, Mosby, St. Louis, MO, pp. 491-502 (1994)).

The microtitre susceptibility testing method employed allowed excellent reproducibility of MIC results within the simultaneous duplicate runs and between assays carried out on different days. Gemifloxacin demonstrated excellent *in vitro* activity against the species tested. The overall MIC range for both Mycoplasma and Ureaplasma strains tested was ≤0.008-0.5 µg/mL, which is well within the range of expected clinical susceptibility. This

range of concentrations of gemifloxacin inhibited all strains investigated. Comparative *in vitro* activities (MIC_{50}s and MIC_{90}s) of gemifloxacin and other antimicrobials against strains of Mycoplasmas and Ureaplasmas except for *M. genitalium* and *M. penetrans* are shown in Table 71. MIC results for isolates of *M. genitalium* and *M. penetrans* are given in Table 72.

For *M. pneumoniae* and *M. genitalium*, MICs for gemifloxacin were comparable to those of grepafloxacin (MICs 0.063-0.125 μg/mL) but they were two- to four-fold lower than those of the other quinolones and tetracycline. Azithromycin and clarithromycin showed the best activity towards these two species, inhibiting all strains at ≤0.008 μg/mL. *M. fermentans* and *M. hominis* had a high degree of susceptibility to gemifloxacin (MIC_{90} ≤0.008 μg/mL). For *M. hominis*, the activity of gemifloxacin was comparable to that of clindamycin and grepafloxacin (MIC_{90} ≤0.016 μg/mL) and was superior to that of tetracycline (3-12 dilutions lower) and the other quinolones (1-5 dilutions lower). Macrolides were not tested because of known resistance. *M. fermentans* was resistant to clarithromycin (MIC_{90} 64 μg/mL), but showed some susceptibility to azithromycin (MIC_{90} 2.0 μg/mL)

U. urealyticum was equally susceptible to gemifloxacin (MIC_{90} 0.250 μg/mL) and trovafloxacin (MIC_{90} 0.125 μg/mL), but the other quinolones were two- to four-fold less active. Gemifloxacin was also more effective against this microorganism than tetracycline (up to 6 dilutions lower) or azithromycin (3-4 dilutions lower). Tetracycline-resistant strains were as susceptible to gemifloxacin and trovafloxacin as tetracycline-susceptible strains. Clarithromycin had the highest activity against *U. urealyticum* at a concentration of ≤0.008μg/mL. Against *M. penetrans*, gemifloxacin (MICs ≤0.008μg/mL) was at least as active as the other compounds tested .

In vitro, gemifloxacin was as active as or more active than tetracycline, clindamycin and the other quinolones against the *Mycoplasma* and *Ureaplasma* species tested. Depending on the *Mycoplasma* species tested, gemifloxacin showed variable results when compared to the macrolides. Although over 300 different strains of six species collected over a 10 year period and from widely differing geographic areas were analysed, no resistance to gemifloxacin was observed.

Fluoroquinolones have proven to be useful in the treatment of mycoplasmal infections because they are potentially effective against pathogenic species including those strains resistant to other drugs, such as tetracycline. The results from this study and other recent

studies (Waites, *et al.*, *Pediatrics*, 83: 84-89 (1989)) indicate that gemifloxacin is a promising drug for the treatment of respiratory and urogenital tract infections caused by *Mycoplasma* and *Ureaplasma* species. In addition, gemifloxacin has been reported to be a highly active fluoroquinolone against a wide range of Gram-negative and Gram-positive pathogens, (Moore, et al., 38th ICAAC, Abstract F-098, p. 257 (1998); Kim, *et al.*, *38th ICAAC*, Abstract F-093 (1998); Critchley, *et al.*, *38th ICAAC*, Abstract F-100, p. 257 (1998); Dubois, *et al.*, *38th ICAAC*, Abstract F-105, p. 258 (1998); Ridgeway, *et al.*, *38th ICAAC*, Abstract F-097, p. 256 (1998)) making it an effective new drug in the treatment of infectious diseases.

Table 69. Origin of *M. hominis* clinical isolates investigated.

No. of isolates	Specimen cultured	Diagnosis
17	Endometrial Biopsy	Pelvic inflammatory disease
13	Urethra	Non-gonococcal urethritis
12	Endometrial Biopsy	Endometritis
1	Wound	unknown

Table 70. Origin of *U. urealyticum* clinical isolates investigated.

No. of isolates	Specimen cultured	Diagnosis
28	Endometrial Biopsy	Pelvic inflammatory disease
26	Urethra	Non-gonococcal urethritis
20	Endometrial Biopsy	Endometritis
12	Endotracheal aspirates	Infant respiratory distress syndrome

Table 71. *In vitro* activities of gemifloxacin and other antimicrobials against *Mycoplasma* species.

Microorganism	MIC (μg/mL)		
Antimicrobial Agent	Range	MIC_{50}	MIC_{90}
M. pneumoniae (n = 130)			
Gemifloxacin	≤0.008–0.125	0.063	0.125
Trovafloxacin	≤0.008–1.0	0.125	0.25
Grepafloxacin	0.063–0.25	0.063	0.125
Levofloxacin	0.031–8.0	0.5	0.5
Tetracycline	0.008–1.0	0.125	0.5
Clarithromycin	≤0.008	≤0.008	≤0.008
Azithromycin	≤0.008	≤0.008	≤0.008

M. fermentans (*n* = 18)			
Gemifloxacin	≤0.008	≤0.008	0.008
Trovafloxacin	≤0.008–0.031	≤0.008	0.016
Grepafloxacin	≤0.008–0.5	≤0.008	0.016
Levofloxacin	≤0.008–0.063	0.016	0.063
Tetracycline	≤0.008–0.125	0.031	0.063
Clarithromycin	≤0.008–128	16	64
Azithromycin	0.063–4.0	0.5	2.0
Clindamycin	0.016–0.125	0.016	0.031
M. hominis (*n* = 32)			
Gemifloxacin	≤0.008	≤0.008	≤0.008
Trovafloxacin	≤0.008–0.031	≤0.008	0.031
Grepafloxacin	≤0.008–0.031	≤0.008	0.016
Levofloxacin	≤0.008–0.25	0.125	0.25
Tetracycline	≤0.008–128	0.063	32
Clindamycin	≤0.008–0.016	≤0.008	0.016
U. urealyticum (*n* = 100)			
Gemifloxacin	≤0.008–0.5	0.125	0.250
Trovafloxacin	≤0.008–0.5	0.063	0.125
Grepafloxacin	0.031–2.0	0.25	1.0
Levofloxacin	0.125–2.0	0.5	1.0
Tetracycline	0.031–128	0.125	16
Clarithromycin	≤0.008–0.25	0.031	0.063
Azithromycin	0.25–4	1	4

Table 72. MIC results for isolates of *M. genitalium* and *M. penetrans*.

Isolates	MICs (μg/mL)							
	Gemiflox-acin	Trovaflo-xacin	Grepafl-oxacin	Levofl-oxacin	Tetrac-ycline	Clarithro-mycin	Azithro-mycin	Clindamy-cin
M. genitalium *(n = 2)*								
JB	0.063	0.063	0.125	1.0	0.125	≤0.008	≤0.008	0.25
G37	0.063	0.063	0.125	0.5	0.063	≤0.008	≤0.008	0.25
M. penetrans *(n = 2)*								
MP	≤0.008	≤0.008	0.016	0.031	0.125	≤0.008	≤0.008	≤0.008
MFDEB	≤0.008	≤0.008	0.5	0.016	0.016	32	0.125	0.031

The invention provides a method for modulating metabolism of *Mycoplasma* pathogenic bacteria. Skilled artisans can readily choose *Mycoplasma* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Mycoplasma* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Mycoplasma* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Mycoplasma* pathogenic bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma penetrans* and *Ureaplasma urealyticum*. Other *Mycoplasma* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Streptococcus pneumoniae*.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Streptococcus pneumoniae* pathogens. An objective of these analyses was to determine the factors contributing to quinolone resistance mechanisms in strains of *Streptococcus pneumoniae* with elevated MICs to ofloxacin.

A further study on which an aspect of this invention was based was performed to characterize the factors contributing to quinolone resistance mechanisms in strains of *Streptococcus pneumoniae* with elevated MICs to ofloxacin. These strains were also tested for their susceptibility to a battery of quinolone antimicrobials, including gemifloxacin.

A collection of *S. pneumoniae* isolates consisting of 27 ofloxacin-susceptible, 18 ofloxacin-intermediate and 53 ofloxacin-resistant strains (ofloxacin MIC <4, 4, >4 μg/ml, respectively) were used in this study. Generally, the ofloxacin susceptible strains had no amino acid substitutions in GyrA, GyrB, ParC or ParE. As the MIC to ofloxacin increased, substitutions appeared first in the quinolone resistance-determining region (QRDR) of ParC, and strains showing the highest MICs also had substitutions in the QRDR of GyrA.

The most common substitutions were ParC → S79F and GyrA → S81F. Additional substitutions were identified within the QRDR region of ParC and outside the QRDR regions of ParC and ParE that did not appear to impact on susceptibility.

The effects of antibiotic efflux pumps were studied by determining MICs against a range of quinolones in the presence and absence of reserpine, an inhibitor of Gram positive efflux pumps. This data indicates that high level resistance due entirely to efflux was seen in a minority of the ofloxacin-resistant *S. pneumoniae* strains.

Susceptibility testing of these quinolone-resistant strains to gemifloxacin, ciprofloxacin, grepafloxacin, norfloxacin, ofloxacin and trovafloxacin revealed that gemifloxacin was least affected by this large variety of resistance mechanisms and was the only quinolone showing MICs of ≤0.5 μg/ml against all strains.

These results indicate that gemifloxacin is highly potent against *S. pneumoniae* and can also be effective against strains resistant to other quinolones.

Resistance to currently available fluoroquinolone (FQ) antibacterials among *Streptococcus pneumoniae* is an increasing worldwide problem. In order to determine the factors contributing to FQ resistance in *Streptococcus pneumoniae,* 98 strains exhibiting a range of ofloxacin susceptibility were investigated.

The targets of FQ antibacterial activity are considered to be the type II topoisomerases, DNA gyrase and DNA topoisomerase IV, (Tankovic, *et al.*, *Antimicrob. Agents. Chemother.*, 40: 2505-2510 (1996); Xiao-Su, *et al.*, *Antimicrob. Agents Chemother.*, 40: 2321-2326 (1996)) which are responsible for topological transformations of DNA. In this study, the QRDR (quinolone resistance-determining region) in the *gyrA* and *gyrB* genes encoding the GyrA and GyrB subunits, respectively, of DNA gyrase, and the *parC* and *parE* genes encoding the ParC and ParE subunits, respectively, of topoisomerase IV were sequenced.

A major mechanism contributing to FQ resistance in certain organisms is drug efflux, and this mechanism of resistance has also been reported in *S. pneumoniae* (Baranova, *et al.*, *Antimicrob. Agents Chemother.*, 41: 1396-1398 (1997); Munoz, *et al.*, *Antimicrob. Agents Chemother.*, 40: 2252-2257 (1996); Zeller, *et al.*, *Antimicrob. Agents Chemother.*, 41: 1973-1987 (1997)). The role of efflux pumps in FQ resistance in these strains was studied by determining MICs against a range of quinolones in the presence and absence of reserpine, an inhibitor of Gram positive efflux pumps.

The following test agents were used in certain studies herein: gemifloxacin, trovafloxacin, ciprofloxacin, reserpine, norfloxacin, ofloxacin and erythromycin.

The *S. pneumoniae* isolates were obtained from the 1997 Alexander Project, the 1996 SENTRY and SPAR surveillance studies, the 1997 ALERT surveillance project and from the University of Iowa.

MICs were determined using NCCLS methodology for broth microdilution. All isolates were tested in Todd Hewitt broth supplemented with %5 Yeast extract (Becton Dickinson, Cockeysville, MD, USA). Reserpine at a concentration of 80 mg/ml was added to inocula to inhibit pmf efflux pump.

Bacterial DNA was isolated from 4 ml broth cultures of *S. pneumoniae*. Target genes were amplified by PCR. PCR products were prepared for automated DNA sequencing with an ABI 377 (USA) by cycle sequencing with BigDye terminator chemistry. The DNA sequences were assembled and edited with Sequencher V3.0 Gene Codes Corp.
Of the 98 isolates reported here, the 12 most susceptible had ofloxacin MICs of 0.125–0.5 μg/ml (Table 73). For the most part, these 12 isolates showed no amino acid changes within the QRDR regions of GyrA, GyrB, ParC or ParE, although two isolates did show a S114G substitution in GyrA and the double substitution S52G + N91D in ParC. These same substitutions were also found in three strains with MICs of 1–4 μg/ml, however, as they appear to have no effect on the MIC of sensitive isolates, they are assumed to be silent. In a similar fashion, a number of seemingly silent substitutions were found outside the QRDR region of ParE in strains of all susceptibilities. The most common of these is I460V, but H351L, H347L and C329S are also found, usually together. Ofloxacin is not affected by efflux to any great extent, but four of these 12 strains showed a reserpine-inhibitable efflux mechanism that generally increased the ofloxacin MIC just two-fold.

Only two of 15 strains with an ofloxacin MIC of 1–2 μg/ml had a substitution in ParC (one S79Y and one D83Y), and all had wild type GyrA, GyrB and ParE. This group was notable for the high incidence of efflux observed. Ofloxacin efflux was observed in about one third of the population overall, but in this MIC group 12 out of 15 strains showed an efflux mechanism which increased the ofloxacin MIC from 0.5 to 1 μg/ml.

No substitutions were found in GyrA, GyrB or ParE in the 26 strains with ofloxacin MICs of 4–8 μg/ml, but most showed a single amino acid substitution in the QRDR of ParC. Six had a S79F substitution, six had S79Y, four had a D83N substitution and four had no detectable substitutions. One strain had the double ParC subtitution of S52G + N91D mentioned above and assumed to have no impact on the MIC. One of the strains with wild type GyrA and ParC did have a D435N sustitution on ParE which can account for the elevated MIC (4 μg/ml) of this strain. For the other three strains with MICs of 4 μg/ml, no

reason for the increased MICs could be determined, and no detectable substitutions in any of the gyrase or topoisomerase proteins were found.

Of the 45 strains with ofloxacin MICs of 16 μg/ml or higher, all but two had single substitutions in both GyrA and ParC. The most common GyrA substitution was S81F, found in 32 isolates, but five strains carried a S81Y and six strains a E85K substitution. The most common ParC substitution was S79F (32 strains), but other substitutions seen were S79Y (nine strains), D83N (three strains) and R95C (one strain). The highest MICs were seen in the two strains showing GyrA and ParC substitutions of S81Y and S79F. Two isolates, with ofloxacin MICs of 32 and 16 μg/ml, showed no substitutions in GyrA and a single S79Y substitution on ParC. No significant efflux of ofloxacin was seen for either of these strains, although efflux of ciprofloxacin was higher for these isolates than for most others.

Generally speaking, amino acid substitutions within the QRDRs of ParC and GyrA cause similar increases in doubling dilutions of the MICs of gemifloxacin, trovafloxacin and ciprofloxacin as they have on ofloxacin. The major differences between antibiotics are the range of MICs and degree of efflux activity.

