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(54) Title: ANTIBIOTIC COMBINATIONS FOR PROVIDING TOTAL SOLUTION TO THE TREATMENT OF INFECTIONS

(57) Abstract: The invention relates to a new pharmaceutical composition, a method of treatment of infection and also a process to prepare the composition. The infectious complications are important causes of morbidity and mortality. Hospital acquired pneumonia (HAP) remains the most severe nosocomial infection in intensive care units. Beta-lactams alone are always considered inadequate when *P. aeruginosa* and / or methicillin-resistant *S. aureus* are implicated as pathogens or copathogens. The present invention provides the desired empirical therapy for control of all bacterial infections. The invention provides antibiotic combination products for delivering at least two different antibiotics, through parenteral dosage form comprising protein-synthesis-inhibiting antibiotic which is amikacin or its sulphate salt and non-protein-synthesis-inhibiting antibiotic which is cefepime or its hydrochloride salt. The invention provides a total solution, against multiresistant *P. aeruginosa*, or *Acinetobacter* spp. and / or methicillin - resistant *S. aureus*, and are useful for intramuscular or intravenous administration as antibiotics for hospitalized patients with acute or serious infections. The pharmaceutical compositions described here normally have the least nephrotoxicity and have better efficacy and safety of cefepime plus amikacin combination.

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TITLE:

**ANTIBIOTIC COMBINATIONS FOR PROVIDING TOTAL SOLUTION TO
THE TREATMENT OF INFECTIONS**

FIELD OF THE INVENTION

The invention relates to antibiotic combination products for delivering at least two different antibiotics, wherein the products are comprised of parenteral dosage form and the two different antibiotics comprise at least one protein synthesis inhibiting antibiotic and at least one non-protein synthesis inhibiting antibiotic/bacterial cell wall synthesis inhibiting antibiotic, where in the first antibiotic amikacin present as sulphate can act synergistically with cefepime present as hydrochloride to provide a total solution against multiresistant *P. aeruginosa*, or *Acinetobacter spp.* and/or methicillin-resistant *S. aureus* are useful for intramuscular or intravenous administration as antibiotics for hospitalized patients with acute or serious infections. The pharmaceutical compositions described herein normally have the least nephrotoxicity and have better efficacy and safety of amikacin plus cefepime combination.

BACKGROUND OF THE INVENTION

Cephalosporins are one of the mainstays of antibiotic therapy, and third-generation cephalosporins are first-line agents for the treatment of many types of serious infections, including those of nosocomial origin. Gaps in activity of currently available third-generation cephalosporins such as cefotaxime, cefoperazone, ceftriaxone, and ceftazidime, and increasing reports of gram-negative bacilli resistance to some of these agents, especially *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*, make it necessary to investigate new compounds. Ceftazidime is a commonly prescribed third-generation cephalosporin used for empiric treatment of serious infections such as pneumonia, urinary tract infection, and skin and skin-structure infection but is found to be less effective alone and to develop resistance easily. Cefepime, a fourth-generation cephalosporin with a wide range of activity against gram-positive and gram-negative bacteria, including multi-resistant strains of enterobacteriaceae, is the drug of choice but has limited use due to multiresistant *P. aeruginosa* or *Acinetobacter spp.* and/or methicillin-resistant *S. aureus*. Hence, a combination of fourth generation cephalosporin along with aminoglycoside is required which has less toxicity and maximum compatability as in the present invention.

The nephrotoxic effects of aminoglycosides (particularly gentamicin and tobramycin) can be increased by the concurrent use of cephalosporins (particularly cefalotin [or cephalothin]). However some cephalosporins (cefuroxime, cefotaxime, ceftazidime and cefipime) appear not to interact adversely. (Plager JE., Cancer 1976;37: 1937-43). In present invention amikacin is used in combination with

cefepime which has lesser nephrotoxicity as compared to other combinations.(Barbhaiya RH et al. Antimicrob Agents in Chemother 1992; 36: 1382-6)

5 Febrile neutropenia is a common consequence of anticancer chemotherapy with a neutrophil count of less than 500 cells/cubic mm (Hughes et al, 1997, *level 2*). Cancer patients receiving myelosuppressive chemotherapy develop severe neutropenia and are at a high risk of developing life-threatening infections (Charnas, Luthi & Ruch, 1997, *level 1*; Cometta et al, 1996). Bacterial infections are a common cause of morbidity and mortality in neutropenic cancer patients (Freifeld & Pizzo, 1997, *level 9*), with a microbiologic cause for the febrile episode being demonstrated in approximately 40% cases (Charnas, 10 Luthi & Ruch, 1997, *level 1*). These patients are at risk of *Enterobacteriaceae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *viridans streptococci* infections (Charnas, Luthi & Ruch, 1997, *level 1*; Patrick, 1997). Since febrile neutropenic patients fail to mount a full inflammatory response, and the current diagnostic tests are not sufficiently rapid, sensitive or specific for identifying or excluding the microbial cause of a febrile episode, they may have to treated 15 empirically. The prompt institution of present invention as suitable antibiotic therapy for febrile neutropenic patients, without waiting 24 to 48 hours for the results of blood cultures, dramatically reduces infection-related morbidity and mortality in the cancer population undergoing chemotherapy.

20 Hospital-acquired pneumonia (HAP) remains the most severe nosocomial infection in intensive care units (ICUs). Some factors influencing mortality have been identified . Bacteraemia and *Pseudomonas aeruginosa* or *Acinetobacter spp.* as causative agents increase mortality. Beta-lactams alone were always considered inadequate when *P. aeruginosa* and/or methicillin-resistant *S. aureus* were implicated as pathogen(s) or copathogen(s).

25 Treatment instituted before knowing the aetiology and antimicrobial sensitivities is empirical. Therefore, present invention provides the desired empirical therapy for control of these bacterial infections in HAP patients.

30 Infectious complications are an important cause of morbidity and mortality, especially in patients with cancer with profound and prolonged neutropenia following intensive chemotherapy for haematological malignancies. Thus, prompt administration of empirical broad-spectrum antibiotics at the onset of fever in neutropenic patients with cancer has been the standard care since the 1971 report by Schimpff et al;(New England Journal of Medicine 284, 1061–5) documenting reduction in mortality rates. Combination therapy with an aminoglycoside plus an anti-pseudomonal β -lactam has commonly been recommended 35 because this approach provides broad-spectrum coverage, bactericidal activity and potential synergic effects, and minimizes the development of resistance during treatment. Piperacillin–tazobactam and ceftazidime have been used in combination with aminoglycoside like gentamycin and tobramycin ,

having nephrotoxicity and lesser efficacy in certain cases. The present invention provides a cutting edge over conventional therapies. (Cometta, A., Zinner, S., De Bock, R., Calandra, T., Gaya, H., Klastersky, J. et al. (1995) *Antimicrobial Agents and Chemotherapy* **39**, 445–52).

