

US 20050042606A9

(48) **Pub. Date:**

(57)

(19) United States(12) Patent Application Publication

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- (54) SPECIFIC AND UNIVERSAL PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY COMMON BACTERIAL PATHOGENS AND ANTIBIOTIC RESISTANCE GENES FROM CLINICAL SPECIMENS FOR ROUTINE DIAGNOSIS IN MICROBIOLOGY LABORATORIES
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- (21) Appl. No.: 10/121,120
- (22) Filed: Apr. 11, 2002

Prior Publication Data

- (15) Correction of US 2003/0180733 A1 Sep. 25, 2003 See Related U.S. Application Data.
- (65) US 2003/0180733 A1 Sep. 25, 2003

Related U.S. Application Data

(63) Continuation of application No. 09/452,599, filed on Dec. 1, 1999, now abandoned.
Continuation of application No. 08/526,840, filed on Sep. 11, 1995, now Pat. No. 6,001,564, which is a continuation-in-part of application No. 08/304,732, filed on Sep. 12, 1994, now abandoned.

Publication Classification

- (51) Int. Cl.⁷ C12Q 1/68; C12P 19/34
- (52) U.S. Cl. 435/6; 435/91.2

ABSTRACT

The present invention relates to DNA-based methods for universal bacterial detection, for specific detection of the common bacterial pathogens *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Staphylococcus saprophyticus, Streptococcus pyogenes, Haemophilus influenzae* and *Moraxella catarrhalis* as well as for specific detection of commonly encountered and clinically relevant bacterial antibiotic resistance genes directly from clinical specimens or, alternatively, from a bacterial colony. The above bacterial species can account for as much as 80% of bacterial pathogens isolated in routine microbiology laboratories.

(10) Pub. No.: US 2005/0042606 A9

CORRECTED PUBLICATION

Feb. 24, 2005

The core of this invention consists primarily of the DNA sequences from all species-specific genomic DNA fragments selected by hybridization from genomic libraries or, alternatively, selected from data banks as well as any oligonucleotide sequences derived from these sequences which can be used as probes or amplification primers for PCR or any other nucleic acid amplification methods. This invention also includes DNA sequences from the selected clinically relevant antibiotic resistance genes.

With these methods, bacteria can be detected (universal primers and/or probes) and identified (species-specific primers and/or probes) directly from the clinical specimens or from an isolated bacterial colony. Bacteria are further evaluated for their putative susceptibility to antibiotics by resistance gene detection (antibiotic resistance gene specific primers and/or probes). Diagnostic kits for the detection of the presence, for the bacterial identification of the abovementioned bacterial species and for the detection of antibiotic resistance genes are also claimed. These kits for the rapid (one hour or less) and accurate diagnosis of bacterial infections and antibiotic resistance will gradually replace conventional methods currently used in clinical microbiology laboratories for routine diagnosis. They should provide tools to clinicians to help prescribe promptly optimal treatments when necessary. Consequently, these tests should contribute to saving human lives, rationalizing treatment, reducing the development of antibiotic resistance and avoid unnecessary hospitalizations.

SPECIFIC AND UNIVERSAL PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY COMMON BACTERIAL PATHOGENS AND ANTIBIOTIC RESISTANCE GENES FROM CLINICAL SPECIMENS FOR ROUTINE DIAGNOSIS IN MICROBIOLOGY LABORATORIES

BACKGROUND OF THE INVENTION

[0001] Classical Identification of Bacteria

[0002] Bacteria are classically identified by their ability to utilize different substrates as a source of carbon and nitrogen through the use of biochemical tests such as the API20ETM system. Susceptibility testing of Gram negative bacilli has progressed to microdilution tests. Although the API and the microdilution systems are cost-effective, at least two days are required to obtain preliminary results due to the necessity of two successive overnight incubations to isolate and identify the bacteria from the specimen. Some faster detection methods with sophisticated and expensive apparatus have been developed. For example, the fastest identification system, the autoSCAN-Walk-Away system[™] identifies both Gram negative and Gram positive from isolated bacterial colonies in 2 hours and susceptibility patterns to antibiotics in only 7 hours. However, this system has an unacceptable margin of error, especially with bacterial species other than Enterobacteriaceae (York et al., 1992. J. Clin. Microbiol. 30:2903-2910). Nevertheless, even this fastest method requires primary isolation of the bacteria as a pure culture, a process which takes at least 18 hours if there is a pure culture or 2 to 3 days if there is a mixed culture.

[0003] Urine Specimens

[0004] A large proportion (40-50%) of specimens received in routine diagnostic microbiology laboratories for bacterial identification are urine specimens (Pezzlo, 1988, Clin. Microbiol. Rev. 1:268-280). Urinary tract infections (UTI) are extremely common and affect up to 20% of women and account for extensive morbidity and increased mortality among hospitalized patients (Johnson and Stamm, 1989; Ann. Intern. Med. 111:906-917). UTI are usually of bacterial etiology and require antimicrobial therapy. The Gram negative bacillus *Escherichia coli* is by far the most prevalent urinary pathogen and accounts for 50 to 60% of UTI (Pezzlo, 1988, op. cit.). The prevalence for bacterial pathogens isolated from urine specimens observed recently at the "Centre Hospitalier de 1'Université Laval (CHUL)" is given in Tables 1 and 2.

[0005] Conventional pathogen identification in urine specimens. The search for pathogens in urine specimens is so preponderant in the routine microbiology laboratory that a myriad of tests have been developed. The gold standard is still the classical semi-quantitative plate culture method in which a calibrated loop of urine is streaked on plates and incubated for 18-24 hours. Colonies are then counted to determine the total number of colony forming units (CFU) per liter of urine. A bacterial UTI is normally associated with a bacterial count of $\geq 10^7$ CFU/L in urine. However, infections with less than 10^7 CFU/L in urine are possible, particularly in patients with a high incidence of diseases or those catheterized (Stark and Maki, 1984, N. Engl. J. Med. 311:560-564). Importantly, close to 80% of urine specimens tested are considered negative (<10⁷ CFU/L; Table 3).

[0006] Accurate and rapid urine screening methods for bacterial pathogens would allow a faster identification of negative results and a more efficient clinical investigation of the patient. Several rapid identification methods (Uriscreen[™], UTIscreen[™], Flash Track[™] DNA probes and others) were recently compared to slower standard biochemical methods which are based on culture of the bacterial pathogens. Although much faster, these rapid tests showed low sensitivities and specificities as well as a high number of false negative and false positive results (Koening et al., 1992. J. Clin. Microbiol. 30:342-345; Pezzlo et al., 1992. J. Clin. Microbiol. 30:640-684).

[0007] Urine specimens found positive by culture are further characterized using standard biochemical tests to identify the bacterial pathogen and are also tested for susceptibility to antibiotics.

[0008] Any Clinical Specimens

[0009] As with urine specimen which was used here as an example, our probes and amplification primers are also applicable to any other clinical specimens. The DNA-based tests proposed in this invention are superior to standard methods currently used for routine diagnosis in terms of rapidity and accuracy. While a high percentage of urine specimens are negative, in many other clinical specimens more than 95% of cultures are negative (Table 4). These data further support the use of universal probes to screen out the negative clinical specimens. Clinical specimens from organisms other than humans (e.g. other primates, mammals, farm animals or live stocks) may also be used.

[0010] Towards the Development of Rapid DNA-Based Diagnostic Tests

[0011] A rapid diagnostic test should have a significant impact on the management of infections. For the identification of pathogens and antibiotic resistance genes in clinical samples, DNA probe and DNA amplification technologies offer several advantages over conventional methods. There is no need for subculturing, hence the organism can be detected directly in clinical samples thereby reducing the costs and time associated with isolation of pathogens. DNAbased technologies have proven to be extremely useful for specific applications in the clinical microbiology laboratory. For example, kits for the detection of fastidious organisms based on the use of hybridization probes or DNA amplification for the direct detection of pathogens in clinical specimens are commercially available (Persing et al, 1993. Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

[0012] The present invention is an advantageous alternative to the conventional culture identification methods used in hospital clinical microbiology laboratories and in private clinics for routine diagnosis. Besides being much faster, DNA-based diagnostic tests are more accurate than standard biochemical tests presently used for diagnosis because the bacterial genotype (e.g. DNA level) is more stable than the bacterial phenotype (e.g. biochemical properties). The originality of this invention is that genomic DNA fragments (size of at least 100 base pairs) specific for 12 species of commonly encountered bacterial pathogens were selected from genomic libraries or from data banks. Amplification primers or oligonucleotide probes (both less than 100 nucleotides in length) which are both derived from the sequence of speciesspecific DNA fragments identified by hybridization from genomic libraries or from selected data bank sequences are used as a basis to develop diagnostic tests. Oligonucleotide primers and probes for the detection of commonly encountered and clinically important bacterial resistance genes are also included. For example, Annexes I and II present a list of suitable oligonucleotide probes and PCR primers which were all derived from the species-specific DNA fragments selected from genomic libraries or from data bank sequences. It is clear to the individual skilled in the art that oligonucleotide sequences appropriate for the specific detection of the above bacterial species other than those listed in Annexes 1 and 2 may be derived from the species-specific fragments or from the selected data bank sequences. For example, the oligonucleotides may be shorter or longer than the ones we have chosen and may be selected anywhere else in the identified species-specific sequences or selected data bank sequences. Alternatively, the oligonucleotides may be designed for use in amplification methods other than PCR. Consequently, the core of this invention is the identification of species-specific genomic DNA fragments from bacterial genomic DNA libraries and the selection of genomic DNA fragments from data bank sequences which are used as a source of species-specific and ubiquitous oligonucleotides. Although the selection of oligonucleotides suitable for diagnostic purposes from the sequence of the species-specific fragments or from the selected data bank sequences requires much effort it is quite possible for the individual skilled in the art to derive from our fragments or selected data bank sequences suitable oligonucleotides which are different from the ones we have selected and tested as examples (Annexes I and II).

[0013] Others have developed DNA-based tests for the detection and identification of some of the bacterial pathogens for which we have identified species-specific sequences (PCT patent application Serial No. WO 93/03186). However, their strategy was based on the amplification of the highly conserved 16S rRNA gene followed by hybridization with internal species-specific oligonucleotides. The strategy from this invention is much simpler and more rapid because it allows the direct amplification of species-specific targets using oligonucleotides derived from the species-specific bacterial genomic DNA fragments.

[0014] Since a high percentage of clinical specimens are negative, oligonucleotide primers and probes were selected from the highly conserved 16S or 23S rRNA genes to detect all bacterial pathogens possibly encountered in clinical specimens in order to determine whether a clinical specimen is infected or not. This strategy allows rapid screening out of the numerous negative clinical specimens submitted for bacteriological testing.

[0015] We are also developing other DNA-based tests, to be performed simultaneously with bacterial identification, to determine rapidly the putative bacterial susceptibility to antibiotics by targeting commonly encountered and clinically relevant bacterial resistance genes. Although the sequences from the selected antibiotic resistance genes are available and have been used to develop DNA-based tests for their detection (Ehrlich and Greenberg, 1994. PCR-based Diagnostics in Infectious Diseases, Blackwell Scientific Publications, Boston, Mass.; Persing et al, 1993. Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.), our approch is innovative as it represents major improvements over current "gold standard" diagnostic methods based on culture of the bacteria because it allows the rapid identification of the presence of a specific bacterial pathogen and evaluation of its susceptibility to antibiotics directly from the clinical specimens within one hour.

[0016] We believe that the rapid and simple diagnostic tests not based on cultivation of the bacteria that we are developing will gradually replace the slow conventional bacterial identification methods presently used in hospital clinical microbiology laboratories and in private clinics. In our opinion, these rapid DNA-based diagnostic tests for severe and common bacterial pathogens and antibiotic resistance will (i) save lives by optimizing treatment, (ii) diminish antibiotic resistance by reducing the use of broad spectrum antibiotics and (iii) decrease overall health costs by preventing or shortening hospitalizations.

SUMMARY OF THE INVENTION

[0017] In accordance with the present invention, there is provided sequence from genomic DNA fragments (size of at least 100 base pairs and all described in the sequence listing) selected either by hybridization from genomic libraries or from data banks and which are specific for the detection of commonly encountered bacterial pathogens (i.e. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Staphylococcus saprophyticus, Streptococcus pyogenes, Haemophilus influenzae and Moraxella catarrhalis) in clinical specimens. These bacterial species are associated with approximately 90% of urinary tract infections and with a high percentage of other severe infections including septicemia, meningitis, pneumonia, intraabdominal infections, skin infections and many other severe respiratory tract infections. Overall, the above bacterial species may account for up to 80% of bacterial pathogens isolated in routine microbiology laboratories.

[0018] Synthetic oligonucleotides for hybridization (probes) or DNA amplification (primers) were derived from the above species-specific DNA fragments (ranging in sizes from 0.25 to 5.0 kilobase pairs (kbp)) or from selected data bank sequences (GenBank and EMBL). Bacterial species for which some of the oligonucleotide probes and amplification primers were derived from selected data bank sequences are Escherichia coli, Enterococcus faecalis, Streptococcus pyogenes and Pseudomonas aeruginosa. The person skilled in the art understands that the important innovation in this invention is the identification of the species-specific DNA fragments selected either from bacterial genomic libraries by hybridization or from data bank sequences. The selection of oligonucleotides from these fragments suitable for diagnostic purposes is also innovative. Specific and ubiquitous oligonucleotides different from the ones tested in the practice are considered as embodiments of the present invention.

[0019] The development of hybridization (with either fragment or oligonucleotide probes) or of DNA amplification protocols for the detection of pathogens from clinical specimens renders possible a very rapid bacterial identification. This will greatly reduce the time currently required for the identification of pathogens in the clinical laboratory

since these technologies can be applied for bacterial detection and identification directly from clinical specimens with minimum pretreatment of any biological specimens to release bacterial DNA. In addition to being 100% specific, probes and amplification primers allow identification of the bacterial species directly from clinical specimens or, alternatively, from an isolated colony. DNA amplification assays have the added advantages of being faster and more sensitive than hybridization assays, since they allow rapid and exponential in vitro replication of the target segment of DNA from the bacterial genome. Universal probes and amplification primers selected from the 16S or 23S rRNA genes highly conserved among bacteria, which permit the detection of any bacterial pathogens, will serve as a procedure to screen out the numerous negative clinical specimens received in diagnostic laboratories. The use of oligonucleotide probes or primers complementary to characterized bacterial genes encoding resistance to antibiotics to identify commonly encountered and clinically important resistance genes is also under the scope of this invention.

DETAILED DESCRIPTION OF THE INVENTION

[0020] Development of Species-Specific DNA Probes

[0021] DNA fragment probes were developed for the following bacterial species: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Haemophilus influenzae and Moraxella catarrhalis. (For Enterofaecalis and Streptococcus coccus pyogenes, oligonucleotide sequences were exclusively derived from selected data bank sequences). These species-specific fragments were selected from bacterial genomic libraries by hybridization to DNA from a variety of Gram positive and Gram negative bacterial species (Table 5).