Ciprofloxacin (0.25–64 μg/ml) and ofloxacin (0.125–>64 μg/ml) displayed the highest MIC ranges, with trovafloxacin (0.008–16 μg/ml) somewhat lower and gemifloxacin (0.004–0.5 μg/ml) the only quinolone to maintain MICs at less than 1 μg/ml against all isolates.

Efflux of trovafloxacin and ofloxacin was low. Only 40% of isolates showed efflux and in most cases the result was a doubling of the MIC. Gemifloxacin was effluxed by 55% of all isolates (Table 74), with two thirds of this group showing a doubling of MIC and the remainder showing a 4- to 8-fold increase in MIC. Ciprofloxacin was effluxed by virtually all isolates (Table 75), with most isolates showing at least a 4-fold increase in MIC attributable to efflux. The four isolates showing the greatest ciprofloxacin efflux were, interestingly, the four strains with ofloxacin MICs of 4 μg/ml and no discernible target mutations. The rank order of susceptibility to efflux was ciprofloxacin = norfloxacin > gemifloxacin > ofloxacin = trovafloxacin = grepafloxacin.

In *S. pneumoniae,* the first step mutation resulting in elevated MIC occurs in ParC for most quinolones, although there is some evidence that GyrA is the primary target for a few quinolones, such as sparfloxacin and gatifloxacin (Heaton, *et al., 39th ICAAC,* Abstract 1394 (1999)). All of the clinical isolates analyzed here showed the first step mutation in the QRDR of ParC, with higher level resistance arising after a second mutation in the QRDR of

GyrA. This does not imply that the primary target for gemifloxacin is ParC. In fact, it is likely to be GyrA.[7] The most effective (and most common) substitutions appear to be S79F in ParC and S81Y in GyrA and the two most resistant isolates in this study carried both of these substitutions.

Virtually all isolates, including most of the highly susceptible ones, showed some evidence of efflux of ciprofloxacin. The majority (41%) showed a 2-fold increase in MIC, but 33% of isolates showed a 4-fold increase and 19% showed increases of 8-fold or greater. In only five isolates was the elevated MIC entirely attributable to efflux. All five of these isolates had an intermediate susceptibility to ofloxacin (MIC 4 μg/ml) with ciprofloxacin MICs ranging from 4–8 μg/ml.

Quinolone resistance in recent clinical isolates of *S. pneumoniae* is mediated primarily by amino acid substitutions in the QRDR regions of ParC and GyrA. Almost all isolates showed some evidence of active efflux of ciprofloxacin, but this was the primary cause of resistance in just five of the isolates examined. The MIC ranges for trovafloxacin, ciprofloxacin and ofloxacin were 0.008–16, 0.25–64 μg/ml and ofloxacin 0.125–>64 μg/ml, respectively. Gemifloxacin was the most potent quinolone tested against all isolates of *S. pneumoniae*, with a MIC range of 0.004–0.5 μg/ml.

Table 73. Number of Isolates Showing Various Target Substitutions by Ofloxacin MIC

Ofloxacin MIC	No. of isolate	Efflux*	Number of strains with:									
			ParC						GyrA			
			WT	S79F	S79Y	D83N	D83Y	R95C	WT	S81F	S81Y	E85K
≤0.5	12	4	12						12			
1	13	11	12		1				13			
2	2	1	1				1		2			
4	18	7	5	4	6	3			18			
8	8	1		2		1			3			
16	11	4		7	1	2		1	1	10		
32	30	9		23	6	1			1	22	3	4
64	3	2		1	2						1	2
>64	1	0		1							1	

*Virtually all strains showed a single dilution increase in MIC

Table 74. Number of Isolates Showing Gemifloxacin Efflux

Gemifloxacin MIC	No. of isolates	Fold increase in MIC

		0	2	4	8
≤0.008	17	3	8	3	
0.015	4	1	6	3	1
0.03	8	9	4	1	2
0.06	14		6	1	1
0.13	7	6	3	1	
0.25	7	14	11	4	
0.5	23	1	3		

Table 75. Number of Isolates Showing Ciprofloxacin Efflux

Ciprofloxacin MIC	No. of strains	Fold increase in MIC				
		0	2	4	8	>8
≤0.5	17		9	6	2	
1	4			3	1	
2	8		2	2	2	2
4	14	3	5	2	4	
8	7	1	3		1	2
16	7	1	2	3		1
32	23	1	16	5		1
64	12		1	9	2	
>64	1			1		

The invention provides a method for modulating metabolism of *Streptococcus pneumoniae* pathogenic bacteria. Skilled artisans can readily choose *Streptococcus pneumoniae* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention can be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Streptococcus pneumoniae* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said

pathogenic bacteria is selected from the group consisting of: *Streptococcus pneumoniae*. Other pathogenic bacteria can also be included in the methods. The skilled artisan can identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against *Streptococcus pneumoniae*.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various *Streptococcus pneumoniae* pathogens. An objective of these analyses was to determine the molecular basis of the enhanced anti-pneumococcal activity of gemifloxacin compared with other fluoroquinolones.

Gemifloxacin was compared to levofloxacin (LEV), ciprofloxacin (CIP), grepafloxacin (GRE), moxifloxacin (MOX) or trovafloxacin (TRO).

Gemifloxacin (SB-265805) is a new fluoroquinolone (FQ) with potent activity against *Streptococcus pneumoniae*. In this study, DNA gyrase (GYR) and topoisomerase IV (TOP) were purified from FQ-susceptible (FQ-S) *S. pneumoniae* C3LN4 and FQ-resistant (FQ-R) *S. pneumoniae* 502226 by a gene cloning and recombination method using *E. coli* as a host, as described previously (38th ICAAC, abst. C-176). The GYR supercoiling and TOP decatenating reactions were inhibited by gemifloxacin (GEM), levofloxacin (LEV), ciprofloxacin (CIP), grepafloxacin (GRE), moxifloxacin (MOX) or trovafloxacin (TRO). Percent inhibition from triplicate experiments was calculated and plotted against drug concentration. The 50% inhibitory concentration (IC_{50}) was deduced from these plots (see Table 76 below).

Table 76

S. pneumoniae		IC_{50} or MIC_{90} (μg/ml)					
Strain		GEM	GRE	TRO	MOX	LEV	CIP
C3LN4	MIC_{90}	0.06	0.5	0.06	0.12	1.0	2.0
(FQ-S)	TOP IC_{50}	1.4	3.5	3.7	3.9	4.0	6.4
	GYR IC_{50}	47.5	42.8	44.0	44.0	49.1	59.2
502226	MIC_{90}	0.5	16.0	2.0	2.0	16.0	>16.0

(FQ-R)	TOP IC_{50}	3.1	18.0	11.0	9.6	15.8	16.1
	GYR IC_{50}	88.0	80.0	61.9	95.5	101.9	142.9

TOP was considerably more FQ sensitive than GYR indicating that TOP is the primary target for these FQs against *S. pneumoniae*. Gemifloxacin was the most potent inhibitor of TOP, with at least 2.5 times more activity against the FQ-S C3LN4 enzyme than the other FQs. In contrast, all the FQs possessed very similar activity against GYR from FQ-S C3LN4. FQ IC_{50}s against TOP and GYR from FQ-R 502226 were higher than IC_{50}s against enzymes from FQ-S C3LN4. However, as with the C3LN4 enzymes, ability to inhibit TOP was a better indicator of FQ MIC than the ability to inhibit GYR. Gemifloxacin retained good potency against TOP from FQ-R 502226, which explains the enhanced potency of Gemifloxacin against this strain. It would appear therefore that the ability to inhibit TOP is a more important determinant for FQ MIC than ability to inhibit GYR, even against a FLQ-R strain with FQ-R GYR and FQ-R TOP.

Fluoroquinolones inhibit the essential bacterial enzymes, DNA gyrase and topoisomerase IV, which alter DNA topology after inserting a double-stranded DNA break. DNA gyrase exists as an A_2B_2 tetramer, encoded by the *gyrA* and *gyrB* genes, and catalyses negative DNA supercoiling. Topoisomerase IV exists as a C_2E_2 tetramer encoded by the *parC* and *parE* genes and is involved in chromosome partitioning. Previous studies have shown that fluoroquinolones are stronger inhibitors of pneumococcal topoisomerase IV than of pneumococcal DNA gyrase (Morrissey, *et al.*, *Antimicrob. Agents Chemother.*, 43, in press (1999); Pan, *et al.*, *Antimicrob. Agents Chemother.*, 43: 1129-1136 (1999)). Furthermore, with *Streptococcus pneumoniae*, fluoroquinolone MICs correlate better with topoisomerase IV IC_{50} than with DNA gyrase IC_{50} (Morrissey, *et al.*, *Antimicrob. Agents Chemother.*, 43, in press (1999)). Topoisomerase IV is the most likely primary target for fluoroquinolones against *S. pneumoniae* (Morrissey, *et al.*, *Antimicrob. Agents Chemother.*, 43, in press (1999)).

A further study on which an aspect of this invention was based was carried out to investigate the molecular basis of the enhanced anti-pneumococcal activity of gemifloxacin compared with other fluoroquinolones.

Bacterial Strains included: *S. pneumoniae* C3LN4 – a ciprofloxacin-sensitive laboratory strain studied previously (Morrissey, *et al.*, *Antimicrob. Agents Chemother.*, 43, in press (1999)), and *S. pneumoniae* 502226 – a ciprofloxacin-resistant (MIC 16 μg/ml)

clinical isolate from the Alexander Project culture collection (Felmingham, *et al.*, *J. Chemotherapy*, 11 (Suppl. 1): 5-21 (1999)).

Construction of over-expressing *E. coli* and purification of topoisomerase subunits

gyrA, gyrB, parC and *parE* subunit PCR products, prepared as previously described, (Morrissey, *et al.*, *Antimicrob. Agents Chemother.*, 43, in press (1999)), were inserted separately into pMAL-c2™ protein fusion vector (New England Biolabs UK Ltd), and used to transform competent *E. coli* DH5. Topoisomerase subunits fused to maltose-binding protein were produced, cleaved and purified as previously described.[1]

Reaction mixtures (20 µL) were set up to contain 0.4 µg of kinetoplast DNA (Topogen Inc., Ohio, USA), 40 mM Tris-HCl, 20 mM KCl, 5mM $MgCl_2$, 50 mg/L bovine serum albumen (BSA), 1mM dithiothreitol (DTT), 0.5 mM ATP and one unit of topoisomerase IV. One unit of topoisomerase IV was defined as the amount of reconstituted ParC and ParE required to decatenate 0.4 µg of kDNA in 1 h at 37°C. After incubation with various fluoroquinolone concentrations at 37°C for 1 h, reactions were stopped by the addition of 5 µL of stopping solution (5% (v/v) sarkosyl, 25% (v/v) bromophenol blue and 25% (v/v) glycerol). The samples were then subjected to 1% agarose gel electrophoresis and the intensity of the decatenated DNA band was analysed using the Gel Doc 1000 system (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK). Inhibition of topoisomerase IV was expressed as a percentage of the intensity of the decatenated band compared to a drug free control. The average of triplicate experiments was used to plot percent decatenation against fluoroquinolone concentration. The fluoroquinolone concentration required to inhibit enzyme activity by 50% (IC_{50}) was estimated from these plots.

Reaction mixtures (20 µL) were set up to contain 20 mM Tris HCl, 20 mM KCl, 8 mM $MgCl_2$, 25 mg/L BSA, 1 mM DTT, 5 mM ATP, 5 mM spermidine, 2.5 µg t-RNA, 0.2 µg relaxed pBR322 DNA and one unit of DNA gyrase. One unit of DNA gyrase was defined as the amount of reconstituted enzyme required to supercoil 0.2 µg of relaxed DNA in 1 h at 37°C. After incubation with various fluoroquinolone concentrations at 37°C for 1 h, reactions were stopped and analysed as described for topoisomerase IV. For DNA gyrase inhibition, IC_{50} values were calculated by comparing the intensity of supercoiled DNA bands.

As examples, Figures 35 and 36 show the results of the inhibition of DNA gyrase and topoisomerase IV from *S. pneumoniae* C3LN4 by ciprofloxacin as deduced from agarose gel analysis, respectively. From similar plots, the remaining fluoroquinolone IC_{50} values were calculated, as summarised in Table 77. The specific activity of the topoisomerase subunits

produced ranged from 4.2 to 7.2 x 10^3 units per mg protein.

The data indicates that gemifloxacin was the most potent inhibitor of topoisomerase IV isolated from both the fluoroquinolone-sensitive and ciprofloxacin-resistant pneumococci tested. In contrast, gemifloxacin was not the most potent inhibitor of DNA gyrase.

It seems likely that the potent anti-pneumococcal activity of gemifloxacin is due to strong activity against pneumococcal topoisomerase IV.

All the fluoroquinolones, with the exception of ciprofloxacin, showed very similar IC_{50} values against DNA gyrase from *S. pneumoniae* C3LN4, despite the fact that these antimicrobials differ in their ability to inhibit the organism. This data supports previous findings that topoisomerase IV is the primary target for fluoroquinolones against *S. pneumoniae* (Morrissey, *et al.*, *Antimicrob. Agents Chemother.*, 43, in press (1999); Pan, *et al.*, *Antimicrob. Agents Chemother.*, 43: 1129-1136 (1999)). The fluoroquinolone-resistant phenotype exhibited by *S. pneumoniae* 502226 appears to be due to reduced inhibition of both DNA gyrase and topoisomerase IV. This agrees with topoisomerase QRDR sequence data which has been shown to have mutations in both DNA gyrase and topoisomerase IV genes (Broskey, *et al.*, *J. Antimicrob. Chemother.*, 44 (Suppl. A): Abstract P391, p. 127 (1999)).

Table 77. Fluoroquinolone Inhibition of DNA Gyrase and Topoisomerase IV Purified from Two Strains of *S. pneumoniae

*Values shown are an average of 3 determinations with standard deviation (n-1) in parenthesis

Strain	Drug	IC_{50} µg/ml (SD(n-1))			
		Topoisomerase IV		DNA gyrase	
S. pneumoniae C3LN4	Gemifloxacin	1.4	(± 0)	47.5	(± 5.6)
	Ciprofloxacin	6.4	(± 0.15)	59.2	(± 1.6)
	Levofloxacin	4.0	(± 0)	49.1	(± 0.5)
	Grepafloxacin	3.5	(± 0.26)	42.8	(± 0.4)
	Moxifloxacin	3.9	(± 0.26)	44.0	(± 0)
	Trovafloxacin	3.7	(± 0.16)	44.0	(± 1.4)

	Gemifloxacin	3.1	(± 0.15)	88.0	(± 2.8)
	Ciprofloxacin	16.1	(± 0.23)	142.9	(± 4.9)
S. pneumoniae 502226	Levofloxacin	15.8	(± 0.29)	101.9	(± 5.1)
	Grepafloxacin	18.0	(± 0.4)	80.0	(± 0)
	Moxifloxacin	9.6	(± 0.4)	95.5	(± 0.92)
	Trovafloxacin	11.0	(± 0.35)	61.9	(± 2.4)

The invention provides a method for modulating metabolism of *Streptococcus pneumoniae* pathogenic bacteria. Skilled artisans can readily choose *Streptococcus pneumoniae* pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by *Streptococcus pneumoniae* pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with *Streptococcus pneumoniae* pathogenic bacteria.