5 Beaucaire G et al . 1999 in *Ann Fr Anesth Reanim*; Feb;18(2):186-95 had studied comparison of cefepime (2 g x 2/day) + amikacin (7.5 mg.kg⁻¹ x 2/day) (= cefe-ami) and ceftazidime (2 g x 3/day) + amikacin (7.5 mg.kg⁻¹ x 2/day) (= cefta-ami) in patients under mechanical ventilation suffering from a nosocomial pneumonia. The efficacy rates of cefe-ami and cefta-ami combinations were similar in ICU patients under mechanical ventilation with a nosocomial pneumonia. However the cefe-ami association

10 was significantly more efficient in the population with a bacteriologically documented pneumonia. Chudanova TV et. Al 2003; *Antibiot Khimioter.*;48(7):29-32 studied the results of the use of cefepime (Maxipime) combination with amikacin vs ceftriaxon combination with amikacin in the treatment of 80 patients with different forms of hemoblastosis are presented. They found that the average period of the treatment with cefepime and amikacin equaled to 13 days (8 to 16). The treatment with cefepime + amikacin was successful in 38 out of 40 patients (95%). The average period of the treatment with

15 ceftriaxon and amikacin equaled to 14 days (7 to 18). The efficacy of the treatment with ceftriaxon + amikacin was 60% (24 patients out of 40).

Miguel A. Sanz et al for the Spanish PETHEMA Group 2002. *J Antimicrob Chemother.* Jul;50(1):79-88. In this prospective multicentre trial, 969 patients with 984 febrile neutropenic episodes were randomized to receive iv amikacin (20 mg/kg every 24 h) combined with either cefepime (2 g every 8 h) or piperacillin-tazobactam (4 g/500 mg every 6 h). Clinical response was determined at 72 h and at completion of therapy. Drug-related adverse events were reported in 10% of cefepime plus amikacin versus 11% of piperacillin-tazobactam plus amikacin patients. Mortality due to infection occurred in a

20 total of 10 patients (two cefepime, eight piperacillin-tazobactam).

Similarly Barbhuiya RH, et al. in their paper "Lack of pharmacokinetic interaction between Cefepime and Amikacin in Humans" (*Antimicrobial Agents and Chemotherapy*, July 1992, pp 1382-6), and Sanz, Miguel A, et al in their paper "Cefepime plus amikacin versus piperacillin-tazobactam plus amikacin..." (*antimicrobial agents and Chemotherapy*, 2002, pp 79-88 have mentioned about use of cefepime and amikacin co-administration.

Co-administering as mentioned in the prior art has a number of disadvantages as stated here:

A) Drugs mentioned as the combinations are administered one after the other.

B) These drugs are not available in a premixed combination. Moreover, one of the drug component

30 is available as liquid (ready to use) and other as dry powder for injection.

- C) There is complexity involved in administration of the drug as more number of pricks are required and the time of administration is also long .
- D) The chances of nephrotoxicity increases in the case of excess administration of aminoglycoside.

5 Some other shortcomings of individual administration or co-administration of amikacin and cefepime as done in the prior art are:

- a) Treatment time is prolonged to about 20 days in case of individual administration of these drugs and to about 13 days in case of co-administration.
- b) Cost to the patient is higher due to increased hospitalization time.
- 10 c) The failure rate is higher due to inconsistency of dose. Like Beaucaire G et al . 1999 used cefepime (2 g x 2/day) + amikacin (7.5 mg.kg⁻¹ x 2/day) where as amikacin (20 mg/kg every 24 h) combined with either cefepime (2 g every 8 h) was used by Miguel A. Sanz et al . 2002.

The individual administration of the amikacin and cefepime components of drugs described in the prior art fails to solve the treatment problem satisfactorily because of following reasons:

- a) The components are administered one after the other and individually in different doses .
- b) The components are administered either in equal proportions or the ratio is undefined and not fixed.
- 20 c) The success rate of such a treatment is not as per the desired levels.

Also, adequate dose is not available to the patient and the chances of development of resistance increases in the case of prior art.

It is therefore submitted that the prior art does not address typical problems to which solutions are provided by the present invention.

The individual doses of amikacin and cefepime of prior art for their defined treatment time, are costlier than the combination of present invention. A working on the cost comparison is provided for reference.

30 Accordingly, there is a need to provide a total solution by providing a pharmaceutical composition of antibiotic composition useful for intramuscular and/or intravenous administration for hospitalization patients with acute or serious bacterial infections, particularly against multiresistant *P. aeruginosa*, or *Acinetobacter supp.* and/or methicillin-resistant *S. aureus*.

35 OBJECTS AND ADVANTAGES OF INVENTION

Accordingly, the objects and advantages of the present invention are described below:

An object of the present invention is to provide an antibiotic combination product effective against acute/ serious bacterial infections.

5 Another object of the present invention is to provide pharmaceutical compositions having better efficacy and safety.

Yet another object of the present invention is to provide a pharmaceutical composition having the least nephrotoxicity.

10 Further object of the present invention is to provide pharmaceutically effective dose for intramuscular and / or intravenous administration for hospitalized patients with acute or serious infections.

Another object of the present invention is to reduce hospitalization time.

15 Yet another object of the present invention is to reduce treatment cost to patient

Other objects and advantages of the present invention will become apparent from the ensuing detailed description of the invention.

20 SUMMARY OF THE INVENTION

The invention relates to a new pharmaceutical composition, a method of treatment of infection and also a process to prepare the composition. The infectious complications are important causes of morbidity and mortality. Hospital acquired pneumonia (HAP) remains the most severe nosocomial infection in intensive care units. Beta-lactams alone are always considered inadequate when *P. aeruginosa* and / or
25 methicillin-resistant *S. aureus* are implicated as pathogens or copathogens. The present invention provides the desired empirical therapy for control of all bacterial infections. The invention provides antibiotic combination products for delivering at least two different antibiotics, through parenteral dosage form comprising protein-synthesis-inhibiting antibiotic which is amikacin or its sulphate salt and non-protein-synthesis-inhibiting antibiotic which is cefepime or its hydrochloride salt. The invention
30 provides a total solution, against multiresistant *P. aeruginosa*, or *Acinetobacter spp.* and / or methicillin – resistant *S. aureus*, and are useful for intramuscular or intravenous administration as antibiotics for hospitalized patients with acute or serious infections. The pharmaceutical compositions described here normally have the least nephrotoxicity and have better efficacy and safety of cefepime plus amikacin combination.

35

DESCRIPTION OF INVENTION

This invention relates to antibiotic compositions and the uses thereof. More particularly, this invention

relates to a composition for the parenteral delivery of two different antibiotics, and the uses of combination thereof.

In many cases, it is desirable to employ two different antibiotics in the treatment of a bacterial infection, in that such antibiotics may have complementary mechanisms of action that facilitate broad-spectrum coverage, bactericidal activity and potential synergistic effects, and to minimize the development of resistance during treatment of the severe or acute bacterial infections.

The terminology "protein synthesis inhibiting antibiotic" means an agent that disrupts the bacterial ribosome cycle through which polypeptide chain initiation and elongation is normally effected. There are multiple points in the ribosome cycle at which this can occur.

The terminology "non-protein synthesis inhibiting antibiotic" means antibiotics other than protein synthesis inhibiting antibiotics.

As non-limiting representative examples of "protein synthesis inhibiting antibiotics" there may be mentioned: the aminoglycosides such as streptomycin, amikacin, netilmicin and tobramycin; the macrolides such as erythromycin and lincomycin; the tetracyclines such as tetracycline, doxycycline, chlortetracycline, and minocycline; the oxalidinones such as linezolid; fusidic acid; and chloramphenicol.