[0022] The chromosomal DNA from each bacterial species for which probes were seeked was isolated using standard methods. DNA was digested with a frequently cutting restriction enzyme such as Sau3AI and then ligated into the bacterial plasmid vector pGEM3Zf (Promega) linearized by appropriate restriction endonuclease digestion. Recombinant plasmids were then used to transform competent E. coli strain DH5a thereby yielding a genomic library. The plasmid content of the transformed bacterial cells was analyzed using standard methods. DNA fragments of target bacteria ranging in size from 0.25 to 5.0 kilobase pairs (kbp) were cut out from the vector by digestion of the recombinant plasmid with various restriction endonucleases. The insert was separated from the vector by agarose gel electrophoresis and purified in low melting point agarose gels. Each of the purified fragments of bacterial genomic DNA was then used as a probe for specificity tests.

[0023] For each given species, the gel-purified restriction fragments of unknown coding potential were labeled with the radioactive nucleotide α -³²P(dATP) which was incorporated into the DNA fragment by the random priming labeling reaction. Non-radioactive modified nucleotides could also be incorporated into the DNA by this method to serve as a label.

[0024] Each DNA fragment probe (i.e. a segment of bacterial genomic DNA of at least 100 bp in length cut out

from clones randomly selected from the genomic library) was then tested for its specificity by hybridization to DNAs from a variety of bacterial species (Table 5). The doublestranded labeled DNA probe was heat-denatured to yield labeled single-stranded DNA which could then hybridize to any single-stranded target DNA fixed onto a solid support or in solution. The target DNAs consisted of total cellular DNA from an array of bacterial species found in clinical samples (Table 5). Each target DNA was released from the bacterial cells and denatured by conventional methods and then irreversibly fixed onto a solid support (e.g. nylon or nitrocellulose membranes) or free in solution. The fixed singlestranded target DNAs were then hybridized with the singlestranded probe. Pre-hybridization, hybridization and posthybridization conditions were as follows: (i) Prehybridization; in 1 M NaCl+10% dextran sulfate+1% SDS (sodium dodecyl sulfate)+1 µg/ml salmon sperm DNA at 650° C. for 15 min. (ii) Hybridization; in fresh pre-hybridization solution containing the labeled probe at 650° C. overnight. (iii) Post-hybridization; washes twice in 3×SSC containing 1% SDS (1×SSC is 0.15M NaCl, 0.015M NaCitrate) and twice in 0.1×SSC containing 0.1% SDS; all washes were at 650° C. for 15 min. Autoradiography of washed filters allowed the detection of selectively hybridized probes. Hybridization of the probe to a specific target DNA indicated a high degree of similarity between the nucleotide sequence of these two DNAs. Species-specific DNA fragments selected from various bacterial genomic libraries ranging in size from 0.25 to 5.0 kbp were isolated for 10 common bacterial pathogens (Table 6) based on hybridization to chromosomal DNAs from a variety of bacteria performed as described above. All of the bacterial species tested (66 species listed in Table 5) were likely to be pathogens associated with common infections or potential contaminants which can be isolated from clinical specimens. A DNA fragment probe was considered specific only when it hybridized solely to the pathogen from which it was isolated. DNA fragment probes found to be specific were subsequently tested for their ubiquity (i.e. ubiquitous probes recognized most isolates of the target species) by hybridization to bacterial DNAs from approximately 10 to 80 clinical isolates of the species of interest (Table 6). The DNAs were denatured, fixed onto nylon membranes and hybridized as described above.

[0025] Sequencing of the Species-Specific Fragment Orobes

[0026] The nucleotide sequence of the totality or of a portion of the species-specific DNA fragments isolated (Table 6) was determined using the dideoxynucleotide termination sequencing method which was performed using Sequenase (USB Biochemicals) or T7 DNA polymerase (Pharmacia). These nucleotide sequences are shown in the sequence listing. Alternatively, sequences selected from data banks (GenBank and EMBL) were used as sources of oligonucleotides for diagnostic purposes for Escherichia coli, Enterococcus faecalis, Streptococcus pyogenes and Pseudomonas aeruginosa. For this strategy, an array of suitable oligonucleotide primers or probes derived from a variety of genomic DNA fragments (size of more than 100 bp) selected from data banks was tested for their specificity and ubiquity in PCR and hybridization assays as described later. It is important to note that the data bank sequences were selected based on their potential of being speciesspecific according to available sequence information. Only

data bank sequences from which species-specific oligonucleotides could be derived are included in this invention.

[0027] Oligonucleotide probes and amplification primers derived from species-specific fragments selected from the genomic libraries or from data bank sequences were synthesized using an automated DNA synthesizer (Millipore). Prior to synthesis, all oligonucleotides (probes for hybridization and primers for DNA amplification) were evaluated for their suitability for hybridization or DNA amplification by polymerase chain reaction (PCR) by computer analysis using standard programs (e.g. Genetics Computer Group (GCG) and Oligo[™] 4.0 (National Biosciences)). The potential suitability of the PCR primer pairs was also evaluated prior to the synthesis by verifying the absence of unwanted features such as long stretches of one nucleotide, a high proportion of G or C residues at the 3' end and a 3'-terminal T residue (Persing et al, 1993. Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

[0028] Hybridization with Oligonucleotide Probes

[0029] In hybridization experiments, oligonucleotides (size less than 100 nucleotides) have some advantages over DNA fragment probes for the detection of bacteria such as ease of preparation in large quantities, consistency in results from batch to batch and chemical stability. Briefly, for the hybridizations, oligonucleotides were 5' end-labeled with the radionucleotide $\gamma^{32}P(ATP)$ using T4 polynucleotide kinase (Pharmacia). The unincorporated radionucleotide was removed by passing the labeled single-stranded oligonucleotide through a Sephadex G50 column. Alternatively, oligonucleotides were labeled with biotin, either enzymatically at their 3' ends or incorporated directly during synthesis at their 5' ends, or with digoxigenin. It will be appreciated by the person skilled in the art that labeling means other than the three above labels may be used.

[0030] The target DNA was denatured, fixed onto a solid support and hybridized as previously described for the DNA fragment probes. Conditions for pre-hybridization and hybridization were as described earlier. Post-hybridization washing conditions were as follows: twice in 3×SSC containing 1% SDS, twice in 2×SSC containing 1% SDS and twice in 1×SSC containing 1% SDS (all of these washes were at 65° C. for 15 min), and a final wash in 0.1×SSC containing 1% SDS at 25° C. for 15 min. For probes labeled with radioactive labels the detection of hybrids was by autoradiography as described earlier. For non-radioactive labels detection may be calorimetric or by chemiluminescence.

[0031] The oligonucleotide probes may be derived from either strand of the duplex DNA. The probes may consist of the bases A, G, C, or T or analogs. The probes may be of any suitable length and may be selected anywhere within the species-specific genomic DNA fragments selected from the genomic libraries or from data bank sequences.

[0032] DNA Amplification

[0033] For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs were derived either from the sequenced species-specific DNA fragments or from data bank sequences or, alternatively, were shortened versions of oligonucleotide probes. Prior to synthesis, the potential primer pairs were analyzed by using

the program oligo^{TM} 4.0 (National Biosciences) to verify that they are likely candidates for PCR amplifications.

[0034] During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the denatured double-stranded target DNA from the bacterial genome are used to amplify exponentially in vitro the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing et al, 1993. Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Briefly, the PCR protocols were as follows. Clinical specimens or bacterial colonies were added directly to the 50 μ L PCR reaction mixtures containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl₂ 0.4μ M of each of the two primers, 200 μ M of each of the four dNTPs and 1.25 Units of Taq DNA polymerase (Perkin Elmer). PCR reactions were then subjected to thermal cycling (3 min at 95° C. followed by 30 cycles of 1 second at 95° C. and 1 second at 55° C.) using a Perkin Elmer 480[™] thermal cycler and subsequently analyzed by standard ethidium bromidestained agarose gel electrophoresis. It is clear that other methods for the detection of specific amplification products, which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after amplification (e.g. TaqMan[™] system from Perkin Elmer or Amplisensor[™] from Biotronics) or liquid hybridization with an oligonucleotide probe binding to internal sequences of the specific amplification product. These novel probes can be generated from our species-specific fragment probes. Methods based on the detection of fluorescence are particularly promising for utilization in routine diagnosis as they are, very rapid and quantitative and can be automated.

[0035] To assure PCR efficiency, glycerol or dimethyl sulfoxide (DMSO) or other related solvents, can be used to increase the sensitivity of the PCR and to overcome problems associated with the amplification of target with a high GC content or with strong secondary structures. The concentration ranges for glycerol and DMSO are 5-15% (v/v) and 3-10% (v\v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and the MgCl₂ are 0.1-1.0 µM and 1.5-3.5 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e. nested PCR) or using more than one primer pair (i.e. multiplex PCR) may also be used (Persing et al, 1993. Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). For more details about the PCR protocols and amplicon detection methods see examples 7 and 8.

[0036] The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), transcription-based amplification systems (TAS), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA) and branched DNA (bDNA) (Persing et al, 1993. Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification methods or any other procedures which may be used to increase rapidity and sensitivity of the tests. Any oligonucleotides suitable for the amplification of nucleic acid by approaches other than PCR and derived from the species-specific fragments and from selected antibiotic resistance gene sequences included in this document are also under the scope of this invention.

[0037] Specificity and Ubiquity Tests for Oligonucleotide Probes and Primers

[0038] The specificity of oligonucleotide probes, derived either from the sequenced species-specific fragments or from data bank sequences, was tested by hybridization to DNAs from the array of bacterial species listed in Table 5 as previously described. Oligonucleotides found to be specific were subsequently tested for their ubiquity by hybridization to bacterial DNAs from approximately 80 isolates of the target species as described for fragment probes. Probes were considered ubiquitous when they hybridized specifically with the DNA from at least 80% of the isolates. Results for specificity and ubiquity tests with the oligonucleotide probes are summarized in Table 6. The specificity and ubiquity of the amplification primer pairs were tested directly from cultures (see example 7) of the same bacterial strains. For specificity and ubiquity tests, PCR assays were performed directly from bacterial colonies of approximately 80 isolates of the target species. Results are summarized in Table 7. All specific and ubiquitous oligonucleotide probes and amplification primers for each of the 12 bacterial species investigated are listed in Annexes I and II, respectively. Divergence in the sequenced DNA fragments can occur and, insofar as the divergence of these sequences or a part thereof does not affect the specificity of the probes or amplification primers, variant bacterial DNA is under the scope of this invention.

[0039] Universal Bacterial Detection

[0040] In the routine microbiology laboratory a high percentage of clinical specimens sent for bacterial identification is negative (Table 4). For example, over a 2 year period, around 80% of urine specimens received by the laboratory at the "Centre Hospitalier de 1' Université Laval (CHUL)" were negative (i.e. $<10^7$ CFU/L) (Table 3). Testing clinical samples with universal probes or universal amplification primers to detect the presence of bacteria prior to specific identification and screen out the numerous negative specimens is thus useful as it saves costs and may rapidly orient the clinical management of the patients. Several oligonucleotides and amplification primers were therefore synthesized from highly conserved portions of bacterial 16S or 23S ribosomal RNA gene sequences available in data banks (Annexes III and IV). In hybridization tests, a pool of seven oligonucleotides (Annex I; Table 6) hybridized strongly to DNA from all bacterial species listed in Table 5. This pool of universal probes labeled with radionucleotides or with any other modified nucleotides is consequently very useful for detection of bacteria in urine samples with a sensitivity range of $\geq 10^7$ CFU/L. These probes can also be applied for bacterial detection in other clinical samples.

[0041] Amplification primers also derived from the sequence of highly conserved ribosomal RNA genes were used as an alternative strategy for universal bacterial detection directly from clinical specimens (Annex IV; Table 7). The DNA amplification strategy was developed to increase the sensitivity and the rapidity of the test. This amplification test was ubiquitous since it specifically amplified DNA from 23 different bacterial species encountered in clinical specimens.

[0042] Well-conserved bacterial genes other than ribosomal RNA genes could also be good candidates for universal bacterial detection directly from clinical specimens. Such genes may be associated with processes essential for bacterial survival (e.g. protein synthesis, DNA synthesis, cell division or DNA repair) and could therefore be highly conserved during evolution. We are working on these candidate genes to develop new rapid tests for the universal detection of bacteria directly from clinical specimens.

[0043] Antibiotic Resistance Genes

[0044] Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of bacterial resistance. Our goal is to provide the clinicians, within one hour, the needed information to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection and the identification of the presence of a specific pathogen in the positive specimens with DNA-based tests for specific bacterial detection, the clinicians also need timely information about the ability of the bacterial pathogen to resist antibiotic treatments. We feel that the most efficient strategy to evaluate rapidly bacterial resistance to antimicrobials is to detect directly from the clinical specimens the most common and important antibiotic resistance genes (i.e. DNA-based tests for the detection of antibiotic resitance genes). Since the sequence from the most important and common bacterial antibiotic resistance genes are available from data banks, our strategy is to use the sequence from a portion or from the entire gene to design specific oligonucleotides which will be used as a basis for the development of rapid DNA-based tests. The sequence from the bacterial antibiotic resistance genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in the sequence listing. Table 8 summarizes some characteristics of the selected antibiotic resistance genes.

EXAMPLES

[0045] The following examples are intended to be illustrative of the various methods and compounds of the invention.

Example 1

[0046] Isolation and cloning of fragments. Genomic DNAs from Escherichia coli strain ATCC 25922, Klebsiella pneumoniae strain CK2, Pseudomonas aeruginosa strain ATCC 27853, Proteus mirabilis strain ATCC 35657, Streptococcus pneumoniae strain ATCC 27336, Staphylococcus aureus strain ATCC 25923, Staphylococcus epidermidis strain ATCC 12228, Staphylococcus saprophyticus strain ATCC 15305, Haemophilus influenzae reference strain Rd and Moraxella catarrhalis strain ATCC 53879 were prepared using standard procedures. It is understood that the bacterial genomic DNA may have been isolated from strains other than the ones mentioned above. (For Enterococcus faecalis and Streptococcus pyogenes oligonucleotide sequences were derived exclusively from data banks). Each DNA was digested with a restriction enzyme which frequently cuts DNA such as Sau3AI. The resulting DNA fragments were ligated into a plasmid vector (pGEM3Zf) to create recombinant plasmids and transformed into competent E. coli cells (DH5 α). It is understood that the vectors and corresponding competent cells should not be limited to the ones herein above specifically examplified. The objective of obtaining recombinant plasmids and transformed cells is to provide an easily reproducible source of DNA fragments useful as probes. Therefore, insofar as the inserted fragments are specific and selective for the target bacterial DNA, any recombinant plasmids and corresponding transformed host cells are under the scope of this invention. The plasmid content of the transformed bacterial cells was analyzed using standard methods. DNA fragments from target bacteria ranging in size from 0.25 to 5.0 kbp were cut out from the vector by digestion of the recombinant plasmid with various restriction endonucleases. The insert was separated from the vector by agarose gel electrophoresis and purified in a low melting point agarose gel. Each of the purified fragments was then used for specificity tests.