While a preferred object of the invention provides a method wherein said *Streptococcus pneumoniae* pathogenic bacteria is selected. Other *Streptococcus pneumoniae* pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The contacting step in any of the methods of the invention may be performed in many ways that will be readily apparent to the skilled artisan. However, it is preferred that the contacting step is a provision of a composition comprising a gemifloxacin compound to a human patient in need of such composition or directly to bacteria in culture medium or buffer.

For example, when contacting a human patient or contacting said bacteria in a human patient or *in vitro*, the compositions comprising a quinolone, particularly a gemifloxacin compound, preferably pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes

among others.

It is also preferred that these compositions be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a compound of the invention, a quinolone, preferably a gemifloxacin compound, and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

Quinolone compounds, particularly gemifloxacin compounds and compostions of the methods of the invention may be employed alone or in conjunction with other compounds, such as bacterial efflux pump inhibtor compounds or antibiotic compounds, particularly non-quinolone compounds, *e.g.*, beta-lactam antibiotic compounds.

In therapy or as a prophylactic, the active agent of a method of the invention is preferably administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably an isotonic one.

Alternatively, the gemifloxacin compounds or compositions in the methods of the invention may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

For administration to mammals, and particularly humans, it is expected that the antibacterially effective amount is a daily dosage level of the active agent from 0.001 mg/kg to 10 mg/kg, typically around 0.1 mg/kg to 1 mg/kg, preferably about 1 mg/kg. A physician, in any event, will determine an actual dosage that is most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention. It is preferred that the dosage is selected to modulate metabolism of the bacteria in such a way as to inhibit or stop growth of said bacteria or by killing said bacteria.

The skilled artisan may identify this amount as provided herein as well as using other methods known in the art, *e.g.* by the application MIC tests.

A further embodiment of the invention provides for the contacting step of the methods to further comprise contacting an in-dwelling device in a patient. In-dwelling devices include, but are not limited to, surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, and continuous ambulatory peritoneal dialysis (CAPD) catheters.

A quinolone, particularly a gemifloxacin compound or composition of the invention may be administered by injection to achieve a systemic effect against a bacteria of the invention, shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections caused by or related to a bacteria of the invention.

In addition to the therapy described above, a gemifloxacin compound or composition used in the methods of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins, particularly those of a bacteria of the invention, exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, a quinolone, particularly a gemifloxacin compound or composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1μg/ml to 10mg/ml for bathing of wounds or indwelling devices.

Preferred embodiments of the invention include, among other things, methods wherein said composition comprises gemifloxacin, or a pharmaceutically acceptable derivative thereof.

All studies provided herein were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. All parts or amounts set out in the following examples are by weight, unless otherwise specified.

Each reference cited herein is hereby incorporated by reference in its entirety. Moreover, each patent application to which this application claims priority is hereby incorporated by reference in its entirety.

What is claimed is:

1. A method for modulating metabolism of pneumococcal pathogenic bacteria comprising contacting pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or antibacterially effective derivatives thereof.

2. The method of claim 1 wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: penicillin-susceptible, intermediate and resistant (including ciprofloxacin-resistant) pneumococci.

3. A method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising administering an antibacterially effective amount of a composition comprising a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

4. The method of claim 3 wherein said pneumococcal pathogenic bacteria is selected from the group consisting of penicillin-susceptible, intermediate and resistant (including ciprofloxacin-resistant) pneumococci.

5. The method of claim 3 wherein said mammal is a human.

6. The method of claim 1 wherein said modulating metabolism is inhibiting growth of said bacteria.

7. The method of claim 1 wherein said modulating metabolism is killing said bacteria.

8. The method of claim 1 wherein said contacting said bacteria comprises the further step of introducing said composition into a mammal.

9. The method of claim 8 wherein said mammal is a human.

10. The method of claim 1 wherein said bacteria is a penicillin-susceptible and intermediate pneumococci..

11. The method of claim 1 wherein said bacteria is a penicillin-resistant (including ciprofloxacin-resistant) pneumococci.

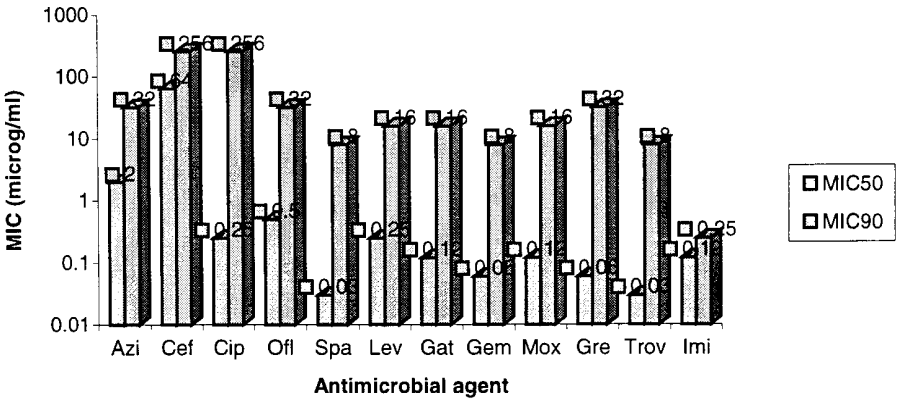


Figure 1

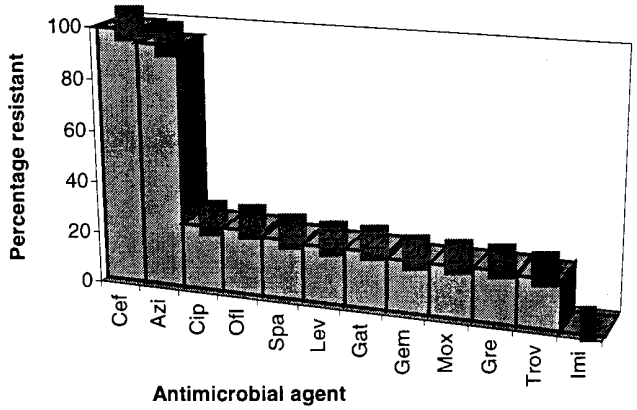


Figure 2

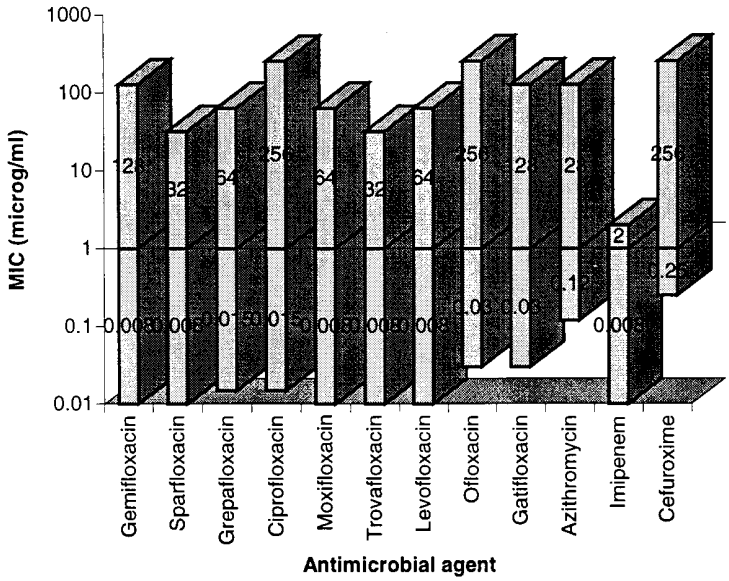

1000
100
10
1
0.1
0.01
MIC (microg/ml)
Gemifloxacin
Sparfloxacin
Grepafloxacin
Ciprofloxacin
Moxifloxacin
Trovafloxacin
Levofloxacin
Ofloxacin
Gatifloxacin
Azithromycin
Imipenem
Cefuroxime
Antimicrobial agent

Figure 3

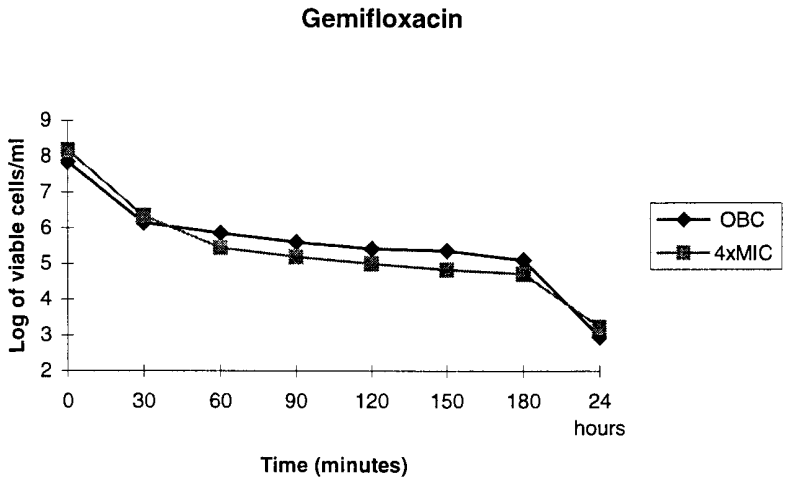


Figure 4a

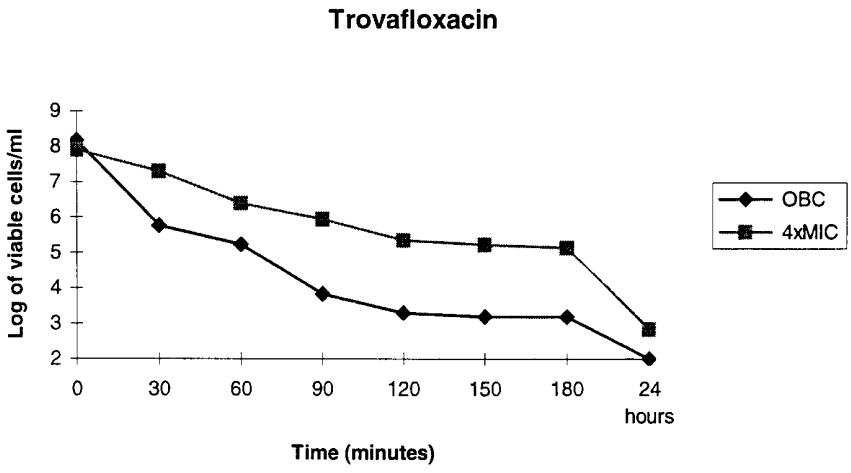


Figure 4b

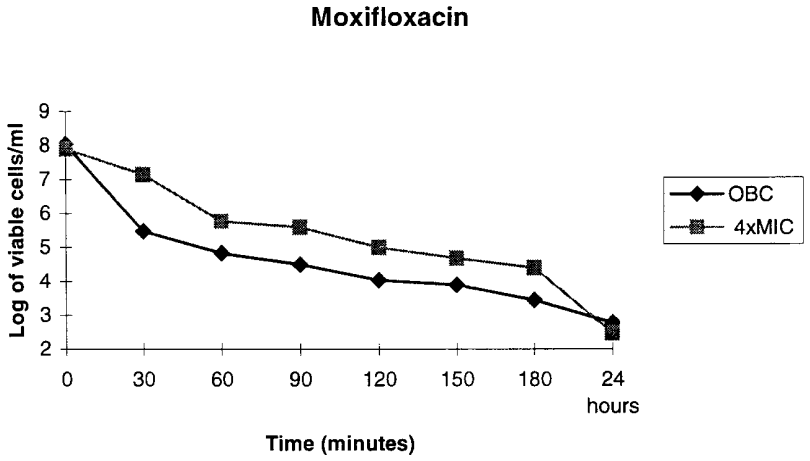


Figure 4c: Bactericidal activity of moxifloxacin against *A. baumannii* ATCC 19606

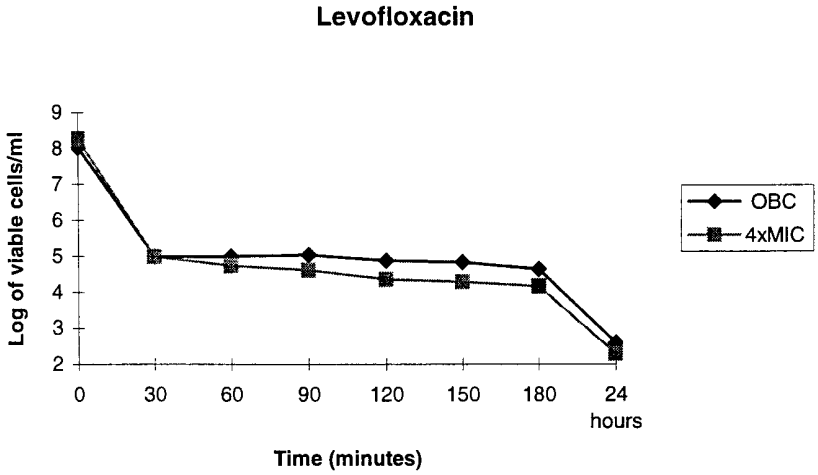


Figure 4d: Bactericidal activity of levofloxacin against *A. baumannii* ATCC 19606

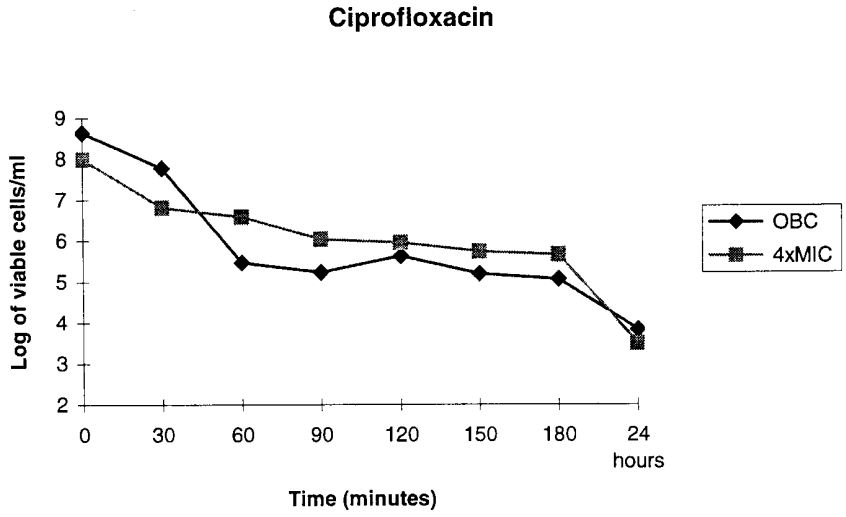


Figure 4e: Bactericidal activity of ciprofloxacin against *A. baumannii* ATCC 19606