As non-limiting representative examples of "non-protein synthesis inhibiting antibiotics", there may be mentioned : the beta-lactam penicillins such as penicillin, dicloxacillin, and ampicillin; the beta lactam cephalosporins such as cefepime, ceftazidime, cefotaxime, cefuroxime, cefaclor, and ceftriaxone; the beta lactam carbapenems such as imipenem and meropenem; the quinolones such as ciprofloxacin, moxifloxacin, and levofloxacin; the sulfonamides such as sulfanilamide and sulfamethoxazole; metronidazole; rifampin; vancomycin; and nitrofurantoin.

The antibiotics may be in the form of a pharmaceutically acceptable salt. Pharmaceutically acceptable salts means which can be generally used as salts of an antibiotic in pharmaceutical industry, including for example, salts of sodium, potassium, calcium and the like, and amine salts of procaine, dibenzylamine, ethylenediamine, ethanolamine, methylglucamine, taurine, and the like, as well as acid addition salts such as hydrochlorides, and basic amino acids and the like.

The different embodiments of the present invention are described below in details.

This invention basically provides an antibiotic combination for providing total solution to the treatment of infections, where said combination is pharmaceutically acceptable, comprising:

- a) first antibiotic and second antibiotic, wherein said first antibiotic is protein-synthesis-inhibiting antibiotic and said second antibiotic is non-protein-synthesis-inhibiting antibiotic;
- b) said combination further comprising a stabilizing agent.

5 In this antibiotic combination, said first antibiotic is selected from the group consisting of the aminoglycosides such as gentamycin, kanamycin, netilmicin, streptomycin, amikacin and tobramycin; and the macrolides such as erythromycin and lincomycin; and the tetracyclines such as tetracycline, doxycycline, chlortetracycline, and minocycline; and the oxalidinones such as linezolid; and fusidic acid; and chloramphenicol.

10

In this antibiotic combination said first antibiotic is preferably aminoglycoside, which is selected from the group consisting of gentamycin, amikacin, tobramycin, kanamycin, and netilmicin, or a pharmaceutically acceptable salt of any of these, more preferably amikacin or a pharmaceutically acceptable salt thereof.

15

In this antibiotic combination said pharmaceutically acceptable salt of amikacin is sulphate salt, that is amikacin sulphate. Amikacin sulfate is a semi-synthetic aminoglycoside antibiotic derived from kanamycin, having a chemical name D-Streptamine, O-3-amino-3-deoxy- α -D-glucopyranosyl(1 $\rightarrow$ 6)-O-[6-amino-6-deoxy- α -D-glucopyranosyl(1 $\rightarrow$ 4)]-N¹-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (S)-, sulfate (1:2)(salt), and has the molecular formula $C_{22}H_{43}N_5O_{13} \cdot 2H_2SO_4$ with a molecular weight of 781.76.

20

In this antibiotic combination, said second antibiotic is selected from the group consisting of the beta-lactam penicillins such as penicillin, dicloxacillin, and ampicillin; the beta lactam cephalosporins such as cefepime, ceftazidime, cefotaxime, cefuroxime, cefaclor, and ceftriaxone; the beta lactam carbapenems

25 such as imipenem and meropenem; the quinolones such as ciprofloxacin, moxifloxacin, and levofloxacin; the sulfonamides such as sulfanilamide and sulfamethoxazole; metronidazole; rifampin; vancomycin; and nitrofurantoin.

30 In this antibiotic combination said beta-lactam cephalosporin is preferably cefepime, more preferably pharmaceutically acceptable salt of cefepime such as hydrochloride salt of cefepime, that is cefepime hydrochloride. Cefepime hydrochloride is a semi-synthetic, broad spectrum, cephalosporin antibiotic for parenteral administration, has a chemical name 1-[[[(6R,7R)-7-[2-(2-amino-4-thiazolyl)-glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-pyrrolidinium chloride, 7²-

35 (Z)-(O-methyloxime), monohydrochloride, monohydrate, and which is a white to pale yellow powder with a molecular formula of $C_{19}H_{25}ClN_6O_5S_2 \cdot HCl \cdot H_2O$ and a molecular weight of 571.5 and is highly soluble in water.

In this antibiotic combination, the weight ratio of said first antibiotic to said second antibiotic is in the range from about 1:5 to about 5:1, preferably from about 1:4 to about 4:1, more preferably from about 1:3 to about 3:1.

5

The antibiotic combination of this invention further comprises a stabilizing agent such as l-arginine wherein the weight of l-arginine, is in the range of about 35% to about 75% of the combined weight of said first antibiotic and second antibiotic .

10 In this said antibiotic combination, the dosage form is a parenteral dosage form. The total said dosage is administered preferably twice a day to a patient, spread over a period of 12 hours in a day depending upon the patient condition and severity of infection.

15 In this antibiotic combination said first antibiotic and said second antibiotic, when released from said parenteral dosage form, reach the maximum serum concentration almost simultaneously with the release.

Alternatively in this antibiotic combination, said first antibiotic and said second antibiotic, when released from said parenteral dosage form, reach the maximum serum concentration in the time-range
20 from about 30 minutes to about 180 minutes, preferably from about 30 minutes to about 90 minutes.

An embodiment of the present invention includes an antibiotic combination, wherein

- 25 a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 50 mg to about 75 mg, calculated as amikacin free acid;
- (b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 250 mg to about 500 mg, calculated as cefepime free acid; and
- 30 (c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount in the range from about 75 mg to about 150 mg; and wherein said antibiotic combination is reconstituted with 3 ml of water for injection.

Another embodiment of the present invention includes an antibiotic combination, wherein

- 35 (a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 100 mg to about 150 mg, calculated as amikacin free acid;
- (b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which

is present in an amount in the range from about 500 mg to about 1 g, calculated as cefepime free acid; and

- (c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount in the range from about 150 mg to about 300 mg; and wherein said antibiotic combination is reconstituted with 5 ml of water for injection.

Yet another embodiment of the present invention includes an antibiotic combination, wherein

- a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 200 mg to about 300 mg, calculated as amikacin free acid;
- b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 1 g to about 2 g, calculated as cefepime free acid; and
- c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount in the range from about 300 mg to about 600 mg; and wherein said antibiotic combination is reconstituted with 10 ml of water for injection.

Still another embodiment of the present invention includes an antibiotic combination, wherein

- a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount of about 400 mg to 600 mg, calculated as amikacin free acid;
- b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount of about 2 g to 4 g, calculated as cefepime free acid; and
- c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount of about 600 mg to 1.2 g; and wherein said antibiotic combination is reconstituted with from about 20 ml of water for injection.

In the present invention, a pharmaceutically effective unit dose of said antibiotic combination, in the form of the concentrate of said dose, is provided in a sealed airtight container which is selected from the group consisting of a vial, a mono vial, an ampoule, a syringe, a packet, a pouch and an auto-injector, wherein said container has a head space volume sufficient for introduction of appropriate volume of an aqueous solvent sufficient to form a unit/ multiple dose in the form of an appropriate reconstituted solution of said antibiotic combination.

In the present invention, said antibiotic combination is provided in the form of a dry sterilized powder, in a sealed airtight container to form a pharmaceutically acceptable required fixed dose combination for reconstitution prior to intramuscular or intravenous administration for the treatment of the acute or serious bacterial infections. The combination dosage form after reconstitution is a sterile, colorless to

light straw colored solution with the pH of the constituted solution being in the range from about 3.5 to about 6.5

5 In the present invention, the antibiotic combination is alternatively provided in a sealed container such as transparent glass vial capped with appropriate halogenated stopper and seal, and is used for reconstitution for intramuscular or intravenous administration for the treatment of acute or serious bacterial infections.