[0047] Labeling of DNA fragment probes. The label used was $\alpha^{32}P(dATP)$, a radioactive nucleotide which can be incorporated enzymatically into a double-stranded DNA molecule. The fragment of interest is first denatured by heating at 95° C. for 5 min, then a mixture of random primers is allowed to anneal to the strands of the fragments. These primers, once annealed, provide a starting point for synthesis of DNA. DNA polymerase, usually the Klenow fragment, is provided along with the four nucleotides, one of which is radioactive. When the reaction is terminated, the mixture of new DNA molecules is once again denatured to provide radioactive single-stranded DNA molecules (i.e. the probe). As mentioned earlier, other modified nucleotides may be used to label the probes.

[0048] Specificity and ubiquity tests for the DNA fragment probes. Species-specific DNA fragments ranging in size from 0.25 to 5.0 kbp were isolated for 10 common bacterial pathogens (Table 6) based on hybridization to chromosomal DNAs from a variety of bacteria. Samples of whole cell DNA for each bacterial strain listed in Table 5 were transferred onto a nylon membrane using a dot blot apparatus, washed and denatured before being irreversibly fixed. Hybridization conditions were as described earlier. A DNA fragment probe was considered specific only when it hybridized solely to the pathogen from which it was isolated. Labeled DNA fragments hybridizing specifically only to target bacterial species (i.e. specific) were then tested for their ubiquity by hybridization to DNAs from approximately 10 to 80 isolates of the species of interest as described earlier. The conditions for pre-hybridization, hybridization and post-hybridization washes were as described earlier. After autoradiography (or other detection means appropriate for the non-radioactive label used), the specificity of each individual probe can be determined. Each probe found to be specific (i.e. hybridizing only to the DNA from the bacterial species from which it was isolated) and ubiquitous (i.e. hybridizing to most isolates of the target species) was kept for further experimentations.

Example 2

[0049] Same as example 1 except that testing of the strains is by colony hybridization. The bacterial strains were inoculated onto a nylon membrane placed on nutrient agar. The membranes were incubated at 37° C. for two hours and then bacterial lysis and DNA denaturation were carried out according to standard procedures. DNA hybridization was performed as described earlier.

Example 3

[0050] Same as example 1 except that bacteria were detected directly from clinical samples. Any biological samples were loaded directly onto a dot blot apparatus and cells were lysed in situ for bacterial detection. Blood samples should be heparizined in order to avoid coagulation interfering with their convenient loading on a dot blot apparatus.

Example 4

[0051] Nucleotide sequencina of DNA fragments. The nucleotide sequence of the totality or a portion of each fragment found to be specific and ubiquitous (Example 1) was determined using the dideoxynucleotide termination sequencing method (Sanger et al., 1977, Proc. Natl. Acad. Sci. USA. 74:5463-5467). These DNA sequences are shown in the sequence listing. Oligonucleotide probes and amplification primers were selected from these nucleotide sequences, or alternatively, from selected data banks sequences and were then synthesized on an automated Biosearch synthesizer (MilliporeTM) using phosphoramidite chemistry.

[0052] Labeling of oliaonucleotides. Each oligonucleotide was 5' end-labeled with γ^{32} P-ATP by the T4 polynucleotide kinase (Pharmacia) as described earlier. The label could also be non-radioactive.

[0053] Specificity test for oligonucleotide probes. All labeled oligonucleotide probes were tested for their specificity by hybridization to DNAs from a variety of Gram positive and Gram negative bacterial species as described earlier. Species-specific probes were those hybridizing only to DNA from the bacterial species from which it was isolated. Oligonucleotide probes found to be specific were submitted to ubiquity tests as follows.

[0054] Ubiquity test for oligonucleotide probes. Specific oligonucleotide probes were then used in ubiquity tests with approximately 80 strains of the target species. Chromosomal DNAs from the isolates were transferred onto nylon membranes and hybridized with labeled oligonucleotide probes as described for specificity tests. The batteries of approximately 80 isolates constructed for each target species contain reference ATCC strains as well as a variety of clinical isolates obtained from various sources. Ubiquitous probes were those hybridizing to at least 80% of DNAs from the battery of clinical isolates of the target species. Examples of specific and ubiquitous oligonucleotide probes are listed in Annex 1.

Example 5

[0055] Same as example 4 except that a pool of specific oligonucleotide probes is used for bacterial identification (i) to increase sensitivity and assure 100% ubiquity or (ii) to identify simultaneously more than one bacterial species. Bacterial identification could be done from isolated colonies or directly from clinical specimens.

Example 6

[0056] PCR amplification. The technique of PCR was used to increase sensitivity and rapidity of the tests. The PCR primers used were often shorter derivatives of the extensive sets of oligonucleotides previously developed for

hybridization assays (Table 6). The sets of primers were tested in PCR assays performed directly from a bacterial colony or from a bacterial suspension (see Example 7) to determine their specificity and ubiquity (Table 7). Examples of specific and ubiquitous PCR primer pairs are listed in annex II.

[0057] Specificity and ubiquity tests for amplification primers. The specificity of all selected PCR primer pairs was tested against the battery of Gram negative and Gram positive bacteria used to test the oligonucleotide probes (Table 5). Primer pairs found specific for each species were then tested for their ubiquity to ensure that each set of primers could amplify at least 80% of DNAs from a battery of approximately 80 isolates of the target species. The batteries of isolates constructed for each species contain reference ATCC strains and various clinical isolates representative of the clinical diversity for each species.

[0058] Standard precautions to avoid false positive PCR results should be taken. Methods to inactivate PCR amplification products such as the inactivation by uracil-N-gly-cosylase may be used to control PCR carryover.

Example 7

[0059] Amplification directly from a bacterial colony or suspension. PCR assays were performed either directly from a bacterial colony or from a bacterial suspension, the latter being adjusted to a standard McFarland 0.5 (corresponds to 1.5×10^8 bacteria/mL). In the case of direct amplification from a colony, a portion of the colony was transferred directly to a 50 µL PCR reaction mixture (containing 50 mM KCl, 10 mM Tris pH 8.3, 2.5 mM MgCl₂, 0.4 µM of each of the two primers, $200 \,\mu\text{M}$ of each of the four dNTPs and 1.25Unit of Taq DNA polymerase (Perkin Elmer)) using a plastic rod. For the bacterial suspension, $4 \mu L$ of the cell suspension was added to 46 μ L of the same PCR reaction mixture. For both strategies, the reaction mixture was overlaid with 50 μ L of mineral oil and PCR amplifications were carried out using an initial denaturation step of 3 min. at 95° C. followed by 30 cycles consisting of a 1 second denaturation step at 95° C. and of a 1 second annealing step at 55° C. in a Perkin Elmer 480[™] thermal cycler. PCR amplification products were then analyzed by standard agarose gel (2%) electrophoresis. Amplification products were visualized in agarose gels containing 2.5 μ g/mL of ethidium bromide under UV at 254 nm. The entire PCR assay can be completed in approximately one hour.

[0060] Alternatively, amplification from bacterial cultures was performed as described above but using a "hot start" protocol. In that case, an initial reaction mixture containing the target DNA, primers and dNTPs was heated at 85° C. prior to the addition of the other components of the PCR reaction mixture. The final concentration of all reagents was as described above. Subsequently, the PCR reactions were submitted to thermal cycling and analysis as described above.

Example 8

[0061] Amplification directly from clinical specimens. For amplification from urine specimens, 4 μ L of undiluted or diluted (1:10) urine was added directly to 46 μ L of the above PCR reaction mixture and amplified as described earlier.

[0062] To improve bacterial cell lysis and eliminate the PCR inhibitory effects of clinical specimens, samples were routinely diluted in lysis buffer containing detergent(s). Subsequently, the lysate was added directly to the PCR reaction mixture. Heat treatments of the lysates, prior to DNA amplification, using the thermocycler or a microwave oven could also be performed to increase the efficiency of cell lysis.

[0063] Our strategy is to develop rapid and simple protocols to eliminate PCR inhibitory effects of clinical specimens and lyse bacterial cells to perform DNA amplification directly from a variety of biological samples. PCR has the advantage of being compatible with crude DNA preparations. For example, blood, cerebrospinal fluid and sera may be used directly in PCR assays after a brief heat treatment. We intend to use such rapid and simple strategies to develop fast protocols for DNA amplification from a variety of clinical specimens.

Example 9

[0064] Detection of antibiotic resistance genes. The presence of specific antibiotic resistance genes which are frequently encountered and clinically relevant is identified using the PCR amplification or hybridization protocols described in previous sections. Specific oligonucleotides used as a basis for the DNA-based tests are selected from the antibiotic resistance gene sequences. These tests can be performed either directly from clinical specimens or from a bacterial colony and should complement diagnostic tests for specific bacterial identification.

Example 10

[0065] Same as examples 7 and 8 except that assays were performed by multiplex PCR (i.e. using several pairs of primers in a single PCR reaction) to (i) reach an ubiquity of 100% for the specific target pathogen or (ii) to detect simultaneously several species of bacterial pathogens.

[0066] For example, the detection of Escherichia coli requires three pairs of PCR primers to assure a ubiquity of 100%. Therefore, a multiplex PCR assay (using the "hot-start" protocol (Example 7)) with those three primer pairs was developed. This strategy was also used for the other bacterial pathogens for which more than one primer pair was required to reach an ubiquity of 100%.

[0067] Multiplex PCR assays could also be used to (i) detect simultaneously several bacterial species or, alternatively, (ii) to simultaneously identify the bacterial pathogen and detect specific antibiotic resistance genes either directly from a clinical specimen or from a bacterial colony.

[0068] For these applications, amplicon detection methods should be adapted to differentiate the various amplicons produced. Standard agarose gel electrophoresis could be used because it discriminates the amplicons based on their sizes. Another useful strategy for this purpose would be detection using a variety of fluorochromes emitting at different wavelengths which are each coupled with a specific oligonucleotide linked to a fluorescence quencher which is degraded during amplification to release the fluorochrome (e.g. TaqManTM, Perkin Elmer).

Example 11

[0069] Detection of amplification Products. The person skilled in the art will appreciate that alternatives other than

standard agarose gel electrophoresis (Example 7) may be used for the revelation of amplification products. Such methods may be based on the detection of fluorescence after amplification (e.g. AmplisensorTM, Biotronics; TaqManTM) or other labels such as biotin (SHARP SignalTM system, Digene Diagnostics). These methods are quantitative and easily automated. One of the amplification primers or an internal oligonucleotide probe specific to the amplicon(s) derived from the species-specific fragment probes is coupled with the fluorochrome or with any other label. Methods based on the detection of fluorescence are particularly suitable for diagnostic tests since they are rapid and flexible as fluorochromes emitting different wavelengths are available (Perkin Elmer).

Example 12

[0070] Species-specific, universal and antibiotic resistance gene amplification primers can be used in other rapid amplification procedures such as the ligase chain reaction (LCR), transcription-based amplification systems (TAS), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA) and branched DNA (bDNA) or any other methods to increase the sensitivity of the test. Amplifications can be performed from an isolated bacterial colony or directly from clinical specimens. The scope of this invention is therefore not limited to the use of PCR but rather includes the use of any procedures to specifically identify bacterial DNA and which may be used to increase rapidity and sensitivity of the tests.

Example 13

[0071] A test kit would contain sets of probes specific for each bacterium as well as a set of universal probes. The kit is provided in the form of test components, consisting of the set of universal probes labeled with non-radioactive labels as well as labeled specific probes for the detection of each bacterium of interest in specific clinical samples. The kit will also include test reagents necessary to perform the prehybridization, hybridization, washing steps and hybrid detection. Finally, test components for the detection of known antibiotic resistance genes (or derivatives therefrom) will be included. Of course, the kit will include standard samples to be used as negative and positive controls for each hybridization test.

[0072] Components to be included in the kits will be adapted to each specimen type and to detect pathogens commonly encountered in that type of specimen. Reagents for the universal detection of bacteria will also be included. Based on the sites of infection, the following kits for the specific detection of pathogens may be developed:

- **[0073]** A kit for the universal detection of bacterial pathogens from most clinical specimens which contains sets of probes specific for highly conserved regions of the bacterial genomes.
- [0074] A kit for the detection of bacterial pathogens retrieved from urine samples, which contains eight specific test components (sets of probes for the

detection of Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Staphylococcus aureus and Staphylococcus epidermidis).

- [0075] A kit for the detection of respiratory pathogens which contains seven specific test components (sets of probes for detecting *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus*).
- [0076] A kit for the detection of pathogens retrieved from blood samples, which contains eleven specific test components (sets of probes for the detection of *Streptococcus pneumoniae, Moraxella catarrhalis, Haemophilus influenzae, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Streptococcus pyogenes* and *Staphylococcus epidermidis*).
- [0077] A kit for the detection of pathogens causing meningitis, which contains four specific test components (sets of probes for the detection of *Haemophilus influenzae, Streptococcus pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*).
- [0078] A kit for the detection of clinically important antibiotic resistance genes which contains sets of probes for the specific detection of at least one of the 19 following genes associated with bacterial resistance : bla_{tem}, bla_{rob}, bla_{shv}, aadB, aacC1, aacC2, aacC3, aacA4, mecA, vanA, vanH, vanX, satA, aacA-aphD, vat, vga, msrA, sul and int.
- [0079] Other kits adapted for the detection of pathogens from skin, abdominal wound or any other clinically relevant kits will be developed.

Example 14

[0080] Same as example 13 except that the test kits contain all reagents and controls to perform DNA amplification assays. Diagnostic kits will be adapted for amplification by PCR (or other amplification methods) performed directly either from clinical specimens or from a bacterial colony. Components required for universal bacterial detection, bacterial identification and antibiotic resistance genes detection will be included.

[0081] Amplification assays could be performed either in tubes or in microtitration plates having multiple wells. For assays in plates, the wells will be coated with the specific amplification primers and control DNAs and the detection of amplification products will be automated. Reagents and amplification primers for universal bacterial detection will be included in kits for tests performed directly from clinical specimens. Components required for bacterial identification and antibiotic resistance gene detection will be included in kits for testing directly from colonies as well as in kits for testing directly from clinical specimens.

[0082] The kits will be adapted for use with each type of specimen as described in example 13 for hybridization-based diagnostic kits.