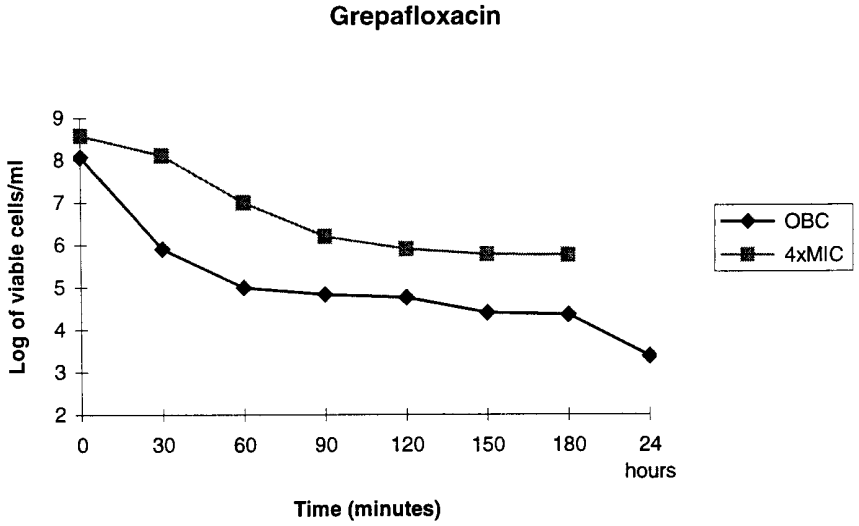


Figure 4f: Bactericidal activity of grepafloxacin against *A. baumannii* ATCC 19606

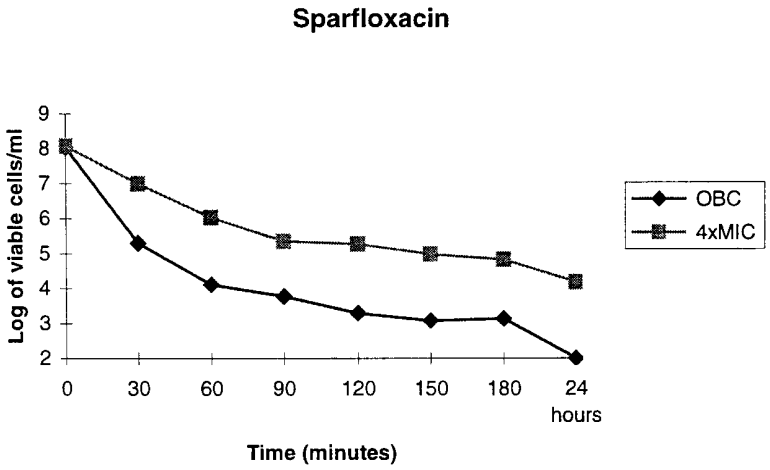


Figure 4g: Bactericidal activity of sparfloxacin against *A. baumannii* ATCC 19606

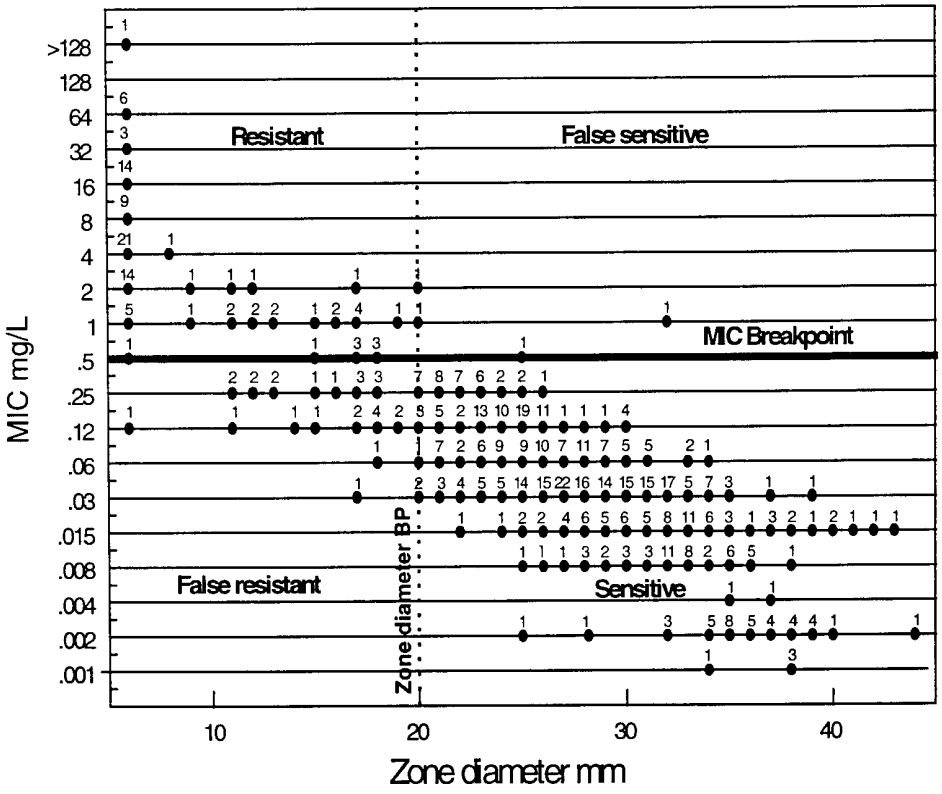

MIC mg/L
>128
128
64
32
16
8
4
2
1
.5
.25
.12
.06
.03
.015
.008
.004
.002
.001
Resistant
False sensitive
MIC Breakpoint
False resistant
Sensitive
Zone diameter BP
10
20
30
40
Zone diameter mm

Figure 5

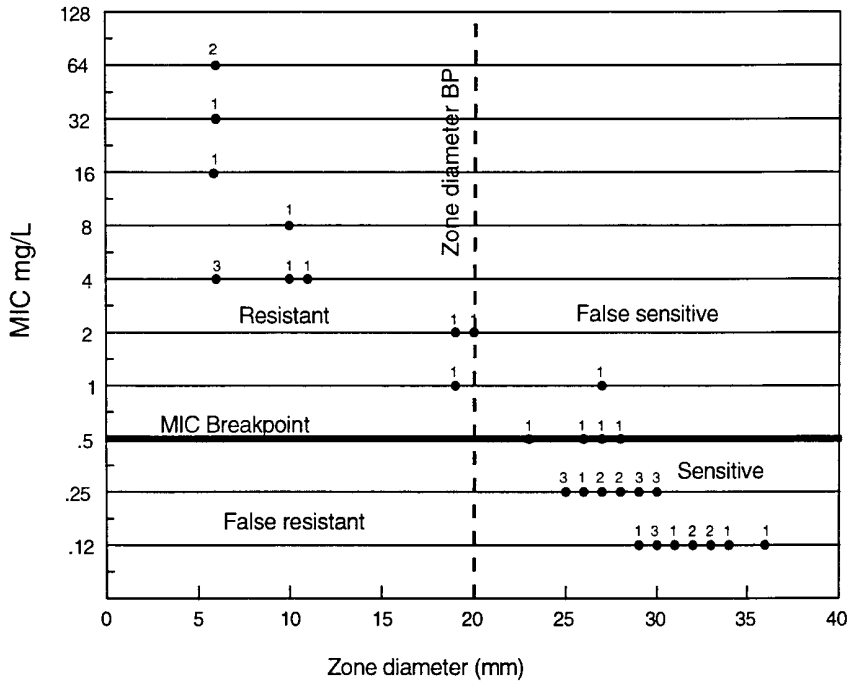

128
64
32
16
8
4
2
1
.5
.25
.12
MIC mg/L
Zone diameter BP
Resistant
False sensitive
MIC Breakpoint
Sensitive
False resistant
0
5
10
15
20
25
30
35
40
Zone diameter (mm)

Figure 6

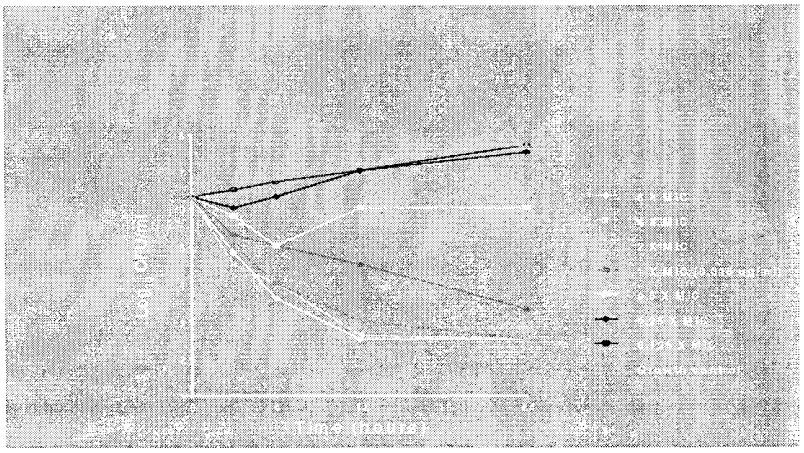
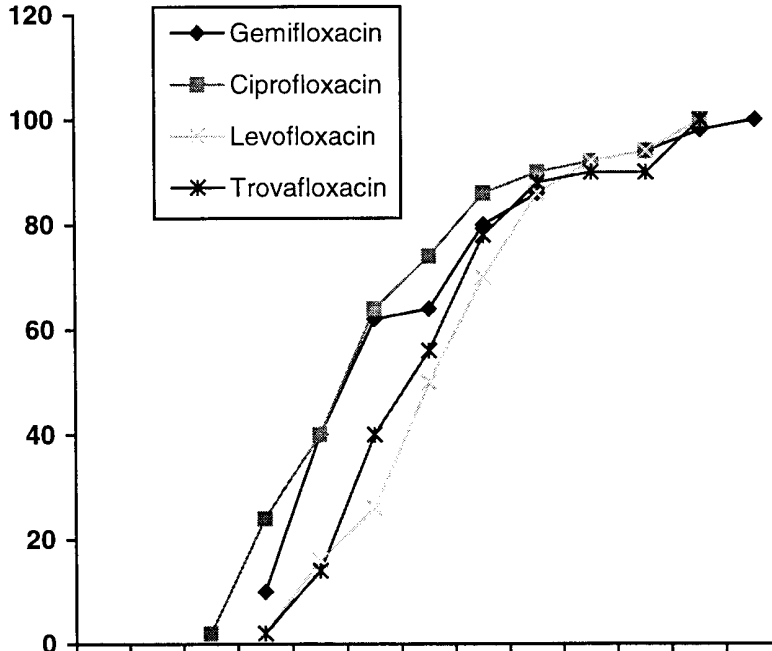
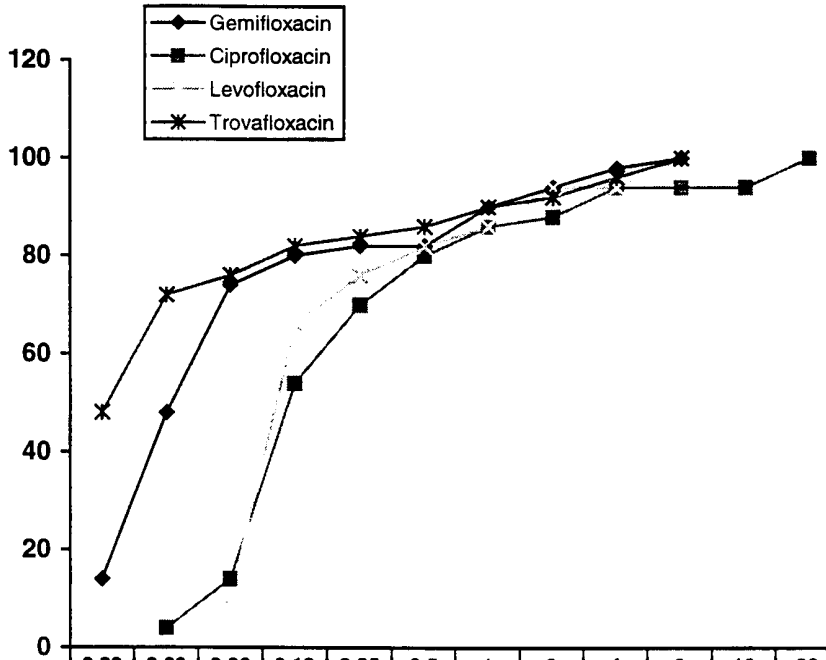


FIGURE 7



	0.02	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	
Gemifloxacin				10	40	62	64	80	86	92	94	98	100		
Ciprofloxacin			2	24	40	64	74	86	90	92	94	100			
Levofloxacin				2	16	26	50	70	86	92	94	100			
Trovafloxacin				2	14	40	56	78	88	90	90	100			

FIGURE 8



	0.02	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32			
Gemifloxacin	14	48	74	80	82	82	90	94	98	100					
Ciprofloxacin		4	14	54	70	80	86	88	94	94	94	100			
Levofloxacin			10	66	76	82	86	94	94	100					
Trovafloxacin	48	72	76	82	84	86	90	92	96	100					

FIGURE 9

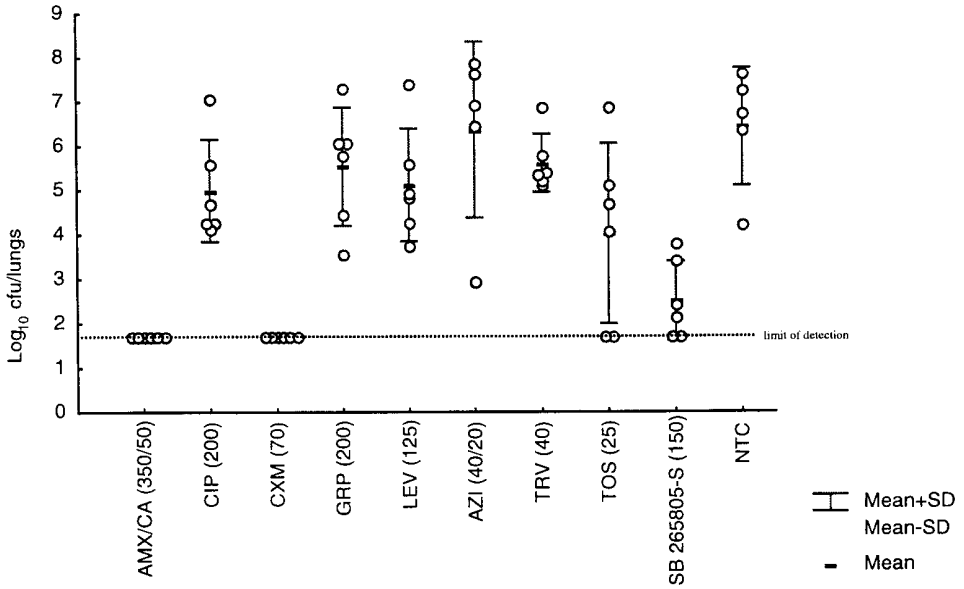

Log10 cfu/lungs
9
8
7
6
5
4
3
2
1
0
limit of detection
AMX/CA (350/50)
CIP (200)
CXM (70)
GRP (200)
LEV (125)
AZI (40/20)
TRV (40)
TOS (25)
SB 265805-S (150)
NTC
Mean+SD
Mean-SD
Mean

FIGURE 10

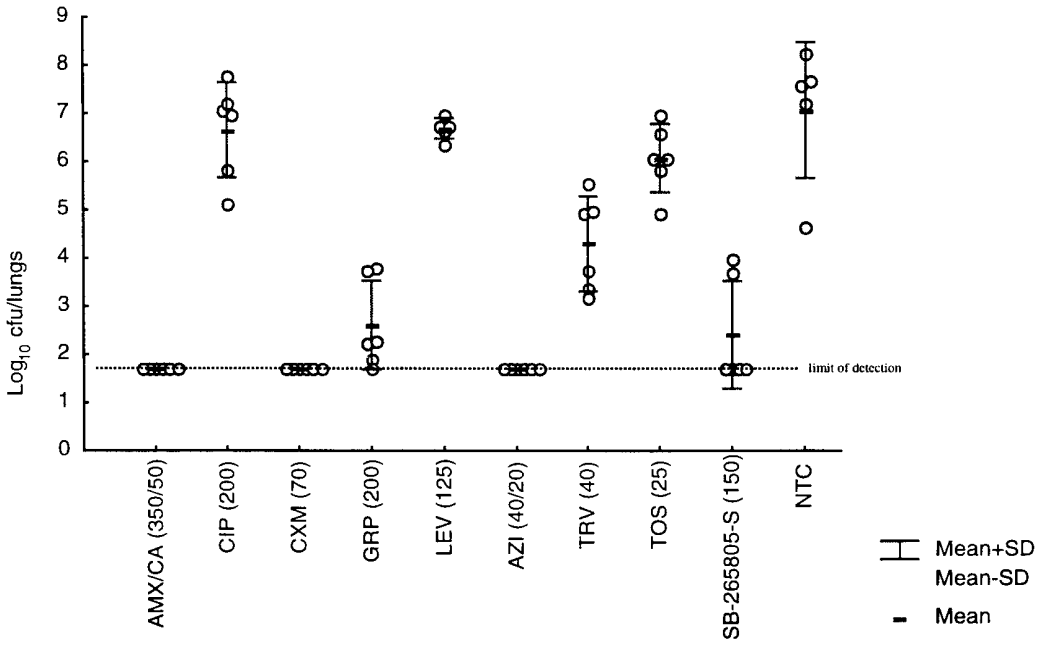

9
8
7
6
5
4
3
2
1
0
Log10 cfu/lungs
limit of detection
AMX/CA (350/50)
CIP (200)
CXM (70)
GRP (200)
LEV (125)
AZI (40/20)
TRV (40)
TOS (25)
SB-265805-S (150)
NTC
Mean+SD
Mean-SD
Mean

Figure 11

Efficacy of SB265805-S compared with grepafloxacin, levofloxacin and trovofloxacin in a respiratory tract infection in rats caused by *S. pneumoniae* 305313.