10 Regarding the fill volume of the sealed airtight container, the antibiotic combination of present invention is provided in a reconstituted form in a sealed airtight container which is selected from the group consisting of a vial, a mono vial, an ampoule, a syringe, a packet, a pouch and an auto-injector, wherein interior space of said container comprises a fill volume occupied by said composition in reconstituted form and a head space volume occupied aseptically by an inert-gas-limited microatmosphere, which comprises essentially one or more inert gas which is selected from the group
15 of noble gases and nitrogen; preferably nitrogen, volume of said nitrogen gas being not more than 5% of said head space volume, and wherein ratio of said fill volume to said head space volume is not less than 1:1.

20 In the present invention of the antibiotic combination, said amikacin or said pharmaceutically acceptable salt thereof, and said cefepime or said pharmaceutically acceptable salt thereof, are present in pharmaceutically effective total amount corresponding to a single unit/multiple dose, in said sealed container, filled aseptically under inert gas blanket.

25 A method of treating a subject, having a condition or disorder, wherein a treatment with amikacin or a pharmaceutically acceptable salt thereof and cefepime or a pharmaceutically acceptable salt thereof, is indicated, which method comprises parenterally administering therapeutically effective amount of the combination of these two antibiotics to mammals.

30 An embodiment of the present invention includes a process for preparing an antibiotic combination for providing total solution to the treatment of acute or serious bacterial infections, comprising the steps of:
(a) sterile filling / blending first and second active ingredients, wherein said first active ingredient comprises a first antibiotic which is amikacin or a pharmaceutically acceptable salt thereof, preferably sulphate salt; and wherein said second ingredient is a second antibiotic which is cefepime or a pharmaceutically acceptable salt thereof, preferably cefepime hydrochloride,
35 (b) sterile adding/blending a stabilizing agent such as l-arginine, wherein the weight of said l-arginine is in the range of about 35% to about 75% of the combined weight of said first antibiotic and said second antibiotic,

- (c) continuing said sterile blending for a period ranging from about 1 hour to about 4 hours;
- (d) proportioning the sterile fill / blend of step (a) to get desired pharmaceutically effective dose in weight ratio of said first active ingredient to said second active ingredient in the range from about 1:5 to about 5:1, preferably from about 1:4 to about 4:1, more preferably from about 1:3 to about 3:1, and.
- (e) capping aseptically with pre-post inert gassing.

In accordance with one preferred embodiment of the invention, the average period of the treatment with amikacin and cefepime equaled to 9 days (8 to 10). The treatment with amikacin and cefepime is successful in more than 95% patients.

In general, the invention is available as sterile fill / blend of two or more dry powders to be reconstituted before injection with suitable solvent.

In a preferred embodiment, the antibiotic product is preferably twice a day product, wherein the administration of the antibiotic product is a concentrate which is diluted before administration in suitable infusions; such as 0.9% Sodium Chloride, 5% Dextrose Injection, 0.5% or 1.0% Lidocaine Hydrochloride, or Sterile Bacteriostatic Water for Injection with Parabens or Benzyl Alcohol. The preferred regimen is that the product is administered twice over a twelve hour period depending upon the patient condition and severity of infection.

One of the key features of the present invention is that both the cefepime and amikacin exist in dry powder form along with a stabilizing agent. The combination is a single product as compared to the prior art which already states about co-administration of two different injection, that is cefepime available as dry powder injection and amikacin available as liquid ready to use injection.

A novel feature of the present invention is the combination of amikacin and cefepime which is being used as a predetermined ratio for the first time. The stability has been worked out. A separate accelerated stability report is being attached for the reference.

Another novel feature of the present invention is that by using the combination of amikacin and cefepime, the duration of treatment of a patient is reduced thereby reducing hospitalization time of patients. Moreover, it increases turnover per bed in hospitals as more number of patients can be treated in same duration.

It has also been observed by the inventor that the combination of amikacin and cefepime of the present invention has higher rate of success than the administration of the components of the combination

individually. Results of comparative susceptibility studies are provided.

It has further been observed by the inventor that there is no organ deformity or toxicity reported after administration of the dose of the present invention. Reference data is provided.

A part of product development data is provided in support of better efficacy against some of the bacterial range. Experimental study details are given in connection with the present invention in tables 1 to 12.

Bacterial Susceptibility Test:

Bacterial Susceptibility Test was performed for amikacin and cefepime with the Batch No. RTA02. The combination of amikacin and cefepime on different microorganisms was taken to analyze the efficiency of combination in comparison with the individual components of the drug. Different concentrations were selected mentioned to as highest, high, low and lowest in the data. Zone size was determined in mm. The activity of amikacin and cefepime is best seen in *E.coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Pseudomonas auriginosa*, *Staphylococcus aureus*. It was found that the combination works better than the individual components.

Ratio determining Test:

Ratio determining Test was performed for amikacin and cefepime with the Batch No. RTA02. The different ratio of amikacin and cefepime on different microorganisms was taken. Bacterial Lytic Zones for different ratios were determined in mm. The quantity of amikacin and cefepime was taken to be 20mcg. Bacterial Lytic Zone was best determined in the combination of Amikacin Cefepime taken in 1:4 ratio.

Stability Tests:

The accelerated stability tests were carried out on amikacin and cefepime combination for a duration of six months. The combination was found to be stable.

Toxicity Tests:

Animal behaviour, acute and sub acute toxicity tests were carried out on rodents. A combination dose up to 30 times higher than proposed human dose was given to the experimental animals. The combination in the proposed dose was found to be non toxic.

Table 1

Bacterial Susceptibility Data, Amikacin Cefepime , Batch No. RTA02
Amikacin Cefepime Combination on *E. coli*

<i>Concentration</i>	<i>Cefepime Zone (mm)</i>	<i>Amikacin Zone (mm)</i>	<i>Combination Zone (mm)</i>
Highest	32.24	30.90	32.85
High	30.16	29.02	31.12
Low	24.20	22.06	24.80
Lowest	16.42	15.02	17.22

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Table 2

Bacterial Susceptibility Data, Amikacin Cefepime , Batch No. RTA02
Amikacin Cefepime Combination on *Klebsiella pneumoniae*

<i>Concentration</i>	<i>Cefepime Zone (mm)</i>	<i>Amikacin Zone (mm)</i>	<i>Combination Zone (mm)</i>
Highest	36.02	34.98	36.62
High	32.35	31.25	33.78
Low	31.96	31.02	32.00
Lowest	30.83	29.98	31.56

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Table 3

Bacterial Susceptibility Data, Amikacin Cefepime Batch No. RTA02
Amikacin Cefepime Combination on *Streptococcus pneumoniae*

<i>Concentration</i>	<i>Cefepime Zone (mm)</i>	<i>Amikacin Zone (mm)</i>	<i>Combination Zone (mm)</i>
Highest	30.32	26.58	32.54
High	28.10	24.28	30.12
Low	23.02	18.34	23.27
Lowest	19.54	12.32	21.66

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Table 4

Bacterial Susceptibility Data, Amikacin Cefepime Batch No. RTA02
Amikacin Cefepime Combination on *Enterococcus faecalis*