Example 15

[0083] It is understood that the use of the probes and amplification primers described in this invention for bacterial detection and identification is not limited to clinical microbiology applications. In fact, we feel that other sectors could also benefit from these new technologies. For example, these tests could be used by industries for quality control of food, water, pharmaceutical products or other products requiring microbiological control. These tests could also be applied to detect and identify bacteria in biological samples from organisms other than humans (e.g. other primates, mammals, farm animals and live stocks). These diagnostic tools could also be very useful for research purposes including clinical trials and epidemiological studies.

TABLE 1

Distribution of urinary isolates from positive urine samples (≥10 ⁷ CFU/L) at the Centre Hospitalier de l'Université Laval (CHUL) for the 1992–1994 period.						
% of isolates						
Organisms	Nov 92 n = 267^a	April 93 n = 265	July 93 n = 238	Jan 94 n = 281		
Escherichia coli	53.2	51.7	53.8	54.1		
Enterococcus faecalis	13.8	12.4	11.7	11.4		
Klebsiella pneumoniae	6.4	6.4	5.5	5.3		
Staphylococcus epidermidis	7.1	7.9	3.0	6.4		
Proteus mirabilis	2.6	3.4	3.8	2.5		
Pseudomonas aeruginosa	3.7	3.0	5.0	2.9		
Staphylococcus saprophyticus	3.0	1.9	5.4	1.4		
Others ^b	10.2	13.3	11.8	16.0		

 ^{a}n = total number of isolates for the indicated month. ^bSee table 2.

[0084]

FABLE	2
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Distribution of uncommon ^a urinary isolates from positive urine
samples ($\geq 10^7$ CFU/L) at the Centre Hospitalier de
I'Université Laval (CHUL) for the 1992-1994 period.

Organisms ^a	Nov 92	April 93	July 93	Jan 94
Staphylococcus aureus	0.4	1.1	1.3	1.4
Staphylococcus spp.	2.2	4.9	1.7	6.0
Micrococcus spp.	0.0	0.0	0.4	0.7
Enterococcus faecium	0.4	0.4	1.3	1.4
Citrobacter spp.	1.4	0.8	0.4	0.7
Enterobacter spp.	1.5	1.1	1.3	1.4
Klebsiella oxytoca	1.1	1.5	2.5	1.8
Serratia spp.	0.8	0.0	0.5	0.0
Proteus spp.	0.4	0.4	0.0	1.1

TABLE 2-continued

Distribution of uncommon ^a urinary isolates from positive u	ırine
samples (${}^{\geqq}10^7~{\rm CFU/L})$ at the Centre Hospitalier de	
l'Université Laval (CHUL) for the 1992-1994 period.	

	% of isolates			
Organisms ^a	Nov 92	April 93	July 93	Jan 94
Morganella and Providencia	0.4	0.8	0.4	0.0
Hafnia alvei	0.8	0.0	0.0	0.0
NFB ^b (Stenotrophomonas,	0.0	0.4	1.3	1.1
Acinetobacter) Candida spp.	0.8	1.9	0.7	0.4

^aUncommon urinary isolates are those identified as "Others" in Table 1. ^bNFB: non fermentative bacilli.

[0085]

TABLE 3

Distribution of positive^a (bacterial count ≥10⁷ CFU/L) and negative (bacterial count <10⁷ CFU/L) urine specimens tested at the Centre Hospitalier de l'Universite Laval (CHUL) for the 1992–1994 period.

	Number of isolates (%)						
Specimens	Nov 92	April 93	July 93	Jan 94			
received: positive: negative:	1383(100) 267(19.3) 1116(80.7)	1338(100) 265(19.8) 1073(80.2)	1139(100) 238(20.9) 901(79.1)	1345(100) 281(20.9) 1064(79.1)			

^aBased on standard diagnostic methods, the minimal number of bacterial pathogens in urine samples to indicate an urinary tract infection is normally 10^7 CFU/L.

[0086]

TABLE 4

Distribution of positive and negative clinical specimens tested in the Microbiology Laboratory of the CHUL.							
Clinical specimens ^a	No. of samples tested	% of positive specimens	% of negative specimens				
Urine	17,981	19.4	80.6				
Haemoculture/marrow	10,010	6.9	93.1				
Sputum	1,266	68.4	31.6				
Superficial pus	1,136	72.3	27.7				
Cerebrospinal fluid	553	1.0	99.0				
Synovial fluid-articular	523	2.7	97.3				
Bronch./Trach./Amyg./Throat	502	56.6	43.4				
Deep pus	473	56.8	43.2				
Ears	289	47.1	52.9				
Pleural and pericardial fluid	132	1.0	99.0				
Peritonial fluid	101	28.6	71.4				

^aSpecimens tested from February 1994 to January 1995.

[0087]

TABLE 5

Bacterial species Bram negative:	Number c strains tested	t Bacterial species Gram positive:	Number of strains tested
Proteus mirabilis	5	Streptococcus pneumoniae	7
Klebsiella pneumoniae	5	Streptococcus salivarius	2
Pseudomonas aeruginosa	5	Streptococcus viridans	2
Escherichia coli	5	Streptococcus pyogenes	2
Moraxella catarrhalis	5	Staphylococcus aureus	2
Proteus vulgaris	2	Staphylococcus epidermidis	2
Morganella morganii	2	Staphylococcus saprophyticus	5
Enterobacter cloacae	2	Micrococcus species	2
Providencia stuartii	1	Corynebacterium species	$\overline{2}$
Providencia species	1	Streptococcus groupe B	$\frac{1}{2}$
Enterobacter agglomerans	2	Staphylococcus simulans	$\frac{\tilde{2}}{2}$
Providencia rettgeri	2	Staphylococcus ludgunensis	1
Veisseria mucosa	1	Staphylococcus capitis	2
Providencia alcalifaciens	1	Staphylococcus haemolyticus	$\frac{\tilde{2}}{2}$
Providencia rustigianii	1	Staphylococcus hominis	$\frac{\tilde{2}}{2}$
Burkholderia cepacia	2	Enterococcus faecalis	$\frac{1}{2}$
Enterobacter aerogenes	2	Enterococcus faecium	1
Stenotrophomonas maltophilia	2	Staphylococcus warneri	1
Pseudomonas fluorescens	1	Enterococcus durans	1
Comamonas acidovorans	2	Streptococcus bovis	1
Pseudomonas putida	2	Diphteroids	2
Jaemophilus influenzae	5	Lactobacillus acidophilus	1
Taemophilus parainfluenzae	2	Euclobucinus uciuopinius	1
Bordetella pertussis	2		
Haemophilus parahaemolyticus	2		
Taemophilus paranaemotyticus	2		
Taemophilus aegyptius	1		
Kingella indologenes	1		
Moraxella atlantae	1		
Noraxena anamae Veisseria caviae	1		
veisseria caviae Veisseria subflava	1		
veisseria suojiava Moraxella urethralis	1		
Moraxena urenrans Shigella sonnei	1		
Shigella flexneri	1		
Klebsiella oxytoca	2		
Siedsieua oxytoca Serratia marcescens	2		
Salmonella typhimurium	1		
ersinia enterocolitica	1		
	1		
Acinetobacter calcoaceticus			
Acinetobacter lwoffi Laftria alusi	$\frac{1}{2}$		
Haftnia alvei	_		
Citrobacter diversus	1		
Citrobacter freundii Salmonella species	1 1		

[0088]

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TABLE	6

	Number of fragment probes ^b			Number of oligonucleotide probes		
Organisms ^a	Tested	Specific	Ubiquitous ^e	Synthesized	Specific	Ubiquitous ^e
E. coli ^d	_	_	_	20	12	9 ^f
E. coli	14	2	2 ^e	_	_	_
K. pneumoniae ^d	_	_	_	15	1	1
K. pneumoniae	33	3	3	18	12	8
P. mirabilis ^d			_	3	3	2
P. mirabilis	14	3	3 ^e	15	8	7
P. aeruginosa ^d		_	_	26	13	9
P. aeruginosa	6	2	2 ^e	6	0	0

Species-specific DNA fragment and oligonucleotide probes for hybridization.							
	Number of fragment probes ^b			Number of	oligonucle	otide probes	
Organisms ^a	Tested	Specific	Ubiquitous ^e	Synthesized	Specific	Ubiquitous ^e	
S. saprophyticus	7	4	4	20	9	7	
H. influenzae ^d		_	_	16	2	2	
H. influenzae	1	1	1	20	1	1	
S. pneumoniae ^d	_	_	_	6	1	1	
S. pneumoniae	19	2	2	4	1	1	
M. catarrhalis	2	2	2	9	8	8	
S. epidermidis	62	1	1	_	_	_	
S. aureus	30	1	1	_	_	_	
Universal probes ^d	—		—	7	_	78	

TABLE 6-continued

^aNo DNA fragment or oligonucleotide probes were tested for *E. faecalis* and *S. pyogenes*. ^bSizes of DNA fragments range from 0.25 to 5.0 kbp. ^cA specific probe was considered ubiquitous when at least 80% of isolates of the target spe-cies (approximately 80 isolates) were recognized by each specific probe. When 2 or more probes are combined, 100% of the isolates are recognized. ^aThese sequences were selected from data banks. ^aUbiquity tested with approximately 10 isolates of the target species. ^fA majority of probes (8/9) do not discriminate *E. coli* and *Shigella* spp. ^gUbiquity tests with a pool of the 7 probes detected all 66 bacterial species listed in Table 5.

5.

[0089]

TABLE 7

P	CR amplification for urine, sputum, bloc				
	Primer pair ^a	Amplicon		DNA ar	nplification
Organism	# (SEQ ID NO)	size (bp)	Ubiquity ^b	from colonies ^c	from specimens ^d
E. coli	l ^e (55–56)	107	75/80	+	+
	$2^{e}(46-47)$	297	77/80	+	+
	3 (42-43)	102	78/80	+	+
	4 (131–132)	134	73/80	+	+
	1 + 3 + 4	_	80/80	+	+
E.faecalis	1 ^e (38–39)	200	71/80	+	+
·	$2^{e}(40-41)$	121	79/80	+	+
	1+2	_	80/80	+	+
K. pneumoniae	1 (67-68)	198	76/80	+	+
•	2 (61-62)	143	67/80	+	+
	$3^{h}(135-136)$	148	78/80	+	$N.T.^{i}$
	4 (137–138)	116	69/80	+	N.T.
	1+2+3	_	80/80	+	N.T.
P. mirabilis	1 (74–75)	167	73/80	+	N.T.
	2 (133–134)	123	80/80	+	N.T.
P. aeruginosa	1 ^e (83–84)	139	79/80	+	N.T.
	2 ^e (85–86)	223	80/80	+	N.T.
S. saprophyticus	1 (98–99)	126	79/80	+	+
St supropriynens	2 (139–140)	190	80/80	+	N.T.
M. catarrhalis	1 (112–113)	157	79/80	+	N.T.
111. Curai mano	2(112-119)	118	80/80	+	N.T.
	3(160-119)	137	80/80	+	N.T.
H. influenzae	$1^{e}(154-155)$	217	80/80	+	N.T.
S. pneumoniae	$1^{e}(156-157)$	134	80/80	+	N.T.
5. pricumonuae	$2^{e}(158-159)$	197	74/80	+	N.T.
	3 (78–79)	175	67/80	+	N.T.
S. epidermidis	1(147-148)	175	80/80	+	N.T.
5. cpiacimiais	2(145-146)	125	80/80	+	N.T.
S. aureus	1(152-153)	108	80/80	+	N.T.
5. uureus	2(149-150)	108	80/80	+	N.T.
	3(149-150)	176	80/80	+	N.T.
S. pyogenes ^f	$1^{e}(149-131)$	213	80/80	+	N.T.
5. pyogenes	$2^{e}(143-144)$	213 157	24/24		N.T.
Universal	1^{e} (126–127)	241	24/24 194/195 ^g	+ +	+

See notes on next page

- [0090] a All primer pairs are specific in PCR assays since no amplification was observed with DNA from 66 different species of both Gram positive and Gram negative bacteria other than the species of interest (Table 5).
- [0091] b The ubiquity was normally tested on 80 strains of the species of interest. All retained primer pairs amplified at least 90% of the isolates. When combinations of primers were used, an ubiquity of 100% was reached.
- [0092] c For all primer pairs and multiplex combinations, PCR amplifications directly performed from a bacterial colony were 100% species-specific.
- [0093] d PCR assays performed directly from urine specimens.

- [0094] e Primer pairs derived from data bank sequences. Primer pairs with no "e" are derived from our species-specific fragments.
- [0095] f For S. pyogenes, primer pair #1 is specific for Group A Streptococci (GAS). Primer pair #2 is specific for the GAS-producing exotoxin A gene (SpeA).
- [0096] g Ubiquity tested on 195 isolates from 23 species representative of bacterial pathogens commonly encountered in clinical specimens.
- [0097] h Optimizations are in progress to eliminate non-specific amplification observed with some bacterial species other than the target species.
- [0098] N.T.: not tested.

TABLE 8

Selected antibiotic resistance genes for diagnostic purposes.									
Genes	Antibiotics	Bacteria ^a	SEQ ID NO						
(bla _{tem}) TEM-1	β-lactams	Enterobacteriaceae, Pseudomonadaceae, Haemophilus, Neisseria	161						
(bla _{rob}) ROB-1	β-lactams	Haemophilus, Pasteurella	162						
(bla _{shv}) SHV-1	β-lactams	Klebsiella and other Enterobacteriaceae	163						
aadB, aacC1, aacC2, aacC3, aacA4	Aminoglycosides	Enterobacteriaceae, Pseudomonadaceae	164, 165, 166 167, 168						
mecA	β-lactams	Staphylococci	169						
vanH, vanA, vanX	Vancomycin	Enterococci	170						
satA	Macrolides	Enterococci	173						
aacA-aphD	Aminoglycosides	Enterococci, Staphylococci	174						
vat	Macrolides	Staphylococci	175						
vga	Macrolides	Staphylococci	176						
msrA	Erythromycin	Staphylococci	177						
Int and Sul conserved sequences	β-lactams, trimethoprim, aminoglycosides, antiseptic,	Enterobacteriaceae, Pseudomonadaceae	171, 172						
conserved sequences	chloramphenicol	1 seudonionadacede							

^aBacteria having high incidence for the specified antibiotic resistance genes. The presence in other bacteria is not excluded.