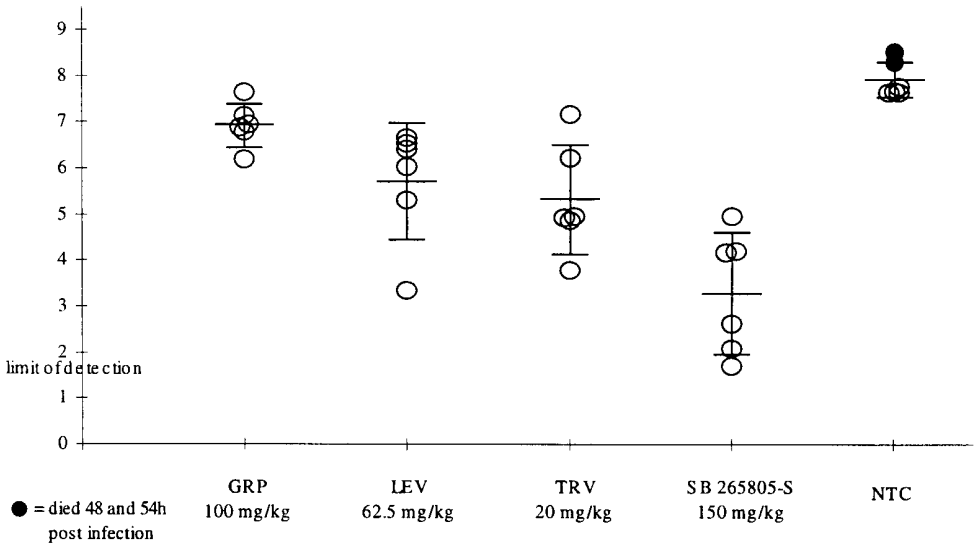


Figure 12

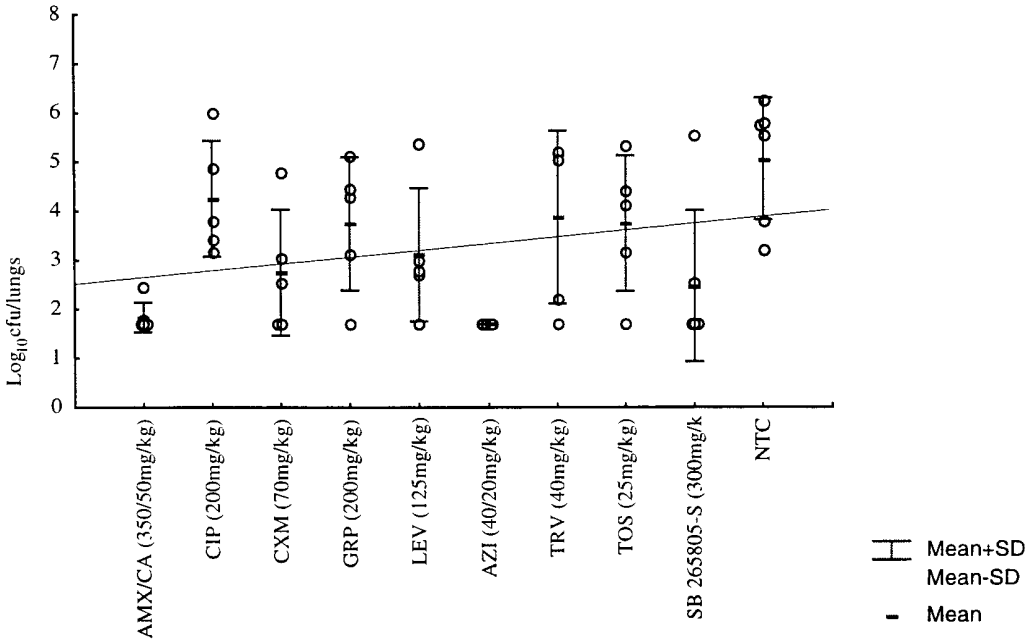

8
7
6
5
4
3
2
1
0
Log10cfu/lungs
AMX/CA (350/50mg/kg)
CIP (200mg/kg)
CXM (70mg/kg)
GRP (200mg/kg)
LEV (125mg/kg)
AZI (40/20mg/kg)
TRV (40mg/kg)
TOS (25mg/kg)
SB 265805-S (300mg/k
NTC
Mean+SD
Mean-SD
Mean

Figure 13

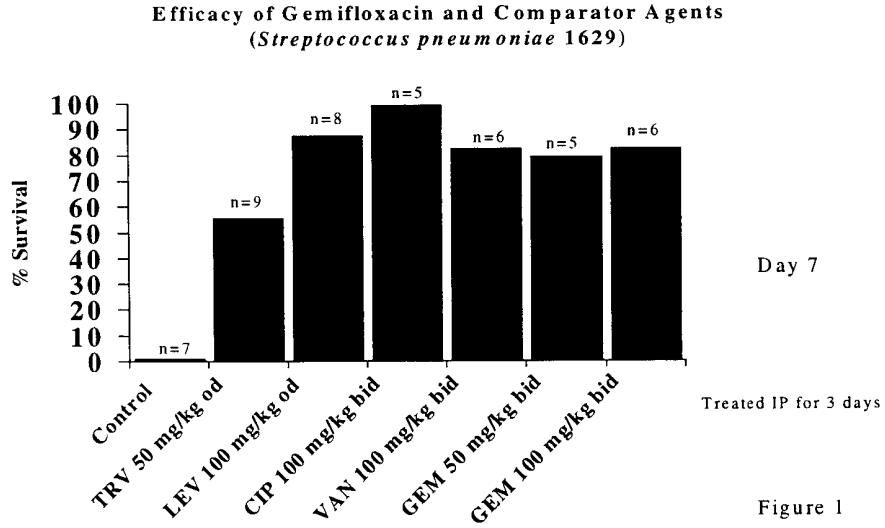


Figure 1

[Illustrator: change TRV...od to TRO (50 mg/kg o.d.); LEV...od to LEV (100 mg/kg o.d.); CIP...bid to CIP (100 mg/kg b.i.d.); VAN...bid to VAN (100 mg/kg b.i.d.); GEM...bid to GEM (50 mg/kg b.i.d.); GEM...bid to GEM (100 mg/kg b.i.d.); % Survival to Survival (%), IP to i.p., n=x to n = x]

FIGURE 14

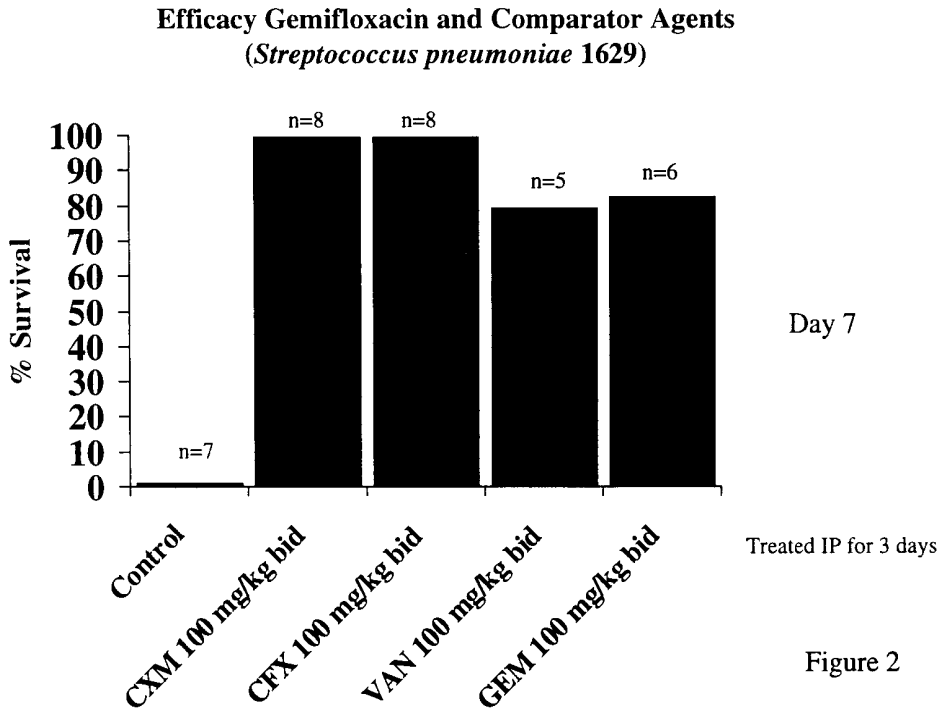


Figure 2

[Illustrator: change CXM...bid to CXM (100 mg/kg b.i.d.); CFX...bid to CFX (100 mg/kg b.i.d.); VAN...bid to VAN (100 mg/kg b.i.d.); GEM...bid to GEM (100 mg/kg b.i.d.); % Survival to Survival (%), IP to i.p., n=x to n = x]

FIGURE 15

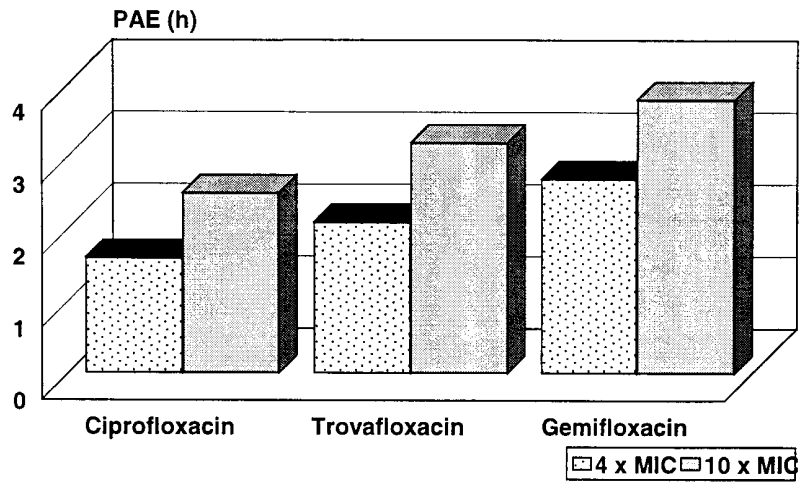

PAE (h)
4
3
2
1
0
Ciprofloxacin
Trovafloxacin
Gemifloxacin
4 x MIC
10 x MIC

Figure 16

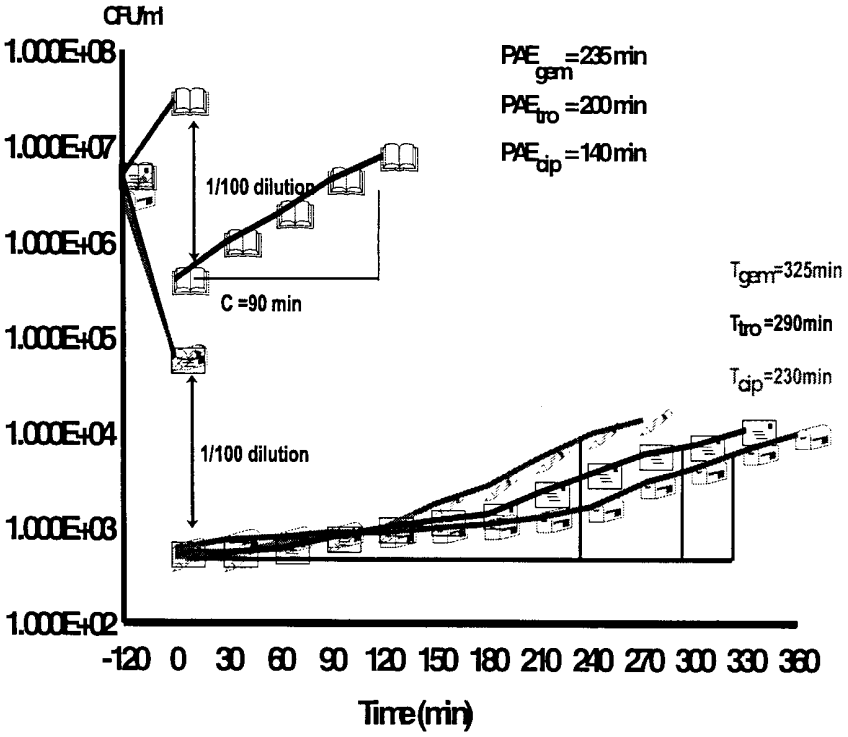

CFU/ml
1.000E+08
1.000E+07
1.000E+06
1.000E+05
1.000E+04
1.000E+03
1.000E+02
PAE gem =235min
PAE tro =200min
PAE cip =140min
1/100 dilution
C =90 min
1/100 dilution
T gem=325min
T tro=290min
T cip=230min
-120 0 30 60 90 120 150 180 210 240 270 300 330 360
Time(min)