<i>Concentration</i>	<i>Cefepime Zone (mm)</i>	<i>Amikacin Zone (mm)</i>	<i>Combination Zone (mm)</i>
Highest	28.98	27.29	30.04
High	25.52	23.24	25.97
Low	19.92	17.97	20.51
Lowest	12.65	08.05	16.98

Table 5

Bacterial Susceptibility Data, Amikacin Cefepime Batch No. RTA02
Amikacin Cefepime Combination on *Pseudomonas auriginosa*

<i>Concentration</i>	<i>Cefepime Zone (mm)</i>	<i>Amikacin Zone (mm)</i>	<i>Combination Zone (mm)</i>
Highest	30.94	26.93	31.06
High	27.56	21.81	29.10
Low	18.81	16.05	20.46
Lowest	12.08	09.81	14.99

Table 6

Bacterial Susceptibility Data, Amikacin Cefepime Batch No. RTA02
Amikacin Cefepime Combination on *Staphylococcus aureus*

<i>Concentration</i>	<i>Cefepime Zone (mm)</i>	<i>Amikacin Zone (mm)</i>	<i>Combination Zone (mm)</i>
Highest	31.06	28.92	32.80
High	28.44	24.42	30.08
Low	21.18	18.79	23.27
Lowest	16.24	10.09	17.41

Table 7
Survival Data, Amikacin Cefepime Batch No. RTA02
Swiss Albino Mice

<i>Group</i>	<i>Control</i>	<i>X</i>	<i>2X</i>	<i>10X</i>	<i>20X</i>	<i>30X</i>
Dose	Diluent	0.9 mg/20g	1.8 mg/20g	9.0 mg/20g	18.0 mg/20g	27.0 mg/20g
Day	No. of surviving mice / Initial no. of mice					
0	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
1	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
2	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
3	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
4	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
5	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	5 of 6
7	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	5 of 6

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Table 8
Ratio Determining Studies showing Bacterial Lytic Zone

<i>Amikacin : Cefepime Ratio</i>	<i>Klebsiella pneumoniae (mm)</i>	<i>Streptococcus pneumoniae</i>	<i>Enterococcus faecalis</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>E.coli (mm)</i>
1:1	26	23	23	21	24	26
1:2	29	25	27	26	26	25
1:3	32	31	29	29	30	28
1:4	35	32	31	31	33	30
4:1	34	27	26	30	32	29
4:2	31	23	27	26	25	28
4:3	29	29	29	27	30	27

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Table 9
Acute Toxicity Data
 Amikacin Cefepime , Batch No. RTA02

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Animal Behavior Data

<i>Day</i>	<i>Control</i>	<i>X</i>	<i>2X</i>	<i>10X</i>	<i>20X</i>	<i>30X</i>	<i>Remarks</i>
Dose		0.9 mg/20g	1.8 mg/20g	9.0 mg/20g	18 mg/20g	27 mg/20g	
1 Day	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Aggressive	OK
2Day	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Aggressive	OK
3 Day	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Aggressive	OK
4Day	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	OK
5 Day	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	OK
6 Day	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	OK
7Day	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	Normal Behavior	OK

Table 10
Acute Toxicity Data
 Amikacin Cefepime , Batch No. RTA02
SUMMARY OF BODY WEIGHT (g) for Swiss Albino Mice

<i>Group</i>	<i>Control</i>	<i>X</i>	<i>2X</i>	<i>10X</i>	<i>20X</i>	<i>30X</i>
Dose	Diluent	0.9 mg/20g	1.8 mg/20g	9.0 mg/20g	18 mg/20g	27 mg/20g
Day						
0	20.52	20.54	20.56	20.57	20.60	20.63
	6	6	6	6	6	6
1	20.50	20.51	20.55	20.56	20.58	20.61
	6	6	6	6	6	6
2	20.50	20.52	21.54	21.54	21.55	21.58
	6	6	6	6	6	6
3	20.51	20.50	20.53	20.52	20.50	20.50
	6	6	6	6	6	6
4	20.51	20.51	20.53	20.48	20.47	20.39
	6	6	6	6	6	6
5	20.45	20.44	20.41	20.39	20.36	20.33
	6	6	6	6	6	6
6	20.44	20.43	20.41	20.37	20.31	20.27
	6	6	6	6	6	5
7	20.43	20.43	20.39	20.37	20.26	20.16
	6	6	6	6	6	5

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Table 11**Average cost comparison of Prior art v/s invention**

<i>Prior art</i>	<i>Invention</i>	<i>Cost Saved</i>
Average hospitalization time 13 days @ Rs-1000/ day= Rs-13000/-	Average hospitalization time 9 days @ Rs-1000/ day= Rs-9000/-	Rs-4000/- per hospital admission
Average cost of Cefepime 2g @250 bd for 13 days and Amikacin 500mg @65 bd for 13 days= 250x2x13= Rs-6500/- +65x2x13= Rs-1690/- Total cost= Rs-8190/-	Average cost of Cefepime Amikacin @350 bd for 9 days= 350x2x9= Rs-6300/- Total cost= Rs- 6300/-	Rs-1890/- per treatment cost

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Table 12
STABILITY DATA

Generic name of product: Cefepime & Amikacin for Inj.

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Batch No. : RTA02

Date of Mfg. : 10/2004 Date of Exp. : 09/2006 Date of initiating: 29.10.2004

Packaging : glass vial

Period (mo)	Storage Conditions	Description	Identification	Particulate matter	pH (3.0-6.0)	BET	Sterility	(a) Assay	
								90% to 110% of labelled amount of Cefepime	90% to 110% of labelled amount of Amikacin
Initial	-	A cream colored powder	Passes test	Passes test	4.52	Passes test	Passes test	100.1	99.8
1.	40°C/75%RH	A dark cream colored powder	-do-	-do-	4.48	-do-	-do-	99.5	99.3
2.	40°C/75%RH	A cream colored powder with yellowish tinge	-do-	-do-	4.41	-do-	-do-	99.0	98.5
3.	40°C/75%RH	A light yellow colored powder	-do-	-do-	4.31	-do-	-do-	98.2	97.6
6.	40°C/75%RH	A light yellow colored powder	-do-	-do-	3.91	-do-	-do-	97.4	96.9

- 10 **REMARKS:** 1. All procedure carried out as per STP.
2. Product as stable for 6 month at 40°/75%RH

15 While the above description contains many specificities, these should not be construed as limitations in the scope of the invention but as exemplifications of embodiments thereof. Many other variations are possible. Accordingly, the scope of the invention should be determined not by the embodiments illustrated, but by the appended claims and their legal equivalents.