[0099]

	Annex I: Specific and ubiquitous oligonucleotides probes for hybridization								
					Origina	ating DNA fraqment			
SEQ ID	NO Nucleotide	e Sequence	e		SEQ ID NO	Nucleotide position			
		Bact	erial speci	es: Escher	ichia coli				
44	5'-CAC CCG	CTT GCG	TGG CAA GC	r gcc c	5ª	213-237			
45	5'-CGT TTO	TGG ATT	CCA GTT CCA	A TCC G	5 ^a	489-513			
48	5'-TGA AGO	ACT GGC	CGA AAT GC	г ссс т	6 ^a	759-783			
49	5'-GAT GTA	A CAG GAT	TCG TTG AAG	G GCT T	6 ^a	898-922			
50	5'-TAG CGA	AGG CGT	AGC AGA AAG	с таа с	7 ^a	1264-1288			
51	5'-GCA ACC	CGA ACT	CAA CGC CGC	G ATT T	7 ^a	1227—1251			
52	5'-ATA CAC	AAG GGT	CGC ATC TG	c GGC C	7ª	1313-1337			

	-continued											
Annex I: Specific and ubiquitous oligonucleotides probes for hybridization												
	Originating DNA fragment											
SEQ ID NC	Nucleotide Sequence	SEQ ID NO Nucleotide position										
53	5'-TGC GTA TGC ATT GCA GAC CTT GTG GC	7 ^a 111–136										
54	5'-GCT TTC ACT GGA TAT CGC GCT TGG G	7 ^a 373–397										
	Bacterial species: Proteus m	nirabilis										
70 ^b	5'-TGG TTC ACT GAC TTT GCG ATG TTT C	12 23–47										
71	5'-TCG AGG ATG GCA TGC ACT AGA AAA T	12 53–77										
72 ^b	5'-CGC TGA TTA GGT TTC GCT AAA ATC TTA TTA	A 12 80-109										
73	5'-TTG ATC CTC ATT TTA TTA ATC ACA TGA CCA	A 12 174–203										

 $^{\rm a}S$ equences from data banks $^{\rm b}T$ hese sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0100]

Annex I: Specific and ubiquitous oligonucleotides probes for hybridization

								Origina	ating DNA fragment				
SEQ ID NO	Nucleotio	de Sequ	lence					SEQ ID NO	Nucleotide position				
	Bacterial species: Proteus mirabilis												
76	5'-CCG CC	СТ ТТА	GCA TTA	ATT	GGT	GTT	TAT AGT	13	246-275				
77	5'-CCT A	IT GCA	GAT ACC	TTA	AAT	GTC	TTG GGC	13	291-320				
80 ^b	5'-TTG AG	GT GAT	GAT TTC	ACT	GAC	TCC	С	14	18-42				
81	5'-GTG AG	GA CAG	TGA TGG	TGA	GGA	CAC	A	15 ^a	1185—1209				
82	5'-TGG T	IG TCA	TGC TGT	TTG	TGT	GAA	AAT	15 ^a	1224-1230				
		Bac	terial	speci	es:	Kleł	osiella p	neumoniae	-				
57	5'-GTG G	IG TCG	TTC AGG	GGT	TTC	AC		8	45—67				
58	5'-GCG A	FA TTC	ACA CCC	TAC	GCA	GCC	A	9	161-185				
59 ^b	5'-GTC G	АА ААТ	GCC GGA	AGA	GGT	ATA	CG	9	203-228				
60 ^b	5'-ACT G	AG CTG	CAG ACC	GGT	ААА	ACT	CA	9	233–258				
63 ^b	5'-CGT G	AT GGA	ТАТ ТСТ	TAA	CGA	AGG	GC	10	250-275				
64 ^b	5'-ACC A	AA CTG	TTG AGC	CGC	CTG	GA		10	201-223				
65	5'-GTG A	rc gcc	CCT CAT	CTG	CTA	СТ		10	77—99				
66	5'-CGC C	CT TCG	TTA AGA	ATA	TCC	ATC	AC	10	249-274				
69	5'-CAG GA	AA GAT	GCT GCA	CCG	GTT	GTT	G	11^{a}	296-320				

^aSequences from data banks

^bThese sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0101]

		Annex	I: 8	Speci	fic a			uitou oridi				cleoti	des p	robes	
											(DNA frag	
SEQ	ID NO	Nucleot		-							~			eotide po	sition
			Bac	teri	al s	speci	Les:	Psei	idomo	ona	as a	ərugin	osa		
87		5 '- AAT	GCG	GCT	GTA	CCT	CGG	CGC	TGG	т	18^{a}		2985	—3009	
88		5 ' - GGC	GGA	GGG	CCA	GTT	GCA	ССТ	GCC	A	18^{a}		2929	-2953	
89		5'-AGC	сст	GCT	сст	CGG	CAG	ССТ	CTG	С	18^{a}		2821·	-2845	
90		5 '- TGG	CTT	TTG	CAA	CCG	CGT	TCA	GGT	т	18^{a}		1079	-1103	
91		5 '- GCG	ccc	GCG	AGG	GCA	TGC	TTC	GAT	G	19^{a}		705—	729	
92		5'-ACC	TGG	GCG	CCA	ACT	ACA	AGT	TCT	A	19^{a}		668—	692	
93		5'-GGC	TAC	GCT	GCC	GGG	CTG	CAG	GCC	G	19^{a}		505—	529	
94		5 '- CCG	ATC	TAG	ACC	ATC	GAG	ATG	GGC	G	20^{a}		1211	-1235	
95		5 '- GAG	CGC	GGC	TAT	GTG	TTC	GTC	GGC	т	$20^{\rm a}$		2111	-2135	
		_	Bact	eria	al sp	pecie	es: 2	Strep	otoco	bco	cus p	pneumo	niae	_	
120		5 ' – TCT	GTG	ста	GAG	ACT	GCC	CCA	TTT	с	30			423-447	
121		5'-CGA	TGT	CTT	GAT	TGA	GCA	GGG	TTA	т	31 ^a			1198—122	2

^aSequences from data banks ^bThese sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0102]

	Ann	ex I: Specific and ubiquitous oligonu for hybridization	ucleotides probes
			Originating DNA fragment
SEQ ID N	NO Nucleot:	ide Sequence	SEQ ID NO Nucleotide position
	-	Bacterial species: Staphylococcus s	saprophyticus
96	5'-CGT 1	TTT TAC CCT TAC CTT TTC GTA CTA CC	21 45-73
97 ^b	5'-TCA	GGC AGA GGT AGT ACG AAA AGG TAA GGG	21 53-82
100	5'-CAC	CAA GTT TGA CAC GTG AAG ATT CAT	22 89–115
101 ^b	5'-ATG A	AGT GAA GCG GAG TCA GAT TAT GTG CAG	23 105–134
102	5'-CGC 5	TCA TTA CGT ACA GTG ACA ATC G	24 20-44
103	5'-CTG (GTT AGC TTG ACT CTT AAC AAT CTT GTC	24 61-90
104 ^b	5'-GAC	GCG ATT GTC ACT GTA CGT AAT GAG CGA	24 19-48
		Bacterial species: Moraxella ca	tarrhalis
108	5'-GCC (CCA AAA CAA TGA AAC ATA TGG T-3'	28 81-105
109	5'-CTG (CAG ATT TTG GAA TCA TAT CGC C-3'	28 126–130
110	5'-TGG 1	TTT GAC CAG TAT TTA ACG CCA T-3'	28 165–189

	-continued										
	Annex I: Specific and ubiquitous oligonucleotides probes for hybridization										
	Originating DNA fragment										
SEQ ID NO) Nucleotide Sequence	SEQ ID NO Nucleotide position									
111	5'-CAA CGG CAC CTG ATG TAC CTT GTA C-3'	28 232–256									
114	5'-TTA CAA CCT GCA CCA CAA GTC ATC A-3'	29 97–121									
115	5'-GTA CAA ACA AGC CGT CAG CGA CTT A-3'	29 139–163									
116	5'-CAA TCT GCG TGT GTG CGT TCA CT-3'	29 178–200									
117	5'-GCT ACT TTG TCA GCT TTA GCC ATT CA-3'	29 287–312									

^aSequences from data banks ^bThese sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0103]

	Annex I: Specific and ubiquitous oligonucleotides probes for hybridization	i 	
		Origina fı	ating DNA cagment
SEQ ID NO	Nucleotide sequence	SEQ ID NO	Nucleotide position
Bacterial species	:Haemophilus influenzae		
105 ^b	5'-GCG TCA GAA AAA GTA GGC GAA ATG AAA G	25	138—165
106 ^b	5'-AGC GGC TCT ATC TTG TAA TGA CAC A	26 ^a	770—794
107 ^b	5'-GAA ACG TGA ACT CCC CTC TAT ATA A	27 ^a	5184-5208
	Universal probes ^c		
122 ^b	5'-ATC CCA CCT TAG GCG GCT GGC TCC A	_	_
123	5'-ACG TCA AGT CAT CAT GGC CCT TAC GAG TAG G	_	_
124 ^b	5'-GTG TGA CGG GCG GTG TGT ACA AGG C	_	-
125 ^b	5'-GAG TTG CAG ACT CCA ATC CGG ACT ACG A	_	-
128 ^b	5'-CCC TAT ACA TCA CCT TGC GGT TTA GCA GAG AG	_	-
129	5'-GGG GGG ACC ATC CTC CAA GGC TAA ATA C	_	_
130 ^b	5'-CGT CCA CTT TCG TGT TTG CAG AGT GCT GTG TT	-	-

^aSequences from data banks

 $^{\mathrm{b}}\mathrm{These}$ sequences are from the opposite DNA strand of the sequences given in the Sequence listing

^cUniversal probes were derived from 16S or 23S ribosomal RNA gene sequences not included in the Sequence listing

[0104]

_	Annex II: Specific and ubiquitous primers for DNA amplification											
						Originating	DNA fragment					
SEQ ID NO Nucleotide Sequence SEQ ID NO Nucleotide positio												
		Bacteri	al spec:	les:	Esc	herichia coli						
42	5 ' - GCT	TTC CAG	CGT CAT	ATT	G	4	177—195					
43 ^b	5 ' - GAT	CTC GAC	AAA ATG	GTG	A	4	260—278					
46	5 ' - TCA	CCC GCT	TGC GTG	GC		5 ^a	212-228					
47 ^b	5 ' - GGA	ACT GGA	ATC CAC	ААА	с	5 ^a	490—508					
55	5 ' - GCA	ACC CGA	ACT CAA	CGC	с	7 ^a	1227—1245					
56 ^b	5 ' - GCA	GAT GCG	ACC CTT	GTG	т	7 ^a	1315-1333					
131	5 ' - CAG	GAG TAC	GGT GAT	TTT	та	3	60—79					
132 ^b	5 ' – ATT	TCT GGT	TTG GTC	ATA	CA	3	174—193					
	Ba	acterial	species	: En	tero	ococcus faecalis	_					
38	5 '- GCA	ATA CAG	GGA AAA	ATG	тc	1 ^a	69—88					
39 ^b	5 ' – CTT	CAT CAA	ACA ATT	AAC	тc	1ª	249—268					
40	5 '- GAA	CAG AAG	AAG CCA	AAA	AA	2ª	569-588					
41 ^b	5 ' - GCA	ATC CCA	AAT AAT	ACG	GT	2 ^a	670—689					

^aSequences from data banks ^b These sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0105]

Anne	Annex II: Specific and ubiquitous primers for DNA amplification											
		Originating DNA fragment										
SEQ ID NC	Nucleotide Sequence	SEQ ID NO Nucleotide position										
Bacterial species: Klebsiella pneumoniae												
61	5'-GAC AGT CAG TTC GTC AGC C	9 37–55										
62 ^b	5'-CGT AGG GTG TGA ATA TCG C	9 161-179										
67	5'-TCG CCC CTC ATC TGC TAC T	10 81-99										
68 ^b	5'-GAT CGT GAT GGA TAT TCT T	10 260-278										
135	5'-GCA GCG TGG TGT CGT TCA	8 40-57										
136 ^b	5'-AGC TGG CAA CGG CTG GTC	8 170–187										
137	5'-ATT CAC ACC CTA CGC AGC CA	A 9 166—185										
138 ^b	5'-ATC CGG CAG CAT CTC TTT G	2 9 262-281										
	Bacterial species: Pro	oteus mirabilis										
74	5'-gaa aca tcg caa agt cag t	12 23-41										
75 ^b	5'-ATA AAA TGA GGA TCA AGT TO	2 12 170-189										

Ann	ex II: Specif	ic and ubiquit	ous primers	for DNA amplification
			0r	iginating DNA fragment
SEQ ID N	0 Nucleotide	Sequence	SEQ I	D NO Nucleotide position
133	5'-CGG GAG	TCA GTG AAA 7	ICA TC 14	17-36
134 ^b	5'-CTA AAA	TCG CCA CAC C	CTC TT 14	120-139

^aSequences from data banks ^bThese sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0106]

	Annex II:	: Specific and ubiquitous primers	for DNA amplif	ication
			Originating	g DNA fragment
SEQ ID N	NO Nucleotid	de Sequence	SEQ ID NO Nuc	leotide position
	Ba	acterial species: Staphylococcus	saprophyticus	
98	5'-CGT TI	TT TAC CCT TAC CTT TTC GTA CT	21	45-70
99 ^b	5'-ATC GA	AT CAT CAC ATT CCA TTT GTT TTT A	21	143-170
139	5'-CTG GI	TT AGC TTG ACT CTT AAC AAT C	24	61—85
140 ^b	5'-TCT TA	AA CGA TAG AAT GGA GCA ACT G	24	226-250
		Bacterial species: Pseudomonas	aeruginosa	
83	5'-CGA GC	CG GGT GGT GTT CAT C	16 ^a	554-572
84 ^b	5'-CAA GI	TC GTG GTG GGA GGG A	16 ^a	674—692
85	5'-тсс ст	TG TTC ATC AAG ACC C	17 ^a	1423—1441
86 ^b	5'-CCG AG	GA ACC AGA CTT CAT C	17 ^a	1627—1645
		Bacterial species: Moraxella c	atarrhalis	
112	5'-GGC AC	CC TGA TGT ACC TTG	28	235-252
113 ^b	5'-AAC AG	GC TCA CAC GCA TT	28	375-391
118	5'-TGT TI	TT GAG CTT TTT ATT TTT TGA	29	41—64
119 ^b	5'-CGC TG	GA CGG CTT GTT TGT ACC A	29	137—158
160	5'-GCT CA	AA ATC AGG GTC AGC	29	22—39
119 ^b	5'-CGC TG	GA CGG CTT GTT TGT ACG A	29	137—158

^aSequences from data banks

 $^{\mathrm{b}}\mathrm{These}$ sequences are from the opposite DNA strand of the sequences given in the Sequence listing

	Annex II: Specific and ubiquitous primers	s for DNA amplification
		Originating DNA fragment
SEQ ID NO	O Nuclectide Sequence	SEQ ID NO Nucleotide position
	Bacterial species: Staphylococcu	15 epidermidis
145	5'-ATC AAA AAG TTG GCG AAC CTT TTC A	36 21-45
146 ^b	5'-CAA AAG AGC GTG GAG AAA AGT ATC A	36 121–145
147	5'-TCT CTT TTA ATT TCA TCT TCA ATT CCA TA	AG 36 448-477
148 ^b	5'-AAA CAC AAT TAC AGT CTG GTT ATC CAT AT	C 36 593–622
	Bacterial species: Staphyloco	ccus aureus
149 ^b	5'-CTT CAT TTT ACG GTG ACT TCT TAG AAG AT	TT 37 409-438
150	5'-TCA ACT GTA GCT TCT TTA TCC ATA CGT TC	EA 37 288–317
149 ^b	5'-CTT CAT TTT ACG GTG ACT TCT TAG AAG AT	TT 37 409-438
151	5'-ATA TTT TAG CTT TTC AGT TTC TAT ATC A	AC 37 263–292
152	5'-AAT CTT TGT CGG TAC ACG ATA TTC TTC AC	CG 37 5—34
153 ^b	5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA AC	CA 37 83-112