FIGURE 17

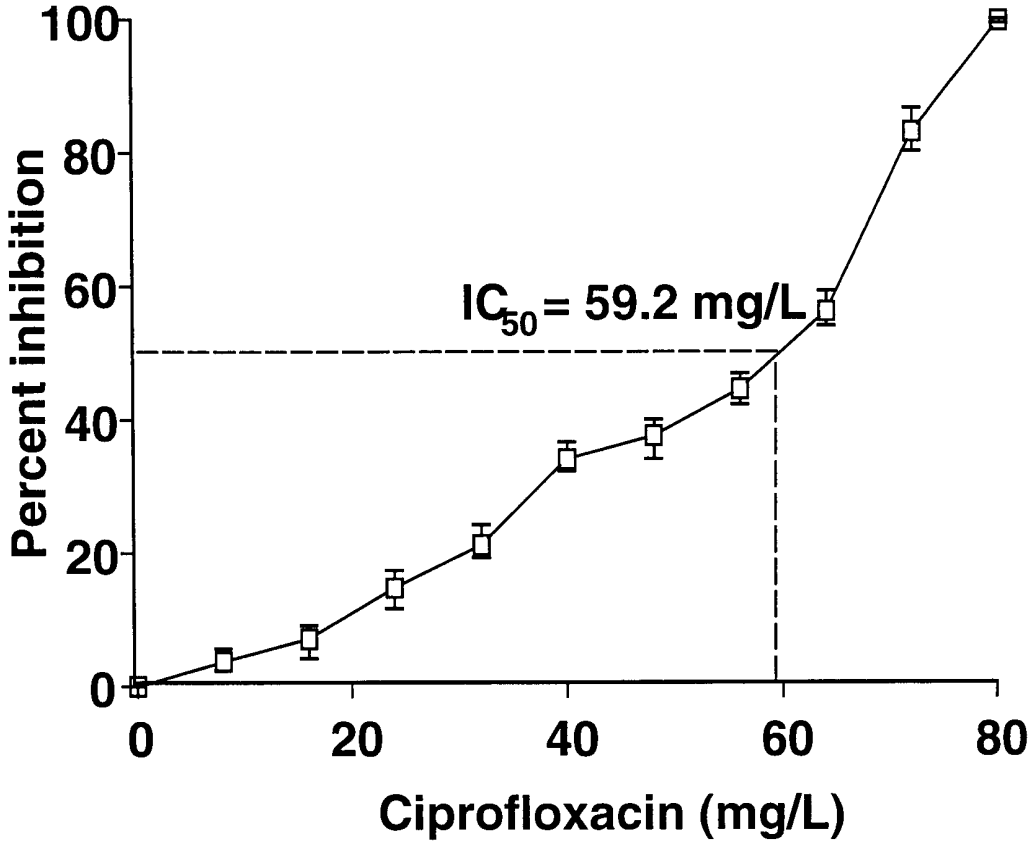

100
80
60
40
20
0
Percent inhibition
IC50 = 59.2 mg/L
0
20
40
60
80
Ciprofloxacin (mg/L)

FIGURE 18

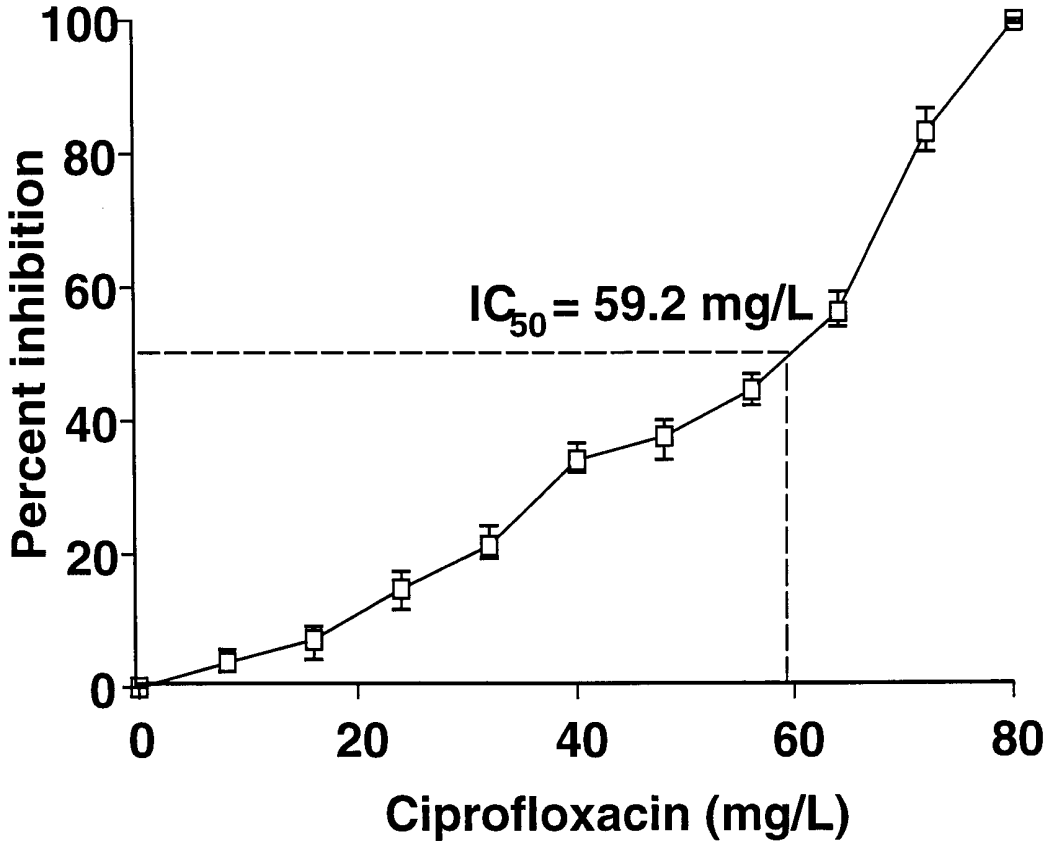

100
80
60
40
20
0
Percent inhibition
IC50 = 59.2 mg/L
0
20
40
60
80
Ciprofloxacin (mg/L)

FIGURE 19

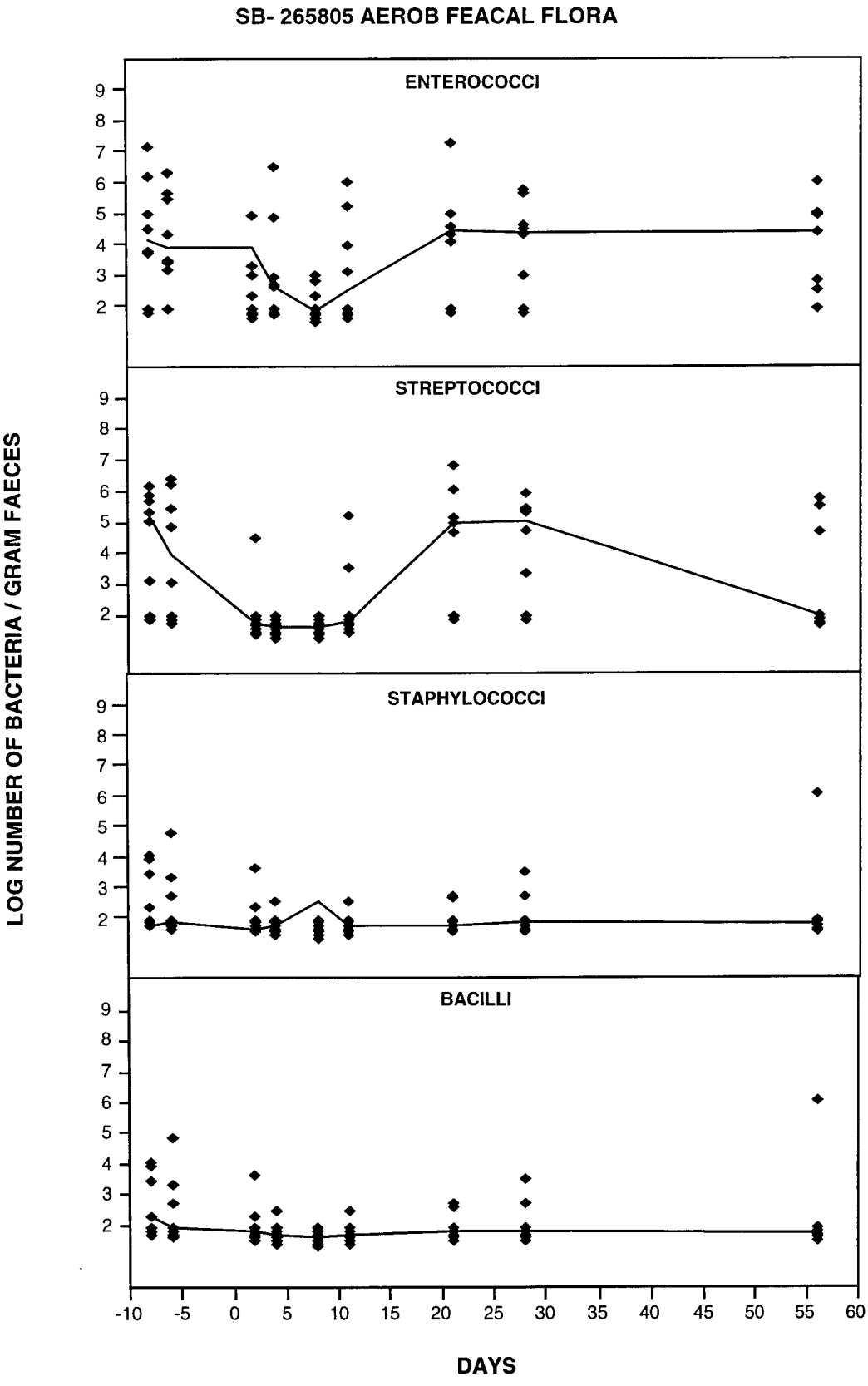

SB- 265805 AEROB FEACAL FLORA
ENTEROCOCCI
STREPTOCOCCI
STAPHYLOCOCCI
BACILLI
LOG NUMBER OF BACTERIA / GRAM FAECES
DAYS
-10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60

FIGURE 20

SB - 265805 ANAEROB FAECAL FLORA

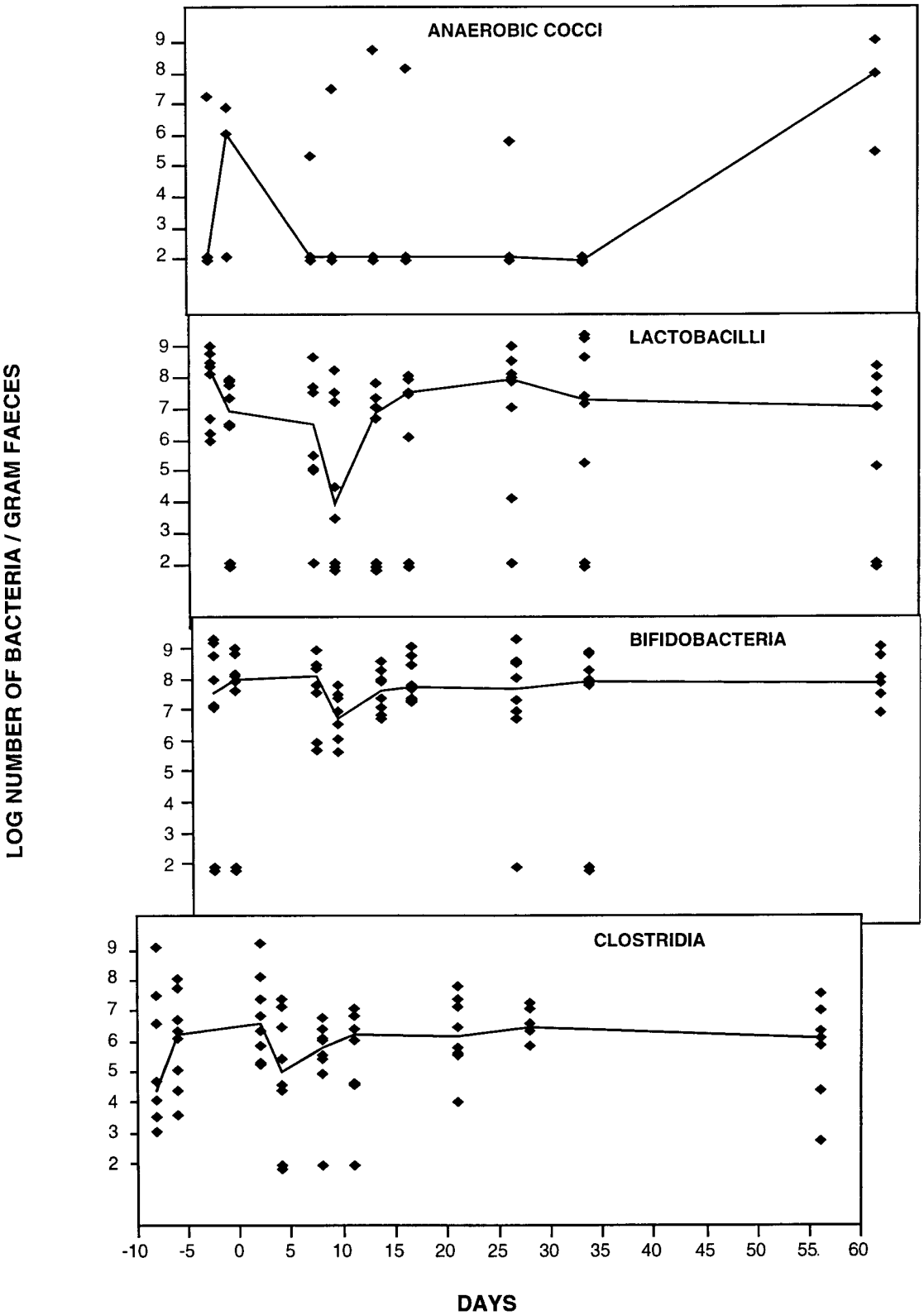


FIGURE 21

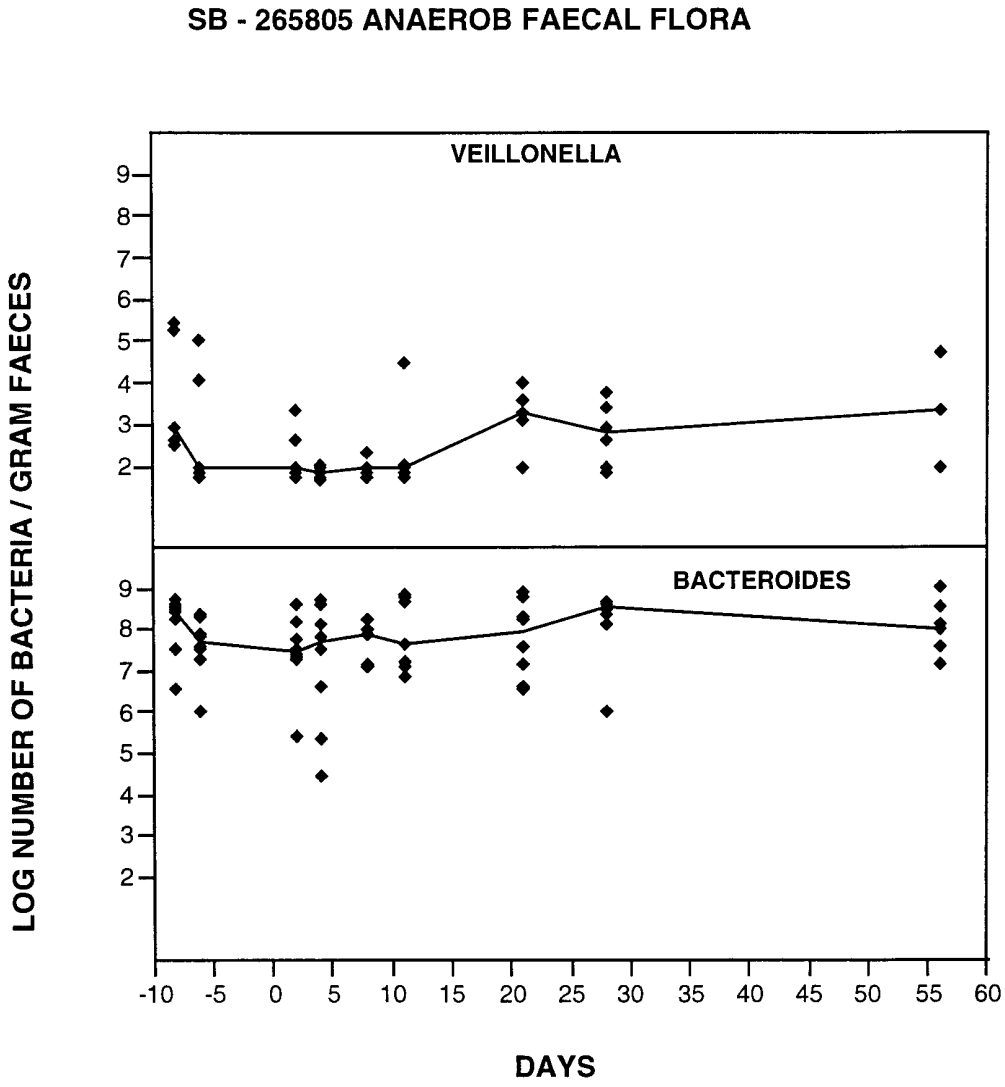

SB - 265805 ANAEROB FAECAL FLORA
VEILLONELLA
BACTEROIDES
LOG NUMBER OF BACTERIA / GRAM FAECES
DAYS
9
8
7
6
5
4
3
2
-10
-5
0
5
10
15
20
25
30
35
40
45
50
55
60