We Claim:

1. An antibiotic combination for providing total solution to the treatment of acute or serious infections, where said combination is pharmaceutically acceptable, comprising :
 - 5 a. first antibiotic and second antibiotic, wherein said first antibiotic is a protein synthesis inhibiting antibiotic and said second antibiotic is non-protein synthesis inhibiting antibiotic;
 - b. a stabilizing agent.
- 10 2. An antibiotic combination, as claimed in Claim 1, wherein said first antibiotic is selected from the group consisting of the aminoglycosides such as netilmicin, kanamycin, gentamycin, streptomycin, amikacin, and tobramycin; and the macrolides such as erythromycin and lincomycin; and the tetracyclines such as tetracycline, doxycycline, chlortetracycline, and minocycline; and the oxalidinones such as linezolid; and fusidic acid; and chloramphenicol.
- 15 3. An antibiotic combination, as claimed in Claims 1 or 2, wherein said second antibiotic is selected from the group consisting of the beta-lactam penicillins such as penicillin, dicloxacillin, and ampicillin; the beta lactam cephalosporins such as cefepime, ceftazidime, cefotaxime, cefuroxime, cefaclor, and ceftriaxone; the beta lactam carbapenems such as imipenem and meropenem; the quinolones such as ciprofloxacin, moxifloxacin, and levofloxacin; the sulfonamides such as sulfanilamide and sulfamethoxazole; metronidazole; rifampin; vancomycin; and nitrofurantoin.
- 20 4. An antibiotic combination, as claimed in Claim 2, wherein said first antibiotic is preferably said aminoglycoside, which is selected from the group consisting of streptomycin, gentamycin, amikacin, tobramycin, kanamycin, and netilmicin, or a pharmaceutically acceptable salt of any of these, more preferably amikacin or a pharmaceutically acceptable salt thereof which is amikacin sulphate, wherein said amikacin sulfate is a semi-synthetic aminoglycoside antibiotic derived from kanamycin, having a chemical name D-Streptamine, O-3-amino-3-deoxy- α -d-glucopyranosyl(1 $\rightarrow$ 6)-O-[6-amino-6-deoxy- α -D-glucopyranosyl(1 $\rightarrow$ 4)]-N¹-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (S)-, sulfate (1:2)(salt), and has the molecular formula C₂₂H₄₃N₅O₁₃ • 2 H₂SO₄ with a molecular weight of 781.76.
- 30 5. An antibiotic combination, as claimed in Claim 3, wherein said beta-lactam cephalosporin is preferably cefepime, more preferably pharmaceutically acceptable salt of cefepime, which is cefepime hydrochloride, wherein said cefepime hydrochloride is a semi-synthetic, broad spectrum, cephalosporin antibiotic for parenteral administration, has a chemical name 1-

[[[(6R,7R)-7-[2-(2 - amino - 4- thiazolyl) - glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-en-3-yl] methyl]-1-methyl-pyrrolidinium chloride, 7²- (Z)-(O-methyloxime), monohydrochloride, monohydrate, and which is a white to pale yellow powder with a molecular formula of C₁₉H₂₅ClN₆O₅S₂•HCl•H₂O and a molecular weight of 571.5 and is highly soluble in water.

6. An antibiotic combination, as claimed in any of Claims 1 to 5, wherein the weight ratio of said first antibiotic to said second antibiotic is in the range from about 1:5 to about 5:1, preferably from about 1:4 to about 4:1, more preferably from about 1:3 to about 3:1 .

7. An antibiotic combination, as claimed in any of Claims 1 to 6 wherein said stabilizing agent is l-arginine wherein the weight of l-arginine is in the range of about 35% to about 75% of the combined weight of said first antibiotic and said second antibiotic.

8. An antibiotic combination, as claimed in any of Claims 1 to 7, wherein a dosage form of said antibiotic combination is a parenteral dosage form, wherein total said dosage is administered preferably twice a day to a patient, spread over a period of 12 hours in a day depending upon the patient condition and severity of infection.

9. An antibiotic combination, as claimed in Claim 8, wherein said first antibiotic and said second antibiotic, when released from said parenteral dosage form, reach the maximum serum concentration almost simultaneously in the time-range from about 30 minutes to about 180 minutes, preferably from about 30 minutes to about 90 minutes.

10. An antibiotic combination, as claimed in any of Claims 1 to 9, wherein

- (a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 50 mg to about 75 mg, calculated as amikacin free acid;
- (b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 250 mg to about 500 mg, calculated as cefepime free acid; and
- (c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount in the range from about 75 mg to about 150 mg; and wherein said antibiotic combination is reconstituted with 3 ml of water for injection.

11. An antibiotic combination, as claimed in any of Claims 1 to 9, wherein

- (a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is

present in an amount in the range from about 100 mg to about 150 mg, calculated as amikacin free acid;

- (b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 500 mg to about 1 g, calculated as cefepime free acid; and
- (c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount in the range from about 150 mg to about 300 mg; and wherein said antibiotic combination is reconstituted with 5 ml of water for injection.

12. An antibiotic combination, as claimed in any of Claims 1 to 9, wherein

- (a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 200 mg to about 300 mg, calculated as amikacin free acid;
- (b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 1 g to about 2 g, calculated as cefepime free acid; and
- (c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount in the range from about 300 mg to about 600 mg; and wherein said antibiotic combination is reconstituted with 10 ml of water for injection.

13. An antibiotic combination, as claimed in any of Claims 1 to 9, wherein

- (a) said first antibiotic is amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount of about 400 mg to 600 mg, calculated as amikacin free acid;
- (b) said second antibiotic is cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount of about 2 g to 4 g, calculated as cefepime free acid; and
- (c) said composition further comprising l-arginine as stabilizing agent, which is present in an amount of about 600 mg to 1.2 g; and wherein said antibiotic combination is reconstituted with from about 20 ml of water for injection.

14. An antibiotic combination, as claimed in any of Claims 1 to 13, wherein a pharmaceutically effective unit/ multiple dose of said combination, in the form of the concentrate of said dose, wherein said dose is provided in a sealed airtight container which is selected from the group consisting of a vial, a mono vial, an ampoule, a syringe, a packet, a pouch and an auto-injector, wherein said container has a head space volume sufficient for introduction of appropriate volume of an aqueous solvent sufficient to form a unit dose in the form of an appropriate reconstituted solution of said combination.

15. An antibiotic combination, as claimed in any of Claims 1 to 14, wherein said combination is provided in the form of a dry sterilized powder, in said sealed airtight container to form a pharmaceutically acceptable required fixed dose combination for reconstitution prior to intramuscular or intravenous administration for the treatment of the acute or serious bacterial infections, wherein the combination dosage form after reconstitution is a sterile, colorless to light straw colored solution with the pH of the constituted solution being in the range from about 3.5 to about 6.5.
16. An antibiotic combination, as claimed in any of Claims 1 to 15, wherein said combination is provided in said sealed container wherein said vial is made of transparent glass and is capped with appropriate halogenated stopper and seal, and is used for reconstitution for intramuscular or intravenous administration for the treatment of the acute or serious bacterial infections in mammals.
17. An antibiotic combination, as claimed in any of Claims 1 to 16, wherein said combination is provided in a reconstituted form in said sealed airtight container which is selected from said group consisting of said vial, said mono vial, said ampoule, said syringe, said packet, said pouch, and said auto-injector, wherein interior space of said container comprises a fill volume occupied by said composition in reconstituted form and a head space volume occupied aseptically by an inert-gas-limited microatmosphere, which comprises essentially one or more inert gas which is selected from the group consisting of noble gases and nitrogen; preferably nitrogen, volume of said nitrogen gas being not more than 5% of said head space volume, and wherein ratio of said fill volume to said head space volume is not less than 1:1.
18. An antibiotic combination, as claimed in any of Claims 1 to 17 wherein said amikacin or said pharmaceutically acceptable salt thereof, and said cefepime or said pharmaceutically acceptable salt thereof, are present in pharmaceutically effective total amount corresponding to a single unit or multiple dose, in said sealed container, filled aseptically under inert gas blanket.
19. A process for preparing an antibiotic combination for providing total solution to the treatment of infections, comprising the steps of:
- (a) sterile filling / blending first and second active ingredients, wherein said first active ingredient comprises a first antibiotic which is amikacin or a pharmaceutically acceptable salt thereof, preferably sulphate salt; and wherein said second active ingredient is a second antibiotic which is cefepime or a pharmaceutically acceptable salt thereof, preferably cefepime hydrochloride, and
 - b) sterile adding/blending a stabilizing agent such as l-arginine, wherein the weight of said l-

arginine is in the range of about 35% to about 75% of the combined weight of said first antibiotic and said second antibiotic,

- c) continuing said sterile blending for a period ranging from about 1 hour to about 4 hours;
- d) proportioning the sterile fill / blend of step (a) to get desired pharmaceutically effective dose in weight ratio of said first active ingredient to said second active ingredient in the range from about 1:5 to about 5:1, preferably from about 1:4 to about 4:1, more preferably from about 1:3 to about 3:1, and
- e) capping aseptically with pre-post inert gassing.