^aSequences from data banks ^bThese sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0108]

Annex II: Specific and ubiquitous primers for DNA amplification							
		Originat	ing DNA fragment				
SEQ ID NO	O Nucleot	ide Sequence SEQ ID NO M	Nucleotide position				
	Bacterial species: Haemophilus influenzae						
154	5 ' – TTT	AAC GAT CCT TTT ACT CCT TTT G 27^{a}	5074-5098				
155 ^b	5 ' - ACT	GCT GTT GTA AAG AGG TTA AAA T 27^{a}	5266-5290				
	Bacterial species: Streptococcus pneumoniae						
78	5 ' - AGT	AAA ATG AAA TAA GAA CAG GAC AG 34	164-189				
79 ^b	5 '- AAA	ACA GGA TAG GAG AAC GGG AAA A 34	314-338				
156	5 ' – ATT	TGG TGA CGG GTG ACT TT 31^{a}	1401-1420				
157 ^b	5 ' - GCT	GAG GAT TTG TTC TTC TT 31 ^a	1515-1534				
158	5 ' - GAG	CGG TTT CTA TGA TTG TA 35 ^a	1342-1361				
159 ^ь	5 ' - ATC	TTT CCT TTC TTG TTC TT 35 ^a	1519—1538				
		Bacterial species: Streptococcus pyogenes	<u>.</u>				
141	5 ' - TGA	AAA TTC TTG TAA CAG GC 32 ^a	286—305				
142 ^b	5 ' –GGC	CAC CAG CTT GCC CAA TA 32 ^a	479-498				

-continued	
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	Annex II: Specific and ubiquitous prime	rs for DNA amplification
		Originating DNA fragment
SEQ ID	NO Nucleotide Sequence	SEQ ID NO Nucleotide position
143	5'-ATA TTT TCT TTA TGA GGG TG	33 ^a 966–985
144 ^b	5'-ATC CTT AAA TAA AGT TGC CA	33 ^a 1103–1122

^aSequences from data banks

 $^{\mathrm{b}}\mathrm{These}$ sequences are from the opposite DNA strand of the sequences given in the Sequence listing

[0109]

Annex II: Specific and ubiquitous primers for DNA amplification					
		Originating DNA fragment			
SEQ ID NO) Nucleotide Sequence	SEQ ID NO Nucleotide position			
	Universal pri	mers ^c			
126	5'-GGA GGA AGG TGG GGA TGA CG	;			
127 ^b	5'-ATG GTG TGA CGG GCG GTG TG				
^a Sequences from data banks ^b These sequences are from the opposite DNA strand of the sequences					

^b These sequences are from the opposite DNA strand of the sequences given in the Sequence listing ^c Universal primers were derived from the 16S ribosomal RNA gene

sequence not included in the Sequence listing

[0110]

Reverse strand of SEQ ID NO: 122 TGGAGCC AGCCGCCTA GGTGGGAT

Streptococcus salivarius		1510 TTT TGGAGCC	AGCCGCCTAA	GGTGGGAT AG	ATGANNGGGG
Proteus vulgaris	TAGCTTAACC	TTC GGGAGGG	CGCTTACCAC	TTTGTGATTC	ATGACTGGGG
Pseudomonas aeruginosa	TAGTCTAACC	GCA AGGGGGA	CGGTTACCAC	GGAGTGAT TC	ATGACTGGGG
Neisseria gonorrhoeae	TAGGGTAACC	GCA AGGAGTC	CGCTTACCAC	GGTATGCT TC	ATGACTGGGG
Streptococcus lactis	TTGCCTAACC	GCA AGGAGGG	CGCTTCCTAA	GGTAAGAC CG	ATGACNNGGG

[0111]

Annex III.	Selec	tion of	univ	ersal	pro	bes by	ali	gnmen	t of	the
sequences	s of b	bacteria	al 165	i and	23S	riboso	mal	RNA o	jenes	

ACGTCAAGTC ATCATGGC CCTTACGAGT AGG

Annex III Selection of Universal Probes by Alignment of the Sequences of Bacterial 16S and 23S Ribosomal RNA Genes.

-co	nt	lr	ıu	ed

Annex III. Selection of universal probes by alignment of the sequences of bacterial 165 and 235 ribosomal RNA genes.						
Neisseria gonorrhoeae	GGTGGGGATG	ACGTCAAGTC	CTCATGGC	CCTTATGACC	AGGGCTTCAC	
Pseudomonas cepacia	GGTNGGGATG	ACGTCAAGTC	CTCATGGC	CCTTATGGGT	AGGGCTTCAC	
Serratia marcescens	GGTGGGGATG	ACGTCAAGTC	ATCATGGC	CCTTACGAGT	AGGGCTACAC	
Escherichia coli	GGTGGGGATG	ACGTCAAGTC	ATCATGGC	CCTTACGACC	AGGGCTACAC	
Proteus vulgaris	GGTGGGGATG	ACGTTAAGTC	GTATCATGGC	CCTTACGAGT	AGGGCTACAC	
Pseudomonas aeruginosa	GGTGGGGATG	ACGTCAAGTC	ATCATGGC	CCTTACGGCN	AGGGCTACAC	
Clostridium perfringens	GGTGGGGATG	ACGTNNAATC	ATCATGCC	CNTTATGTGT	AGGGCTACAC	
Mycoplasma hominis	GGTGGGGATG	ACGTCAAATC	ATCATGCC	TCTTACGAGT	GGGGCCACAC	
Helicobacter pylori	GGTGGGGACG	ACGTCAAGTC	ATCATGGC	CCTTACGCCT	AGGGCTACAC	
Mycoplasma pneumoniae	GGAAGGGATG	ACGTCAAATC	ATCATGCC	CCTTATGTCT	AGGGCTGCAA	

[0112]

Annex III. Selection of u sequences of bacterial				he
Reverse of the probe SEQ ID NO: 124		GCCTTGTACA	CACCGCCCGT	CACAC
Escherichia coli	1451 ACGTTCCCGG	GCCTTGTACA	CACCGCCCGT	1490 CACACCATGG
Neisseria gonorrhoeae	ACGTTCCCNG	NNCTTGTACA	CACCGCCCGT	CACACCATGG
Pseudomonas cepacia	ACGTTCCCGG	GTCTTGTACA	CACNGCCCGT	CACACCATGG
Serratia marcescens	ACGTTCCCGG	GCCTTGTACA	CACCGCCCGT	CACACCATGG
Proteus vulgaris	ACGTTCCCGG	GCCTTGTACA	CACCGCCCGT	CACACCATGG
Haemophilus influenzae	ACGTTCCCGG	GCNTTGTACA	CACCGCCCGT	CACACCATGG
Pseudomonas aeruginosa	ACGTTCCCGG	GCCTTGTACA	CACCGCCCGT	CACACCATGG
Clostridium perfringens	ACGTTCCCNG	GTCTTGTACA	CACCGCNCGT	CACACCATGA
Mycoplasma hominis	ACGTTCTCGG	GTCTTGTACA	CACCGCCCGT	CACACCATGG
Helicobacter pylori	ACGTTCCCGG	GTCTTGTACT	CACCGCCCGT	CACACCATGG
Mycoplasma pneumoniae	ACGTTCTCGG	GTCTTGTACA	CACCGCCCGT	CAAACTATGA

[0113]

Annex III. Selection of universal probes by alignment of the sequences of bacterial 16S and 23S ribosomal RNA genes.						
Reverse strand of SEQ ID NO 125:	TCG TAGTCCGGAT TGGAGTCTGC	AACTC				
Escherichia coli	1361 AAGTGCGTCG TAGTCCGGAT TGGAGTCTGC	1400 AACTCGACTC				
Neisseria gonorrhoeae	AAACCGATCG TAGTCCGGAT TGCACTCTGC	AACTCGAGTG				

-continued					
Annex III. Selection of universal probes by alignment of the sequences of bacterial 16S and 23S ribosomal RNA genes.					
Pseudomonas cepacia	AAACCGATCG TAGTCCGGAT TGCACTCTGC AACTCGAGTG				
Serratia marcescens	AAGTATGTCG TAGTCCGGAT TGGAGTCTGC AACTCGACTC				
Proteus vulgaris	AAGTCTGTCG TAGTCCGGAT TGGAGTCTGC AACTCGACTC				
Haemophilus influenzae	AAGTACGTCT AAGTCCGGAT TGGAGTCTGC AACTCGACTC				
Pseudomonas aeruginosa	AAACCGATCG TAGTCCGGAT CGCAGTCTGC AACTCGACTG				
Clostridium perfringens	AAACCAGTCT CAGTTCGGAT TGTAGGCTGA AACTCGCCTA				
Mycoplasma hominis	AAGCCGATCT CAGTTCGGAT TGGAGTCTGC AATTCGACTC				
Helicobacter pylori	ACACCTCT CAGTTCGGAT TGTAGGCTGC AACTCGCCTG				
Mycloplasma pneumoniae	AAGTTGGTCT CAGTTCGGAT TGAGGGCTGC AATTCGTCCT				

[0114]

Annex III. Selection of universal probes by alignment of the sequences of bacterial 16S and 23S ribosomal RNA genes.					
Reverse strand of SEQ ID NO: 128	CT	CTCTGCTAAA CCGCAAGGTG ATGTATAGGG			
Lactobacillus lactis	1991 AAACACAGCT	2040 CTCTGCTAAA CCGCAAGGTG ATGTATAGGG GGTGACGCCT			
Escherichia coli	AAACACAGCA	CTGTGCAAAC ACGAAAGTGG ACGTATACGG TGTGACGCCT			
Pseudomonas aeruginosa	AAACACAGCA	CTCTGCAAAC ACGAAAGTGG ACGTATAGGG TGTGACGCCT			
Pseudomonas cepacia	AAACACAGCA	CTCTGCAAAC ACGAAAGTGG ACGTATAGGG TGTGACGCCT			
Bacillus stearothermophilus	AAACACAGGT	CTCTGCGAAG TCGTAAGGCG ACGTATAGGG GCTGACACCT			
Micrococcus luteus	AAACACAGGT	CCATGCGAAG TCGTAAGACG ATGTATATGG ACTGACTCCT			
SEQ ID NO: 129		GGGGGGACC ATCCTCCAAG GCTAAATAC			
Escherichia coli	481 TGTCTGAATA	530 TGGGGGGGACC ATCCTCCAAG GCTAAATACT CCTGACTGAC			
Pseudomonas aeruginosa	TGTCTGAACA	TGGGGGGGACC ATCCTCCAAG GCTAAATACT ACTGACTGAC			
Pseudomonas cepacia	TGTCTGAAGA	TGGGGGGGACC ATCCTCCAAG GCTAAATACT CGTGATCGAC			
Lactobacillus lactis	AGTTTGAATC	CGGGAGGACC ATCTCCCAAC CCTAAATACT CCTTAGTGAC			
Micrococcus luteus	CGTGTGAATC	TGCCAGGACC ACCTGGTAAG CCTGAATACT ACCTGTTGAC			

[0115]

Annex III. Selection of universal probes by alignment of the sequences of bacterial 16S and 23S ribosomal RNA genes.

Reverse strand of SEQ ID NO: 130	AACACAGCA C	TCTGCAAAC ACGAAAGTGG ACG
	1981	2030
Pseudomonas aeruginosa	TGTTTATTAA AAACACAGCA C	TCTGCAAAC ACGAAAGTGG ACGTATAGGG

21

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Annex III. Selection of universal probes by alignment of the sequences of bacterial 16S and 23S ribosomal RNA genes.					
Escherichia coli	TGTTTATTAA AAACACAGCA CTGTGCAAAC ACGAAAGTGG ACGTATACG				
Pseudomonas cepacia	TGTTTAATAA AAACACAGCA CTCTGCAAAC ACGAAAGTGG ACGTATAGG				
Bacillus stearothermophilus	TGTTTATCAA AAACACAGGT CTCTGCGAAG TCGTAAGGCG ACGTATAGG				
Lactobacillus lactis	TGTTTATCAA AAACACAGCT CTCTGCTAAA CCACAAGGTG ATGTATAGG				
Micrococcus luteus	TGTTTATCAA AAACACAGGT CCATGCGAAG TCGTAAGACG ATGTATATG				

[0116]

Annex IV. Selection		versal PCR S ribosomal	primers by alignment of RNA gene	the	bacterial	
SEQ ID NO: 126	GGAGGAA	GGTGGGGATG	ACG			
Reverse strand of SEQ ID NO: 127				CA	CACCGCCCGT	CACACCAT
Escherichia coli	1241 ACTGGAGGAA	GGTGGGGATG	12701461 ACGTCAAGTCGCCTTGT	'ACA	CACCGCCCGT	1490 CACACCATGG
Neisseria gonorrhoeae	GCCGGAGGAA	GGTGGGGATG	ACGTCAAGTCNNCTTGT	'ACA	CACCGCCCGT	CACACCATGG
Pseudomonas cepacia	ACCGGAGGAA	GGTNGGGATG	ACGTCAAGTCGTCTTGT	ACA	CACNGCCCGT	CACACCATGG
Seratia marcescens	ACTGGAGGAA	GGTGGGGATG	ACGTCAAGTCGCCTTGT	ACA	CACCGCCCGT	CACACCATGG
Proteus vulgaris	ACCGGAGGAA	GGTGGGGATG	ACGTTAAGTCGCCTTGT	ACA	CACCGCCCGT	CACACCATGG
Haemophilus influenzae	ACTGGAGGAA	GGTNGGGATG	ACGTCAAGTCGCNTTGT	ACA	CACCGCCCGT	CACACCATGG
Legionella pneumophila	ACCGGAGGAA	GGCGGGGATG	ACGTCAAGTCGCCTTGT	ACA	CACCGCCCGT	CACACCATGG
Pseudomonas aeruginos	ACCGGAGGAA	GGTGGGGATG	ACGTCAAGTCGCCTTGT	ACA	CACCGCCCGT	CACACCATGG
Clostridium perfringens	CCAGGAGGAA	GGTGGGGATG	ACGTNNAATCGTCTTGT	ACA	CACCGCNCGT	CACACCATGA
Mycoplasma hominis	CTGGGAGGAA	GGTGGGGATG	ACGTCAAATCGTCTTGT	ACA	CACCGCCCGT	CACACCATGG
Helicobacter pylori	GGAGGAGGAA	GGTGGGGACG	ACGTCAAGTCGTCTTGT	ACT	CACCGCCCGT	CACACCATGG
Mycoplasma pneumoniae	ATTGGAGGAA	GGAAGGGATG	ACGTCAAATCGTCTTGT	ACA	CACCGCCCGT	CAAACTATGA

[0117]

SEQUENCE LISTING

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46

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47

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67

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What is claimed is

1. A method using probes (fragments and/or oligonucleotides) and/or amplification primers which are specific, ubiquitous and sensitive for determining the presence and/or amount of nucleic acids from bacterial species selected from the group consisting of Escherichia coli, Klebsiells pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus pneumoniae, Staphlvococcus aureus, Staphvlococcus epidermidis, Enterococcus faecalis, Staphlyococcus saprophyticus, Streptococcus pyogenes, Haemophilus influenzae and Moraxells catarrhalis in a any sample suspected of containing said bacterial nucleic acid, wherein said bacterial nucleic acid or variant or part thereof comprises a selected target region hybridizable with said probes or primers; said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes and/or amplified products as an indication of the presence and/or amount of said bacterial species.