FIGURE 22

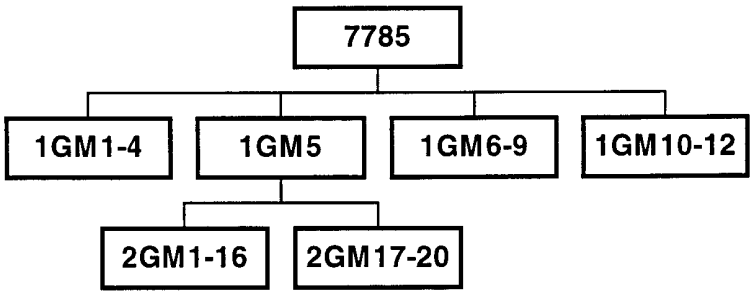
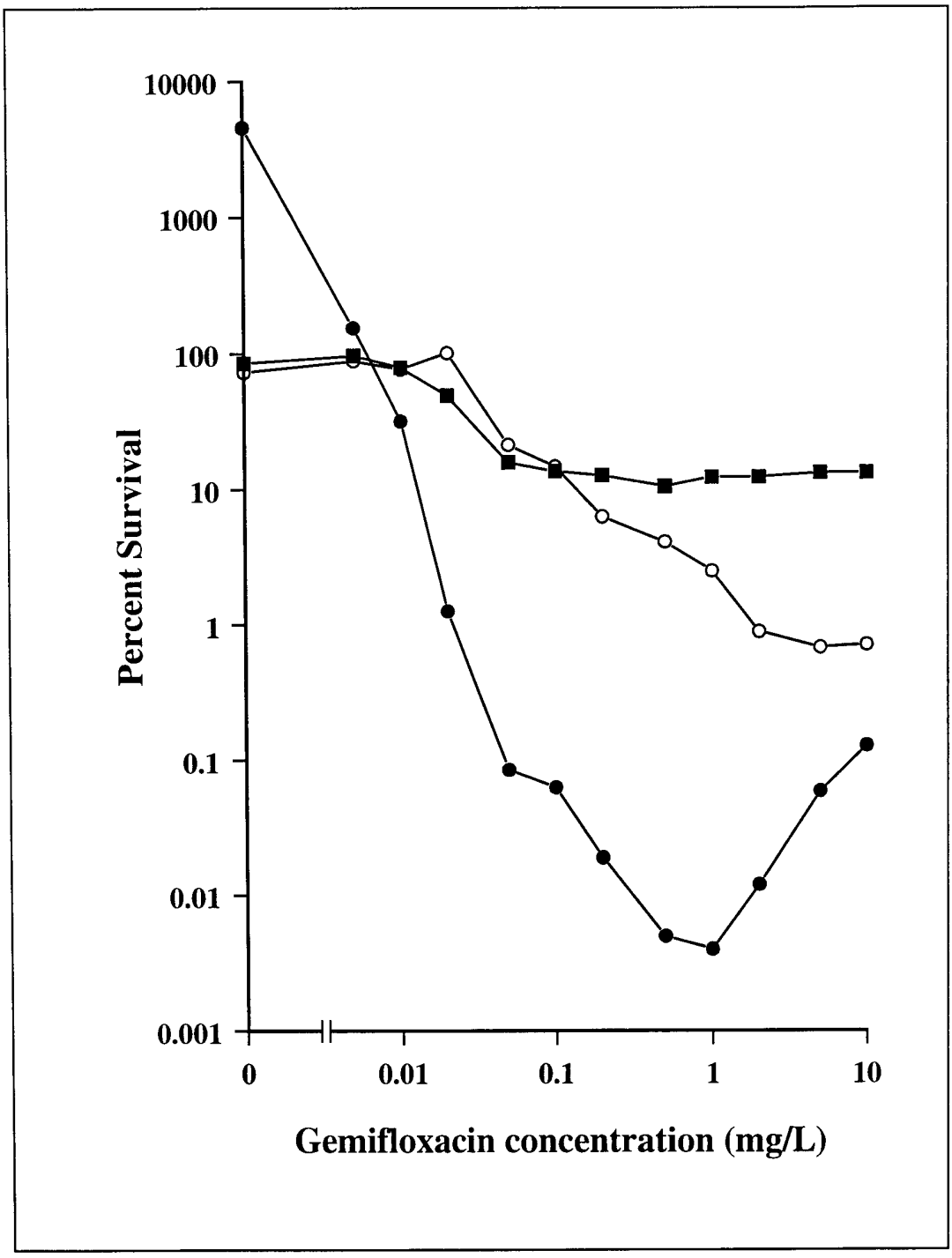
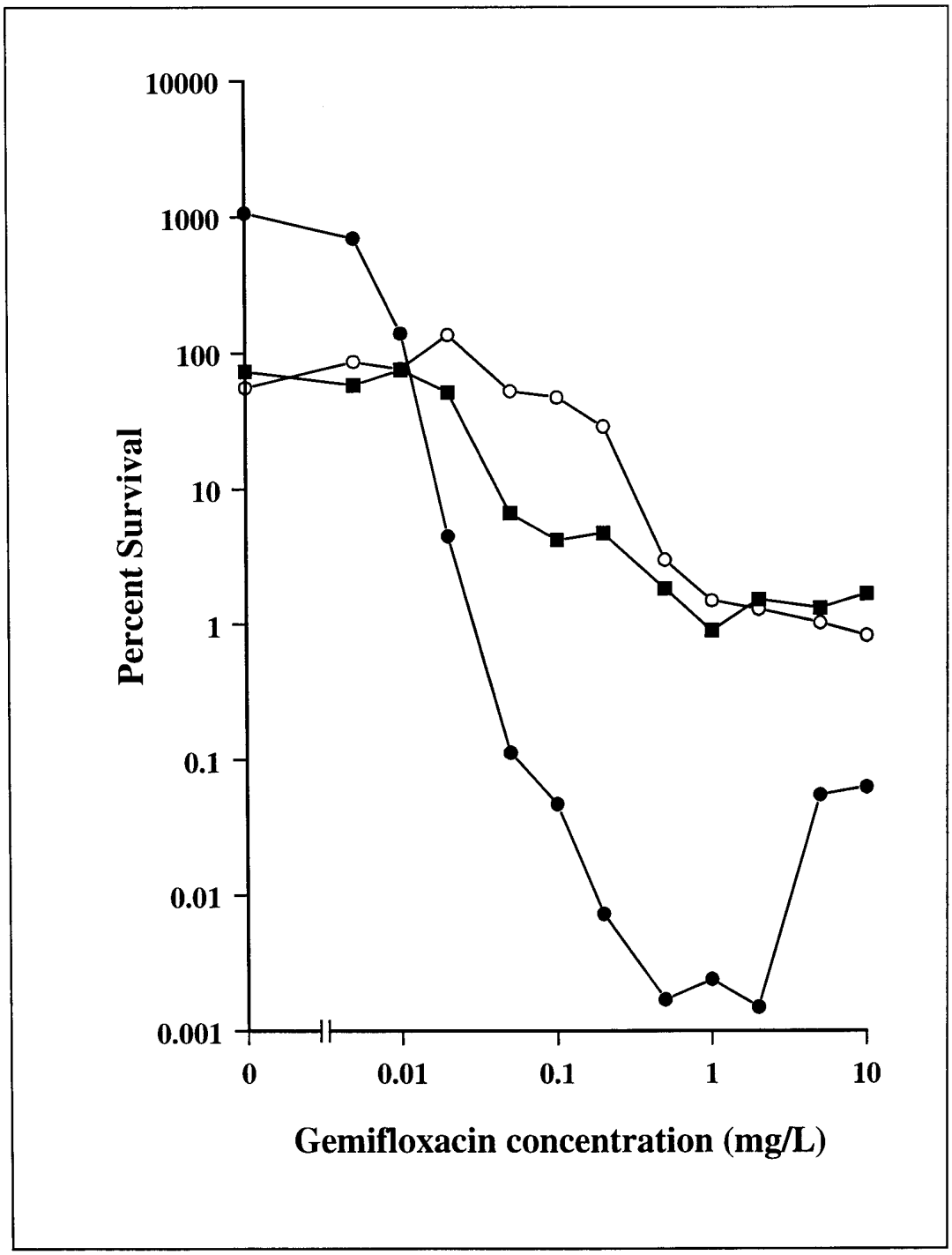


FIGURE 23



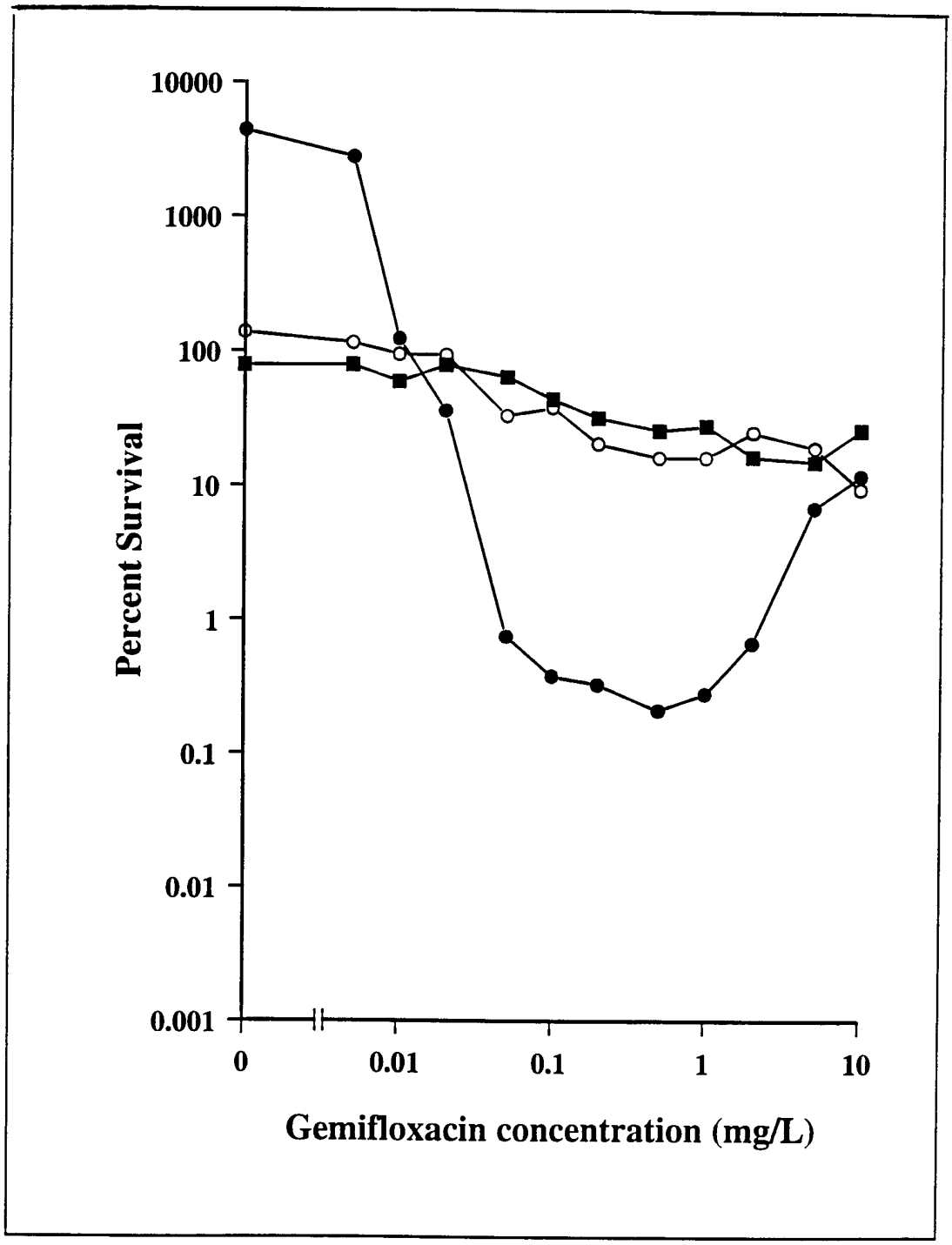
Survival of *Escherichia coli* KL16 treated with gemifloxacin for 3 h at 37 °C. ● = nutrient broth, ○ = nutrient broth & 20 µg/ml chloramphenicol, ■ = phosphate-buffered saline.

FIGURE 24



Survival of *Staphylococcus aureus* E3T treated with gemifloxacin for 3 h at 37 °C. ● = nutrient broth, ○ = nutrient broth & 20 µg/ml chloramphenicol, ■ = phosphate-buffered saline.

FIGURE 25



Survival of *Streptococcus pneumoniae* C3LN4 treated with gemifloxacin for 3 h at 37 °C. ● = nutrient broth plus 7% laked horse blood (blood broth), ○ = blood broth & 20 µg/ml chloramphenicol, ■ = phosphate-buffered saline plus 7% horse serum.