- 10 20. An antibiotic combination for providing total solution to the treatment of acute or serious infections, substantially as herein described and illustrated.
- 21. A process for preparing an antibiotic combination for providing total solution to the treatment of infections, substantially as herein described and illustrated.
- 15 22. A method of treating a subject having a condition or disorder wherein a treatment with amikacin and cefepime is indicated, substantially as herein described and illustrated.

AMENDED CLAIMS**received by the International Bureau on 29 May 2006 (29.05.2006)**

We claim:

1. A pharmaceutical composition suitable for parenteral injection for use as antimicrobial fixed dose combination therapy for a mammal including a human being, comprising a dry powder premix for injection ready after reconstitution with a volume of fluid preferably by water for injection, of an improved efficacy combination, preferably a synergistic combination, of antibiotics further comprising:
 - a. at least one protein synthesis inhibiting antibiotic,
 - b. which is mixed in a weight ratio of about 1:5 to about 5:1, preferably from about 1:4 to about 4:1, more preferably from about 1:3 to about 3:1 with at least another compatible antibiotic that is not a protein synthesis inhibiting antibiotic, excluding cefsulodin sodium
 - c. with addition of one or more of a stabilizing agents including amino acids comprising Arginine, L-Lysine, Histidine, Leucine, Cysteine and the like.
2. A composition of claim 1 wherein:
 - a. the said protein synthesis inhibiting antibiotic is selected from a group of aminoglycosides comprising netilmicin, kanamycin, gentamycin, streptomycin, amikacin, and tobramycin; the macrolides comprising as erythromycin and lincomycin; the tetracyclines comprising tetracycline, doxycycline, chlortetracycline, and minocycline; the oxaxolidinones comprising linezolid; fusidic acid;
 - b. the said another compatible antibiotic that is not a protein synthesis inhibiting antibiotic is selected from a beta-lactam antibiotic comprising a beta-lactam penicillins further comprising

penicillin, dicloxacillin, and ampicillin; a beta lactam cephalosporins further comprising cefepime, ceftazidime, cefotaxime, cefuroxime, cefaclor, and ceftriaxone; a beta lactam carbapenem further comprising imipenem and meropenem; a quinolone further comprising ciprofloxacin, moxifloxacin, levofloxacin; the sulfonamides further comprising sulfanilamide and sulfamethoxazole;; vancomycin; or nitrofurantoin,

- c. the said stabilizing agent is l-arginine wherein the weight of l-arginine is in the range of about 35% to about 75% of the combined weight of the antibiotics in the composition.

3. A composition of claim 2 wherein:

- a. the preferred protein synthesis inhibiting antibiotic is preferably an aminoglycoside comprising streptomycin, gentamycin, amikacin, tobramycin, kanamycin, and netilmicin, or a pharmaceutically acceptable salt of any of these; more preferably amikacin or a pharmaceutically acceptable salt thereof which is amikacin sulphate, wherein said amikacin sulfate is a semi-synthetic aminoglycoside antibiotic derived from kanamycin, having a chemical name D-Streptamine, O-3-amino-3-deoxy-a-b-glucopyranosyl(1>6)-O-[6-amino-6-deoxy-a-D-glucopyranosyl(1>4)]-N1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-(S)-,sulfate (1:2)(salt), and has the molecular formula $C_{22}H_{43}N_5O_{13} \cdot 2H_2SO_4$ with a molecular weight of 781.75,
- b. the preferred antibiotic having no protein synthesis inhibiting activity is a beta-lactam cephalosporin, more preferably cefepime, further more preferably pharmaceutically acceptable salt of cefepime, which is cefepime hydrochloride, wherein said cefepime hydrochloride is a semi-synthetic, broad spectrum, cephalosporin antibiotic for parenteral administration, has a chemical name 1-

[[[(6R,7R)-7-[2-(2-amino-4-thiazolyl) -glyoxylamido] -2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 72_ (Z)-(O-methyloxime), monohydrochloride, monohydrate, and which is a white to pale yellow powder with a molecular formula of C₁₉H₂₅ClN₆O₅S₂•HCl•H₂O, a molecular weight of 571.5 and is highly soluble in water.

4. A single dose composition of claim 3 wherein:

- a. amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 50 mg to about 75 mg, calculated as amikacin free acid;
- b. cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 250 mg to about 500 mg, calculated as cefepime free acid;
- c. l-arginine as stabilizing agent, which is present in an amount in the range from about 75 mg to about 150 mg.

5. A multiple dose composition of claim 3 wherein:

- a. amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 100 mg to about 150 mg, calculated as amikacin free acid;
- b. cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 500 mg to about 1g, calculated as cefepime free acid;
- c. l-arginine as stabilizing agent, which is present in an amount in the range from about 150 mg to about 300 mg.

6. A multiple dose composition of claim 3 wherein:

- a. amikacin or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 400 mg to about 600 mg, calculated as amikacin free acid;
- b. cefepime or a pharmaceutically acceptable salt thereof, which is present in an amount in the range from about 2g to about 4g, calculated as cefepime free acid;
- c. l-arginine as stabilizing agent, which is present in an amount in the range from about 600 mg to about 1200 mg.

7. A method of treating bacterial infections in mammals including a human being by injecting a composition of claim 1, or 2, or 3, or 4 or 5 or 6 parenterally, either intravenously or intramuscularly.

8. A composition of claim 1, or 2 or 3 or 4 or 5 or 6 packed and sealed in a sterile container under blanket of inert gas, where the said container is one or more of a vial, an ampoule, a syringe, a packet, a pouch, and an auto-injector, wherein interior space of said container comprises a fill volume occupied by said composition in reconstituted form and a head space volume occupied aseptically by an inert-gas-limited microatmosphere, which comprises essentially one or more inert gas which is selected from the group consisting of noble gases and nitrogen; preferably nitrogen, volume of said nitrogen gas being not more than 5% of said head space volume, and wherein ratio of said fill volume to said head space volume is not less than 1:1.

9. A process for preparing a premixed antibiotic combination of composition of claim 1, or 2, or 3, or 4 or 5 or 6 comprising the steps of:

- (a) sterile filling / blending the antibiotic ingredients or pharmaceutically acceptable salts thereof,

- (b) sterile adding/blending a stabilizing agent such as l-arginine
- (c) continuing said sterile adding / filling / blending for a period ranging from about 1 hour to about 4 hours,
- (d) proportioning the sterile fill / blend, and
- (e) capping aseptically with pre-post inert gassing.