2. A method as defined in claim 1 further using probes (fragments and/or oligonucleotides) and/or amplification primers which are universal and sensitive for determining the presence and/or amount of nucleic acids from any bacteria from any sample suspected of containing said bacterial nucleic acid, wherein said bacterial nucleic acid or variant or part thereof comprises a selected target region hybridizable with said probes or primers; said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes and/or amplified products as an indication of the presence and/or amount of said any bacteria.

3. A method as defined in claim 1 further using probes (fragments and/or oligonucleotides) and/or amplification primers which are specific, ubiquitous and sensitive for determining the presence and/or amount of nucleic acids from an antibiotic resistance gene selected from the group consisting of blatem, Blarob, Blashv, aadB, aacC1, aacC2, aacC3, aacA4, mecA, vanA, vanH, vanX, satA, aacA-aphD, vat, vga, msrA, sul and int in any sample suspected of containing said bacterial nucleic acid, wherein said bacterial nucleic acid or variant or part thereof comprises a selected target region hybridizable with said probes or primers; said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes and/or amplified products as an indication of the presence and/or amount of said antibiotic resistance gene.

4. The method of any one of claims 1, 2 and 3 which is performed directly on a sample obtained from human patients, animals, environment or food.

5. The method of any one of claims 1, 2 and 3 which is performed directly on a sample consisting of one or more bacterial colonies.

6. The method of any one of claims 1 to 5, wherein the bacterial nucleic acid is amplified by a method selected from the group consisting of:

- a) polymerase chain reaction (PCR),
- b) ligase chain reaction,
- c) nucleic acid sequence-based amplification,
- d) self-sustained sequence replication,
- e) strand displacement amplification,
- f) branched DNA signal amplification,
- g) nested PCR, and
- h) multiplex PCR.

7. The method of claim 6 wherein said bacterial nucleic acid is amplified by PCR.

8. The method of claim 7 wherein the PCR protocol is modified to determine within one hour the presence of said bacterial nucleic acids by performing for each amplification cycle an annealing step of only one second at 55° C. and a denaturation step of only one second at 95° C. without any elongation step.

9. A method for the detection, identification and/or quantification of *Escherichia coli* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
- inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic

acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Escherichia coli*, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and

c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Escherichia coli* in said test sample.

10. A method as defined in claim 9, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-227 nucleotides in length which sequence is comprised in SEQ ID NO: 3 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-278 nucleotides in length which sequence is comprised in SEQ ID NO: 4 or a complementary sequence thereof,
- 3) an oligonucleotide of 12-1596 nucleotides in length which sequence is comprised in SEQ ID NO: 5 or a complementary sequence thereof,
- an oligonucleotide of 12-2703 nucleotides in length which sequence is comprised in SEQ ID NO: 6 or a complementary sequence thereof,
- 5) an oligonucleotide of 12-1391 nucleotides in length which sequence is comprised in SEQ ID NO: 7 or a complementary sequence thereof, and
- variants thereof which specifically and ubiquitously anneal with strains and representatives of *Escherichia coli*.

11. The method of claim 10, wherein the probe for detecting nucleic acid sequences from *Escherichia coli* is selected from the group consisting of SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 and a sequence complementary thereof.

12. A method for detecting the presence and/or amount of *Escherichia coli* in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Escherichia coli* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Escherichia coli* in said test sample.

13. The method of claim 12, wherein said at least one pair of primers is selected from the group consisting of:

- a) SEQ ID NO: 42 and SEQ ID NO: 43,
- b) SEQ ID NO: 46 and SEQ ID NO: 47,
- c) SEQ ID NO: 55 and SEQ ID NO: 56, and
- d) SEQ ID NO: 131 and SEQ ID NO: 132.

14. A method for the detection, identification and/or quantification of *Klebsiella pneumoniae* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
- inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of Klebsiella pneumoniae, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Klebsiella pneumoniae* in said test sample.

15. A method as defined in claim 14, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-238 nucleotides in length which sequence is comprised in SEQ ID NO: 8 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-385 nucleotides in length which sequence is comprised in SEQ ID NO: 9 or a complementary sequence thereof,
- 3) an oligonucleotide of 12-462 nucleotides in length which sequence is comprised in SEQ ID NO: 10 or a complementary sequence thereof,

- 4) an oligonucleotide of 12-730 nucleotides in length which sequence is comprised in SEQ ID NO: 11 or a complementary sequence thereof, and
- variants thereof which specifically and ubiquitously anneal with strains and representatives of *Klebsiella pneumoniae*.

16. The method of claim 15, wherein the probe for detecting nucleic acid sequences from *Klebsiella pneumoniae* is selected from the group consisting of SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 69 and a sequence complementary thereof.

17. A method for detecting the presence and/or amount of *Klebsiella pneumoniae* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Klebsiella pneumoniae* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Klebsiella pneumoniae* in said test sample.

18. The method of claim 17, wherein said at least one pair of primers is selected from the group consisting of:

- a) SEQ ID NO: 61 and SEQ ID NO: 62,
- b) SEQ ID NO: 67 and SEQ ID NO: 68,
- c) SEQ ID NO: 135 and SEQ ID NO: 136, and
- d) SEQ ID NO: 137 and SEQ ID NO: 138.

19. A method for the detection, identification and/or quantification of *Proteus mirabilis* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
- inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13,

SEQ ID NO: 14, SEQ ID NO: 15, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Proteus mirabilis*, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and

c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Proteus mirabilis* in said test sample.

20. A method as defined in claim 19, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-225 nucleotides in length which sequence is comprised in SEQ ID NO: 12 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-402 nucleotides in length which sequence is comprised in SEQ ID NO: 13 or a complementary sequence thereof,
- 3) an oligonucleotide of 12-157 nucleotides in length which sequence is comprised in SEQ ID NO: 14 or a complementary sequence thereof,
- 4) an oligonucleotide of 12-1348 nucleotides in length which sequence is comprised in SEQ ID NO: 15 or a complementary sequence thereof, and
- variants thereof which specifically and ubiquitously anneal with strains and representatives of *Proteus mirabilis*.

21. The method of claim 20, wherein the probe for detecting nucleic acid sequences from *Proteus mirabilis* is selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82 and a sequence complementary thereof.

22. A method for detecting the presence and/or amount of *Proteus mirabilis* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Proteus mirabilis* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Proteus mirabilis* in said test sample.

23. The method of claim 22, wherein said at least one pair of primers is selected from the group consisting of:

a) SEQ ID NO: 74 and SEQ ID NO: 75, and

b) SEQ ID NO: 133 and SEQ ID NO: 134.

24. A method for the detection, identification and/or quantification of *Staphylococcus saprophyticus* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
- inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of Staphylococcus saprophyticus, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Staphylococcus saprophyticus* in said test sample.

25. A method as defined in claim 24, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-172 nucleotides in length which sequence is comprised in SEQ ID NO: 21 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-155 nucleotides in length which sequence is comprised in SEQ ID NO: 22 or a complementary sequence thereof,
- 3) an oligonucleotide of 12-145 nucleotides in length which sequence is comprised in SEQ ID NO: 23 or a complementary sequence thereof,
- 4) an oligonucleotide of 12-265 nucleotides in length which sequence is comprised in SEQ ID NO: 24 or a complementary sequence thereof, and
- variants thereof which specifically and ubiquitously anneal with strains and representatives of *Staphylococcus saprophyticus*.

26. The method of claim 25, wherein the probe for detecting nucleic acid sequences from *Staphylococcus saprophyticus* is selected from the group consisting of SEQ

ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104 and a sequence complementary thereof.

27. A method for detecting the presence and/or amount of *Staphylococcus saprophyticus* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Staphylococcus saprophyticus* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, and SEQ ID NO: 24;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Staphylococcus saprophyticus* in said test sample.

28. The method of claim 27, wherein said at least one pair of primers is selected from the group consisting of:

a) SEQ ID NO: 98 and SEQ ID NO: 99, and

b) SEQ ID NO: 139 and SEQ ID NO: 140.

29. A method for the detection, identification and/or quantification of *Moraxella catarrhalis* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 28, SEQ ID NO: 29, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Moraxella catarrhalis*, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said label-

ling means, said first reactive member reacting with a second reactive member present on said probe; and

c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Moraxella catarrhalis* in said test sample.

30. A method as defined in claim 29, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-526 nucleotides in length which sequence is comprised in SEQ ID NO: 28 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-466 nucleotides in length which sequence is comprised in SEQ ID NO: 29 or a complementary sequence thereof, and
- variants thereof which specifically and ubiquitously anneal with strains and representatives of *Moraxella catarrhalis*.

31. The method of claim 30, wherein the probe for detecting nucleic acid sequences from *Moraxella catarrhalis* is selected from the group consisting of SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117 and a sequence complementary thereof.

32. A method for detecting the presence and/or amount of *Moraxella catarrhalis* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Moraxella catarrhalis* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 28 and SEQ ID NO: 29;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Moraxella catarrhalis* in said test sample.

33. The method of claim 32, wherein said at least one pair of primers is selected from the group consisting of:

a) SEQ ID NO: 112 and SEQ ID NO: 113,

b) SEQ ID NO: 118 and SEQ ID NO: 119, and

c) SEQ ID NO: 160 and SEQ ID NO: 119.

34. A method for the detection, identification and/or quantification of *Pseudomonas aeruginosa* directly from a test sample or from bacterial colonies, which comprises the following steps:

a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or

- inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of Pseudomonas aeruginosa, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Pseudomonas aeruginosa* in said test sample.

35. A method as defined in claim 34, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-2167 nucleotides in length which sequence is comprised in SEQ ID NO: 16 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-1872 nucleotides in length which sequence is comprised in SEQ ID NO: 17 or a complementary sequence thereof,
- 3) an oligonucleotide of 12-3451 nucleotides in length which sequence is comprised in SEQ ID NO: 18 or a complementary sequence thereof,
- 4) an oligonucleotide of 12-744 nucleotides in length which sequence is comprised in SEQ ID NO: 19 or a complementary sequence thereof,
- 5) an oligonucleotide of 12-2760 nucleotides in length which sequence is comprised in SEQ ID NO: 20 or a complementary sequence thereof, and
- variants thereof which specifically and ubiquitously anneal with strains and representatives of *Pseudomo*nas aeruginosa.

36. The method of claim 35, wherein the probe for detecting nucleic acid sequences from *Pseudomonas aeruginosa* is selected from the group consisting of SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95 and a sequence complementary thereof.

37. A method for detecting the presence and/or amount of *Pseudomonas aeruginosa* in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Pseudomonas aeruginosa* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Pseudomonas aeruginosa* in said test sample.

38. The method of claim 37, wherein said at least one pair of primers is selected from the group consisting of:

a) SEQ ID NO: 83 and SEQ ID NO: 84, and

b) SEQ ID NO: 85 and SEQ ID NO: 86.

39. A method for the detection, identification and/or quantification of *Staphylococcus epidermidis* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 36, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Staphylococcus epidermidis*, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Staphylococcus epidermidis* in said test sample.

40. A method as defined in claim 39, wherein said probe is selected from the group consisting of an oligonucleotide of 12-705 nucleotides in length which sequence is comprised in SEQ ID NO: 36 and variants thereof which specifically and ubiquitously anneal with strains and representatives of *Staphylococcus epidermidis*.

41. A method for detecting the presence and/or amount of *Staphylococcus epidermidis* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Staphylococcus epidermidis* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the following sequence: SEQ ID NO: 36;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Staphylococcus epidermidis* in said test sample.

42. The method of claim 41, wherein said at least one pair of primers is selected from the group consisting of:

a) SEQ ID NO: 145 and SEQ ID NO: 146, and

b) SEQ ID NO: 147 and SEQ ID NO: 148.

43. A method for the detection, identification and/or quantification of *Staphylococcus aureus* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 37, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Staphylococcus aureus*, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and

c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Staphylococcus aureus* in said test sample.

44. A method as defined in claim 43, wherein said probe is selected from the group consisting of an oligonucleotide of 12-442 nucleotides in length which sequence is comprised in SEQ ID NO: 37 and variants thereof which specifically and ubiquitously anneal with strains and representatives of *Staphylococcus aureus*.

45. A method for detecting the presence and/or amount of *Staphylococcus aureus* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Staphylococcus aureus* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the following sequence: SEQ ID NO: 37;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Staphylococcus aureus* in said test sample.

46. The method of claim 45, wherein said at least one pair of primers is selected from the group consisting of:

- a) SEQ ID NO: 149 and SEQ ID NO: 150,
- b) SEQ ID NO: 149 and SEQ ID NO: 151, and
- c) SEQ ID NO: 152 and SEQ ID NO: 153.

47. A method for the detection, identification and/or quantification of *Haemophilus influenzae* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Haemophilus influenzae*, under conditions such that

the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and

c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Haemophilus influenzae* in said test sample.

48. A method as defined in claim 47, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-845 nucleotides in length which sequence is comprised in SEQ ID NO: 25 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-1598 nucleotides in length which sequence is comprised in SEQ ID NO: 26 or a complementary sequence thereof,
- 3) an oligonucleotide of 12-9100 nucleotides in length which sequence is comprised in SEQ ID NO: 27 or a complementary sequence thereof, and
 - variants thereof which specifically and ubiquitously anneal with strains and representatives of *Haemophilus influenzae*.

49. The method of claim 48, wherein the probe for detecting nucleic acid sequences from *Haemophilus influenzae* is selected from the group consisting of SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107 and a sequence complementary thereof.

50. A method for detecting the presence and/or amount of *Haemophilus influenzae* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Haemophilus influenzae* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 25, SEQ ID NO: 26 and SEQ ID NO: 27;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Haemophilus influenzae* in said test sample.

51. The method of claim 50, wherein said at least one pair of primers comprises the following pair: SEQ ID NO: 154 and SEQ ID NO: 155.