FIGURE 26

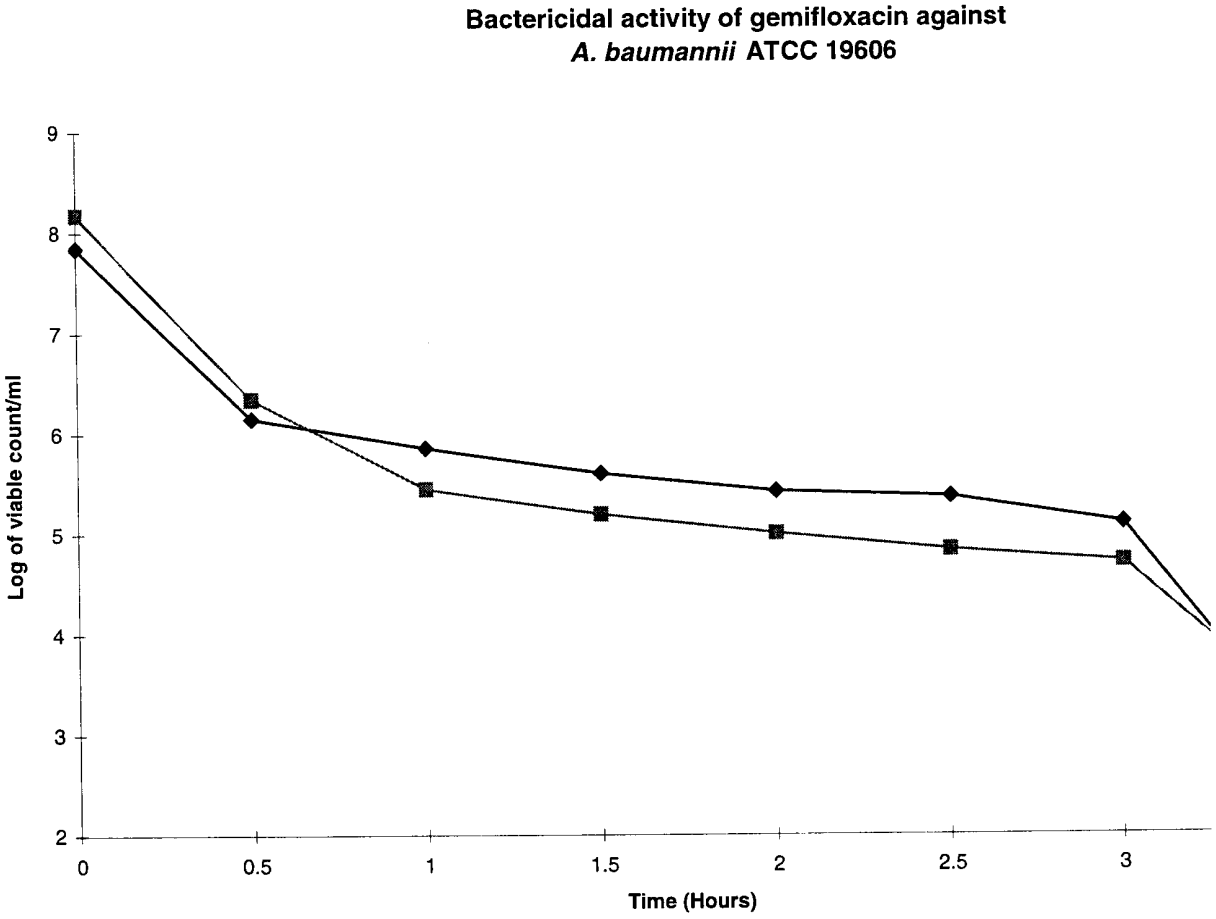

Bactericidal activity of gemifloxacin against
A. baumannii ATCC 19606
Log of viable count/ml
9
8
7
6
5
4
3
2
0
0.5
1
1.5
2
2.5
3
Time (Hours)

FIGURE 27

Bactericidal activity of trovafloxacin against
A. baumannii ATCC 19606

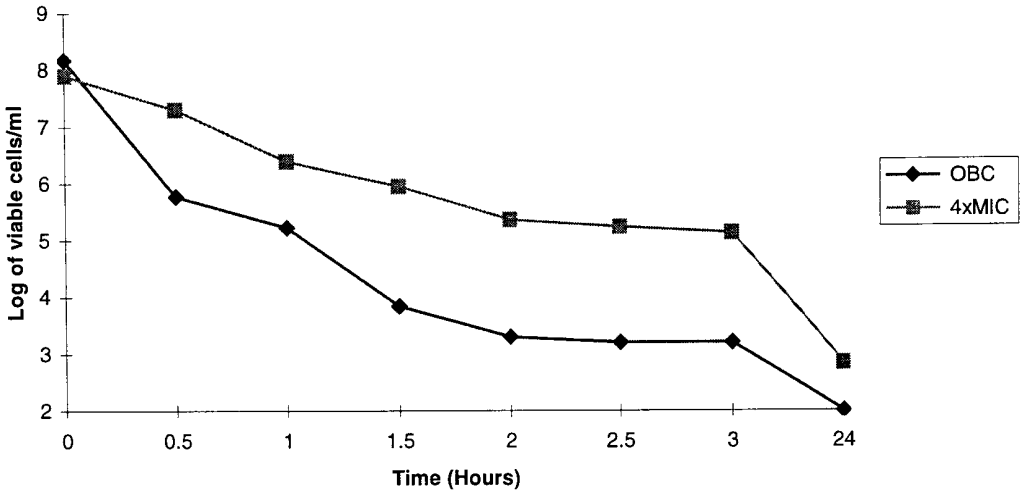


Figure 4. Bactericidal activity of moxifloxacin against *A. baumannii* ATCC 19606

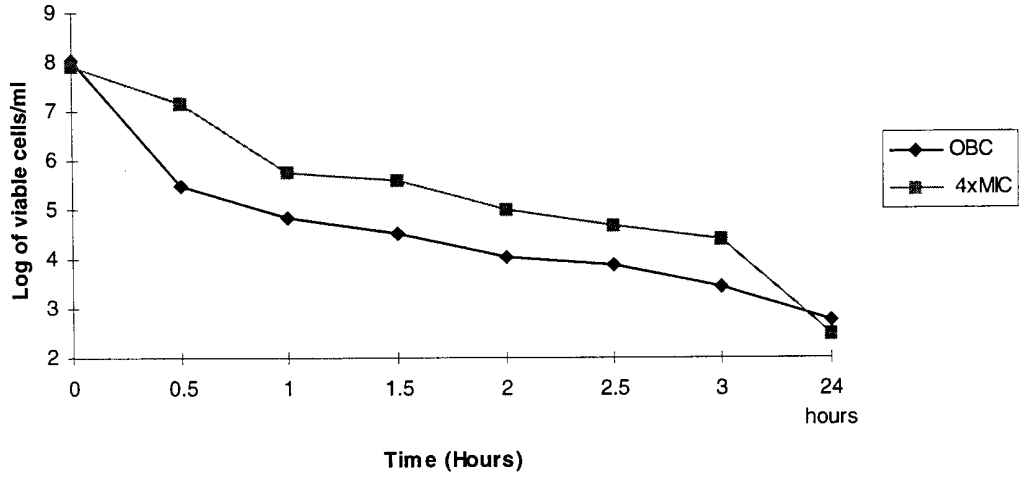


FIGURE 28

Bactericidal activity of levofloxacin against *A. baumannii* ATCC 19606.

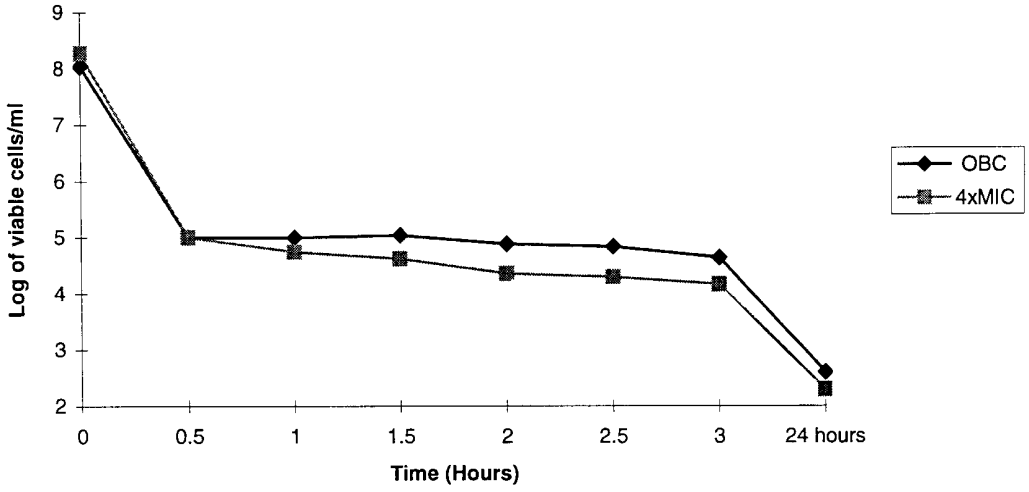


Figure 6. Bactericidal activity of ciprofloxacin against *A. baumannii* ATCC 19606

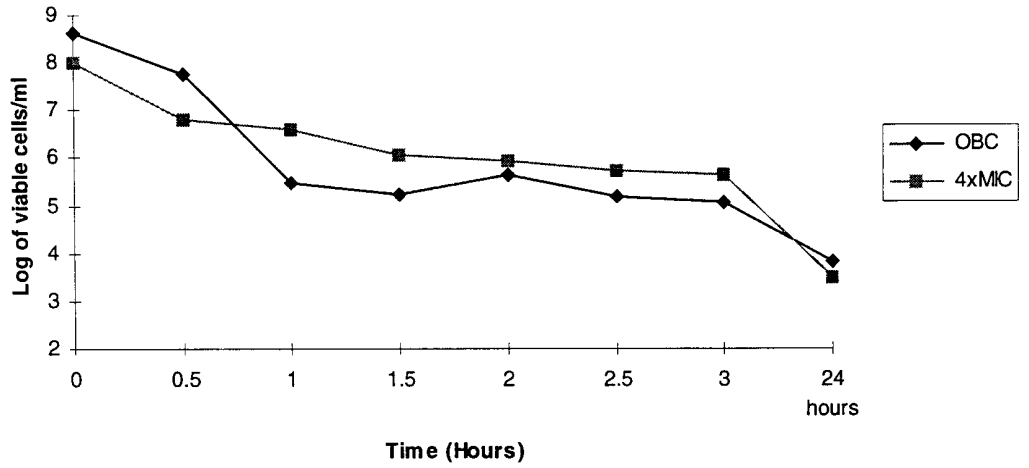
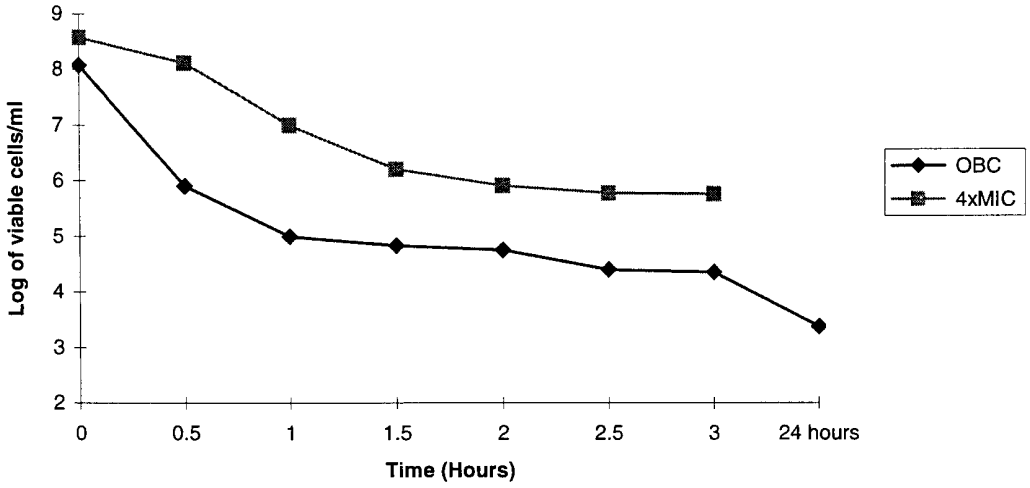


FIGURE 29

Bactericidal activity of grepafloxacin against *A. baumannii* ATCC 19606



Bactericidal activity of sparfloxacin against *A. baumannii* ATCC 19606

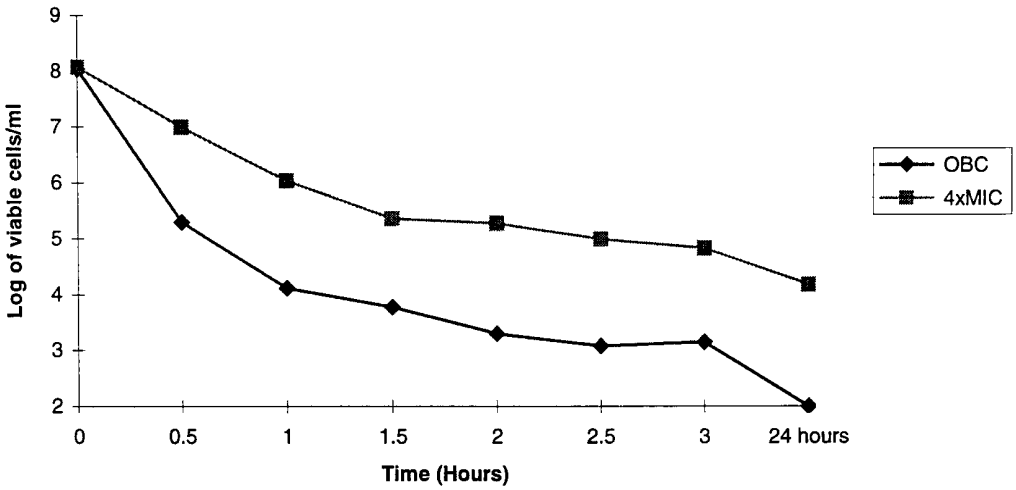


FIGURE 30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23883

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/44
US CL :514/300

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/300

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database DRUGU on STN, Derwent Information Ltd., No. 2000-05866, KING, A. et al. 'The comparative in vitro activity of gemifloxacin, a new fluoroquinolone against selected clinical isolates,' abstract, J. Antimicrob. Chemother. (44, Suppl. A, 147, July 1999.	1-11
X	Database DRUGU on STN, Derwent Information Ltd., No. 2000-05860, HARDY, D. et al. 'Comparative in vitro activity of gemifloxacin, moxifloxacin, trovafloxacin, sparfloxacin, grepafloxacin, ofloxacin, ciprofloxacin and other antimicrobial agents agsinst bloodstream isolates of Gram-positive cocci,' abstract, J. Antimicrob. Chemother. (44, Suppl. A, 146, July 1999.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 SEPTEMBER 2000	Date of mailing of the international search report 19 OCT 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer WILLIAM JARVIS Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23883

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database BIOSIS on STN, No. 1999:404565, HEATON, V. et al. 'Gemifloxacin is highly active against both penicillin and ciprofloxacin resistant Streptococcus pneumoniae,' abstract, J. Antimicrob. Chemother. (July 1999), Vol. 44, No. Suppl. A page 140.	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23883

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

BRS WEST (US PATENTS, JPO ABSTRACTS, EPO ABSTRACTS, DWPI)
STN (REGISTRY, CHEM. ABSTRACTS, BIOSIS, MEDLINE, DERWENT DRUG FILE, EMBASE)
search terms: gemifloxacin, pneumonia, pneumococci