Statement under Article 19(1) Rule 46

Following statement explains the details of amendments in the filed Claims.

AMENDMENT IN CLAIMS.

> **Reason for Amendment:** - Amendment has been carried out in view of the observation made by the International Search Authority (European Patent office) in Re Item V.2.1, V.2.2 V.2.3 & V.2.4 of its written opinion with regards to novelty, inventive step and industrial applicability.

Amendment: Presently pending Claims 1-22 have been replaced by amended Claims 1-9.

Explanation: It is very clear from the documents cited that despite the knowledge of combined efficacy of the antibiotics claimed in the composition of the invention, and knowledge on their synergistic efficacy, for a long period of 1981 to the date of this patent application, established practice was to administer these antibiotics invariably separately in separate pricks, and there was not a single report of use of them of injecting in a single prick as a single premixed composition mainly because of lack of knowledge on safe dosages and on how to ensure the compatibility and stability of the ingredients in such a premix.

Such a knowledge was not an obvious one and was a subject matter of an invention, this invention has been done for the first time to produce a technical effect of improved efficacy in lesser pricks and to bring convenience in treatment and an early and better relief to a patient, the kind of which was never achieved in the past with prior art methods.

In order to reflect this inventive step, the claims are redrafted and presented in proper context. This has been done by replacing the entire text of claims with new ones, which are annexed separately with this response.

Effect of Amendment:

With this explanation, and amendments in wording, where the word "combination" has been replaced by the phrase *"A pharmaceutical composition suitable for parenteral injection for use as antimicrobial fixed dose combination therapy..."* in Claim 1 which is also further qualified and

defined in further part of Claim 1, the principle claim now specifically points out to a product as a fixed dose premix to be administered through a single prick.

Further, the amended Claim nos. 2 to 9, are dependent on Claim 1. If principle claim does not infringe upon a document, dependent claims also do not infringe upon that document. Thus, none of the amended claims infringe any of the cited documents i.e. D1-14.

In view of the above amendments carried out in Claims 1 - 9 it now appears that the same qualify the criterion of novelty and inventive step.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2005/000415

A. CLASSIFICATION OF SUBJECT MATTER A61K31/7036 A61K31/427 A61K31/7048 A61K31/7016 A61K31/7004 A61K31/65 A61K31/5377 A61K31/57 A61K31/165 A61K31/43 A61K31/431 A61K31/545 A61K31/546 A61K31/4025 A61K31/496 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) A61K A61P 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 practical, search terms used) EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, FSTA		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BARBHAIYA R H ET AL: "LACK OF PHARMACOKINETIC INTERACTION BETWEEN CEFEPIME AND AMIKACIN IN HUMANS" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 36, no. 7, 1992, pages 1382-1386, XP002369387 ISSN: 0066-4804 cited in the application the whole document	1-9, 12, 20-22
X	US 4 263 280 A (KRADOLFER ET AL) 21 April 1981 (1981-04-21) column 1, lines 4-9, 27-37, 53-60 column 2, lines 3-6, 66, 67 column 4, lines 1-11, 62-68 column 5, lines 12-18, 28-36, 41-46 column 6, lines 6-14	1-4, 6, 8, 9, 14-17, 20-22
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *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) *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 *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 *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 *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. * & * document member of the same patent family		
Date of the actual completion of the international search 24 February 2006		Date of mailing of the international search report 30/03/2006
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer HOUYVET, C

INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2005/000415

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CORDONNIER C ET AL: "Cefepime/amikacin versus ceftazidime/amikacin as empirical therapy for febrile episodes in neutropenic patients: a comparative study. The French Cefepime Study Group." CLINICAL INFECTIOUS DISEASES : AN OFFICIAL PUBLICATION OF THE INFECTIOUS DISEASES SOCIETY OF AMERICA. JAN 1997, vol. 24, no. 1, January 1997 (1997-01), pages 41-51, XP009062427 ISSN: 1058-4838 abstract	1-22
Y	SANZ MIGUEL A ET AL: "Cefepime plus amikacin versus piperacillin-tazobactam plus amikacin for initial antibiotic therapy in haematology patients with febrile neutropenia: Results of an open, randomized, multicentre trial" JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol. 50, no. 1, July 2002 (2002-07), pages 79-88, XP002369388 ISSN: 0305-7453 cited in the application abstract page 80, column 2, paragraphs 4,5	1-22
Y	BEAUCAIRE G ET AL: "Phare study. Comparative study of the association cefepime-amikacin versus the association ceftazidime-amikacin in the treatment of nosocomial pneumonias in ventilated patients" ANNALES FRANCAISES D'ANESTHESIE ET DE REANIMATION, vol. 18, no. 2, February 1999 (1999-02), pages 186-195, XP002369389 ISSN: 0750-7658 cited in the application abstract page 187, column 2, paragraph 2 page 188, column 2, paragraphs 2,3 page 194, column 1, paragraph 3	1-22
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2005/000415

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FORD C W ET AL: "IN VIVO ACTIVITIES OF U-100592 AND 1-100466, NOVEL OXAZOLIDINONE ANTIMICROBIAL AGENTS, AGAINST EXPERIMENTAL BACTERIAL INFECTIONS" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 40, no. 6, 1 June 1996 (1996-06-01), pages 1508-1513, XP000654572 ISSN: 0066-4804 abstract page 1509 page 1511, column 1, paragraph 4 - page 1512, column 2, paragraph 2	1-22
Y	WO 01/34128 A (PHARMACIA & UPJOHN COMPANY; BATTS, DONALD, H) 17 May 2001 (2001-05-17) page 1, lines 6,7,21-25,32 - page 7, line 10	1-22
Y	US 5 795 563 A (KALLICK ET AL) 18 August 1998 (1998-08-18) column 4, paragraphs 4,5 column 7, paragraphs 3,4 column 8, paragraph 4 column 9, paragraph 2-4	1-22
Y	US 5 641 514 A (CHO ET AL) 24 June 1997 (1997-06-24) column 1, lines 5-12 column 2, lines 35-38 table 2	1-22
Y	US 5 217 493 A (RAAD ET AL) 8 June 1993 (1993-06-08) column 2, line 30 - column 3, line 11 column 5, lines 40-50 column 6, lines 30-63	1-22
Y	FR 2 546 406 A (MICHAUX JEAN) 30 November 1984 (1984-11-30) the whole document	1-22
Y	WO 03/082248 A (RANBAXY LABORATORIES LIMITED; DABRE, RAHUL; NAGAPRASAD, VISHNUBHOTLA;) 9 October 2003 (2003-10-09) page 2, lines 22,23 page 3, lines 5-10 page 4, lines 4-6,14-16 page 8, line 22 - page 9, line 2	1-22
Y	US 2003/055022 A1 (BONNER ERNEST L ET AL) 20 March 2003 (2003-03-20) page 1, paragraphs 2,3,10,13,14 page 2, paragraph 15	1-22
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2005/000415

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BUI KHANH Q ET AL: "In vitro and in vivo influence of adjunct clarithromycin on the treatment of mucoid Pseudomonas aeruginosa" JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol. 45, no. 1, January 2000 (2000-01), pages 57-62, XP002369390 ISSN: 0305-7453 abstract -----	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2005/000415

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 22 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IN2005/000415

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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