52. A method for the detection, identification and/or quantification of *Streptococcus pneumoniae* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 34, SEQ ID NO: 35, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of Streptococcus pneumoniae, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Streptococcus pneumoniae* in said test sample.

53. A method as defined in claim 52, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-631 nucleotides in length which sequence is comprised in SEQ ID NO: 30 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-3754 nucleotides in length which sequence is comprised in SEQ ID NO: 31 or a complementary sequence thereof,
- 3) an oligonucleotide of 12-841 nucleotides in length which sequence is comprised in SEQ ID NO: 34 or a complementary sequence thereof,
- 4) an oligonucleotide of 12-4500 nucleotides in length which sequence is comprised in SEQ ID NO: 35 or a complementary sequence thereof, and
 - variants thereof which specifically and ubiquitously anneal with strains and representatives of *Streptococcus pneumoniae*.

54. The method of claim 53, wherein the probe for detecting nucleic acid sequences from *Streptococcus pneumoniae* is selected from the group consisting of SEQ ID NO: 120, SEQ ID NO: 121 and a sequence complementary thereof.

55. A method for detecting the presence and/or amount of *Streptococcus pneumoniae* in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Streptococcus pneumoniae* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 34 and SEQ ID NO: 35;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Streptococcus pneumoniae* in said test sample.

56. The method of claim 55, wherein said at least one pair of primers is selected from the group consisting of:

a) SEQ ID NO: 78 and SEQ ID NO: 79,

b) SEQ ID NO: 156 and SEQ ID NO: 157, and

c) SEQ ID NO: 158 and SEQ ID NO: 159.

57. A method for the detection, identification and/or quantification of *Streptococcus pyogenes* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 33, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Streptococcus pyogenes*, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Streptococcus pyogenes* in said test sample.

58. A method as defined in claim 57, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-1337 nucleotides in length which sequence is comprised in SEQ ID NO: 32 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-1837 nucleotides in length which sequence is comprised in SEQ ID NO: 33 or a complementary sequence thereof, and
 - variants thereof which specifically and ubiquitously anneal with strains and representatives of *Streptococcus pyogenes*.

59. A method for detecting the presence and/or amount of *Streptococcus pyogenes* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Streptococcus pyogenes* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 32 and SEQ ID NO: 33;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Streptococcus pyogenes* in said test sample.
 60. The method of claim 59, wherein said at least one pair
- of primers is selected from the group consisting of:
 - a) SEQ ID NO: 141 and SEQ ID NO: 142, and

b) SEQ ID NO: 143 and SEQ ID NO: 144.

61. A method for the detection, identification and/or quantification of *Enterococcus faecalis* directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, a sequence complementary thereof, a part thereof and a variant thereof, which specifically and ubiquitously

anneals with strains or representatives of *Enterococcus faecalis*, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and

c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of *Enterococcus faecalis* in said test sample.

62. A method as defined in claim 61, wherein said probe is selected from the group consisting of:

- 1) an oligonucleotide of 12-1817 nucleotides in length which sequence is comprised in SEQ ID NO: 1 or a complementary sequence thereof,
- 2) an oligonucleotide of 12-2275 nucleotides in length which sequence is comprised in SEQ ID NO: 2, and
 - variants thereof which specifically and ubiquitously anneal with strains and representatives of *Enterococcus faecalis*.

63. A method for detecting the presence and/or amount of *Enterococcus faecalis* in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of *Enterococcus faecalis* DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within one of the following sequences: SEQ ID NO: 1 and SEQ ID NO: 2;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of *Enterococcus faecalis* in said test sample.

64. The method of claim 63, wherein said at least one pair of primers is selected from the group consisting of:

a) SEQ ID NO: 38 and SEQ ID NO: 39, and

b) SEQ ID NO: 40 and SEQ ID NO: 41.

65. A method for the detection of the presence and/or amount of any bacterial species directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this

sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,

- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a universal probe which sequence is selected from the group consisting of SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130 and a sequence complementary thereof, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being means, said first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of the presence and/or amount of said any bacterial species in said test sample.

66. A method for detecting the presence and/or amount of any bacterial species in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing a pair of universal primers which sequence is defined in SEQ ID NO: 126 and SEQ ID NO: 127, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said any bacterial species DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of said any bacterial species in said test sample.

67. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene bla_{tem} (TEM-1) directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;

- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 161, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for a β -lactamase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene TEM-1.

68. A method as defined in claim 67, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 161.

69. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene bla_{tem} (TEM-1) in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for a β -lactamase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 161;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to β-lactam antibiotics mediated by the bacterial antibiotic resistance gene TEM-1.

70. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene bla_{rob} (ROB-1) directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,

said bacterial DNA being in a substantially single stranded form;

- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 162, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for a β -lactamase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to β-lactam antibiotics mediated by the bacterial antibiotic resistance gene ROB-1.

71. A method as defined in claim 70, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 162.

72. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene bla_{rob} (ROB-1) in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for a β -lactamase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 162;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to β-lactam antibiotics mediated by the bacterial antibiotic resistance gene ROB-1.

73. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene bla_{shv} (SHV-1) directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,

said bacterial DNA being in a substantially single stranded form;

- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 163, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for a β -lactamase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to β-lactam antibiotics mediated by the bacterial antibiotic resistance gene SHV-1.

74. A method as defined in claim 73, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 163.

75. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene bla_{shv} (SHV-1) in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for a β -lactamase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 163;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to β-lactam antibiotics mediated by the bacterial antibiotic resistance gene SHV-1.

76. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aadB directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,

said bacterial DNA being in a substantially single stranded form;

- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 164, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an aminoglycoside adenylyltransferase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aadB.

77. A method as defined in claim 76, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 164.

78. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aadB in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an aminoglycoside adenylyltransferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 164;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aadB.

79. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC1 directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this

sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,

- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 165, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an aminoglycoside acetyltransferase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC1.

80. A method as defined in claim 79, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 165.

81. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aaccl in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an aminoglycoside acetyl-transferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 165;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC1.

82. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC2 directly from a test sample or from bacterial colonies, which comprises the following steps:

a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or

- inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
- said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 166, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an aminoglycoside acetyltransferase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC2.

83. A method as defined in claim 82, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 166.

84. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC2 in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an aminoglycoside acetyl-transferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 166;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC2.

85. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC3 directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 167, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an aminoglycoside acetyltransferase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC3.

86. A method as defined in claim 85, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 167.

87. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC3 in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an aminoglycoside acetyl-transferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 167;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacC3.

88. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial anti-

biotic resistance gene aacA4 directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 168, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an aminoglycoside acetyltransferase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacA4.

89. A method as defined in claim 88, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 168.

90. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacA4 in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an aminoglycoside acetyl-transferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 168;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance

to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacA4.

91. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene mecA directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 169, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for a penicillin-binding protein, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to β-lactam antibiotics mediated by the bacterial antibiotic resistance gene mecA.

92. A method as defined in claim 91, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 169.

93. A method for evaluating a bacterial resistance to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene mecA in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for a penicillin-binding protein that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 169;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance

to β -lactam antibiotics mediated by the bacterial antibiotic resistance gene mecA.

94. A method for evaluating a bacterial resistance to vancomycin mediated by the bacterial antibiotic resistance genes vanH, vanA and vanX directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 170, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance genes coding for vancomycinresistance proteins, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe: and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to vancomycin mediated by the bacterial antibiotic resistance genes vanH, vanA and vanX.

95. A method as defined in claim 94, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 170.

96. A method for evaluating a bacterial resistance to vancomycin mediated by the bacterial antibiotic resistance genes vanH, vanA and vanX in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance genes coding for vancomycin-resistance proteins that contain a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 170;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to vancomycin mediated by the bacterial antibiotic resistance genes vanH, vanA and vanX.

97. A method for evaluating a bacterial resistance to streptogramin A mediated by the bacterial antibiotic resistance gene satA directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 173, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for a streptogramin A acetyltransferase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to streptogramin A mediated by the bacterial antibiotic resistance gene satA.

98. A method as defined in claim 97, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 173.

99. A method for evaluating a bacterial resistance to streptogramin A mediated by the bacterial antibiotic resistance gene satA in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for streptogramin A acetyltransferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 173;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to streptogramin A mediated by the bacterial antibiotic resistance gene satA.

100. A method for evaluating a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacA-aphD directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 174, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an aminoglycoside acetyltransferase-phosphotransferase under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacA-aphD.

101. A method as defined in claim 100, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 174.

102. A method for evaluating a bacterial resistance to aminoglycoside; antibiotics mediated by the bacterial antibiotic resistance gene aacA-aphD in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an aminoglycoside acetyltransferase-phosphotransferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 174;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to aminoglycoside antibiotics mediated by the bacterial antibiotic resistance gene aacA-aphD.

103. A method for evaluating a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vat directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 175, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for a virginiamycin acetyltransferase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vat.

104. A method as defined in claim 103, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 175.

105. A method for evaluating a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vat in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for a virginiamycin acetyltransferase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said

at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 175;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vat.

106. A method for evaluating a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vga directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 176, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an ATP-binding protein, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vga.

107. A method as defined in claim 106, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 176.

108. A method for evaluating a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vga in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an ATP-binding protein that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which

contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 176;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to virginiamycin mediated by the bacterial antibiotic resistance gene vga.

109. A method for evaluating a bacterial resistance to erythromycin mediated by the bacterial antibiotic resistance gene msrA directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 177, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an erythromycin resistance protein under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of a bacterial resistance to erythromycin mediated by the bacterial antibiotic resistance gene msrA.

110. A method as defined in claim 109, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 177.

111. A method for evaluating a bacterial resistance to erythromycin mediated by the bacterial antibiotic resistance gene msrA in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an erythromycin resistance protein that contains a target sequence, and the other of

said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 177;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance to erythromycin mediated by the bacterial antibiotic resistance gene msrA.

112. A method for evaluating potential bacterial resistance to β -lactams, aminoglycosides, chloramphenicol and/or trimethoprim mediated by the bacterial antibiotic resistance gene int directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 171, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for an integrase, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of potential bacterial resistance to β -lactams, aminogly-cosides, chloramphenicol and/or trimethoprim mediated by the bacterial antibiotic resistance gene int.

113. A method as defined in claim 112, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 171.

114. A method for evaluating potential bacterial resistance to β -lactams, aminoglycosides, chloramphenicol and/or trimethoprim mediated by the bacterial antibiotic resistance gene int in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for an integrase that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 171;

- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of potential bacterial resistance to β -lactams, aminoglycosides, chloramphenicol and/or trimethoprim mediated by the bacterial antibiotic resistance gene int.

115. A method for evaluating potential bacterial resistance to β -lactams, aminoglycosides, chloramphenicol and/or trimethoprim mediated by the bacterial antibiotic resistance gene sul directly from a test sample or from bacterial colonies, which comprises the following steps:

- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogenous population of bacteria isolated from this sample, or
 - inoculating said sample or said substantially homogenous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA,
 - said bacterial DNA being in a substantially single stranded form;
- b) contacting said single stranded DNA with a probe, said probe comprising at least one single stranded nucleic acid which nucleotidic sequence is selected from the group consisting of SEQ ID NO: 172, a sequence complementary thereof, a part thereof and a variant thereof, which specifically anneals with said bacterial antibiotic resistance gene coding for a sulfonamide resistance protein under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed, said complex being detected by labelling means, the label being present on said probe or the label being present on a first reactive member of said labelling means, said first reactive member reacting with a second reactive member present on said probe; and
- c) detecting the presence or the intensity of said label on said inert support or in said solution as an indication of potential bacterial resistance to β -lactams, aminogly-cosides, chloramphenicol and/or trimethoprim mediated by the bacterial antibiotic resistance gene sul.

116. A method as defined in claim 115, wherein said probe comprises an oligonucleotide of at least 12 nucleotides in length which hybridizes to SEQ ID NO: 172.

117. A method for evaluating potential bacterial resistance to β -lactams, aminoglycosides, chloramphenicol and/or tri-

methoprim mediated by the bacterial antibiotic resistance gene sul in a test sample which comprises the following steps:

- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene coding for a sulfonamide resistance protein that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from within the sequence defined in SEQ ID NO: 172;
- b) synthesizing an extension product of each of said primers which extension products contain the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of potential bacterial resistance to β -lactams, aminoglycosides, chloramphenicol and/or trimethoprim mediated by the bacterial antibiotic resistance gene sul.

118. A nucleic acid having the nucleotide sequence of any one of SEQ ID NOs: 1 to 37, SEQ ID NOs: 161 to 177, a part thereof and variants thereof which, when in single stranded form, ubiquitously and specifically hybridize with a target bacterial DNA as a probe or as a primer.

119. An oligonucleotide having a nucleotidic sequence of any one of SEQ ID NOs: 38 to 160.

120. A recombinant plasmid comprising a nucleic acid as defined in claim 118.

121. A recombinant host which has been transformed by a recombinant plasmid according to claim 120.

122. A recombinant host according to claim 121 wherein said host is *Escherichia coli*.

123. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial species defined in any one of claims 9, 14, 19, 24, 29, 34, 39, 43, 47, 52, 57 and 61, comprising any combination of probes defined therein.

124. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial species defined in any one of claims 10, 11, 15, 16, 20, 21, **25, 26, 30, 31, 35, 36, 40, 44, 48, 49, 53, 54, 58, 62** and **65**, comprising any combination of oligonucleotide probes defined therein.

125. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial species defined in any one of claims 12, 13, 17, 18, 22, 23, 27, 28, 32, 33, 37, 38, 41, 42, 45, 46, 50, 51, 55, 56, 59, 60, 63, 64 and 66 comprising any combination of primers defined therein.

126. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes defined in any one of claims 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100, 103, 106 and 109 comprising any combination of probes defined therein.

127. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes defined in any one of claims 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107 and 110 comprising any combination of oligonucleotide probes defined therein.

128. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes defined in any one of claims 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 99, 102, 105, 108 and 111 comprising any combination of primers defined therein.

129. A diagnostic kit for the simultaneous detection and quantification of nucleic acids of any combination of the bacterial species defined in claim 123, comprising any combination of the bacterial probes defined therein and any combination of the probes to the antibiotic resistance genes defined in any one of SEQ ID NOs: 161 to 177 in whole or in part.

130. A diagnostic kit for the simultaneous detection and quantification of nucleic acids of any combination of the bacterial species defined in claim 124, comprising any combination of the bacterial oligonucleotide probes defined therein and any combination of oligonucleotide probes that hybridize to the antibiotic resistance genes defined in any one of SEQ ID NOs: 161 to 177.

131. A diagnostic kit for the simultaneous detection and quantification of nucleic acids of any combination of the bacterial species defined in claim 125, comprising any combination of the primers defined therein and any combination of primers that anneal to the antibiotic resistance genes defined in any one of SEQ ID NOs: 161 to 177.

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