

sample using culture, but this technique is negatively affected by considerable turn around time and suboptimal sensitivity, specificity and predictive value. It also can require more extensive handling of the organism, which can be particularly undesirable for some organisms such as security sensitive biological agents.

[0008] Alternative diagnostic techniques available include the use of polymerase chain reaction (PCR), particularly in conjunction with culture. However, a problem with such a technique is a lack of trust in early positive PCR results in the absence of culture results or relevant clinical or physical symptoms. Another problem with such a technique is, like with culture, a lack of sensitivity and specificity when attempting to detect small quantities of microbial nucleic acid in a background of host nucleic acid.

[0009] Hence, there is a recognized need for rapid and reliable techniques for accurate diagnosis of bacteria, yeast organisms and/or filamentous fungi.

SUMMARY

[0009a] In one aspect, the present disclosure refers to a method of identifying, partially identifying or classifying at least one bacterium selected from the group consisting of *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae* and *Streptococcus pyogenes* in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of single nucleotide polymorphisms (SNPs), wherein the SNPs in the at least a portion of the bacterial 16S rRNA gene or gene product are at positions corresponding to position 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the sample is biological sample comprising sputum, blood, cerebrospinal fluid or urine; and wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the SNPs

[0009b] In another aspect, the present disclosure refers to a method of identifying at least one bacterium selected from the group consisting of *Acinetobacter calcoaceticus*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Listeria monocytogenes*; *Clostridium perfringens*; *Corynebacterium jeikeium*; *Bacteroides fragilis*; *Neisseria meningitidis*; *Haemophilus influenzae* and *Salmonella sp.* in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of nucleotide polymorphisms (SNPs) at positions corresponding to positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the sample is a biological sample comprising sputum, blood, cerebrospinal fluid or urine, and wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the SNPs as follows:

Bacterial species	SNP position in the 16S rRNA gene as set forth in SEQ ID NO:1										
	273	378	412	440	488	647	653	737	755	762	776
<i>Escherichia coli</i>	T	G	A	C	C	C	T	C	G	T	G
<i>Streptococcus pneumoniae</i>	A	A	A	T	T	T	T	C	G	C	G
<i>Streptococcus agalactiae</i>	A	A	A	T	A	A	T	C	G	C	G
<i>Streptococcus pyogenes</i>	A	G	A	T	A	A	T	C	G	T	G
<i>Proteus mirabilis</i>	T	G	A	C	C	T	A	C	G	T	G
<i>Enterobacter cloacae</i>	T	G	A	C	C	C	G	C	G	T	G
<i>Klebsiella pneumoniae</i>	T	G	A	C	C	C	A	C	G	T	G
<i>Pseudomonas aeruginosa</i>	A	G	A	A	C	T	A	A	A	T	G
<i>Acinetobacter calcoaceticus</i>	A	G	A	A	C	A	A	A	G	T	A
<i>Enterococcus faecalis</i>	A	A	A	T	T	G	T	C	G	C	G
<i>Listeria monocytogenes</i>	A	A	A	T	T	A	G	C	G	T	G
<i>Staphylococcus aureus</i>	A	G	T	T	C	A	T	T	G	T	G
<i>Clostridium perfringens</i>	A	G	T	C	C	T	A	C	G	C	G
<i>Corynebacterium jeikeium</i>	A	G	T	C	C	C	A	C	G	A	G
<i>Bacteroides fragilis</i>	A	G	T	T	A	C	T	C	A	C	T
<i>Neisseria meningitidis</i>	A	G	A	T	T	G	C	T	G	C	T
<i>Haemophilus influenzae</i>	A	G	A	C	C	G	A	C	G	C	G
<i>Serratia marcescens</i>	T	G	A	C	T	C	A	C	G	T	G
<i>Salmonella sp.</i>	A	G	A	C	C	C	T	C	G	T	G
<i>Staphylococcus epidermidis</i>	A	G	T	T	C	A	T	T	G	T	G

[0009c] In another aspect, the present disclosure refers to a method of identifying at least one bacterium selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum* type A, *Clostridium botulinum* type B, *Clostridium botulinum* type C, *Clostridium botulinum* type D, *Clostridium botulinum* type G, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of nucleotide polymorphisms (SNPs) at positions corresponding to positions 737, 755, 762 and 776 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the sample is a biological sample comprising sputum, blood, cerebrospinal fluid or urine, and wherein the at least one bacterium in the sample is identified based on the presence or absence of the SNPs, wherein the bacterium is: *Bacillus anthracis* when there is a T at position 737, A at position 755, C at position 762 and G at position 776; *Clostridium botulinum* type A or *Clostridium botulinum* type B when there is a T at position 737, G at position 755, C at position 762 and T at position 776; *Clostridium botulinum* type C when there is a T at position 737, A at position 755, T at position 762 and T at position 776; *Clostridium botulinum* type D when there is a C at position 737, A at position 755, T at position 762 and T at position 776; *Clostridium botulinum* type G when there is a T at position 737, G at position 755, C at position 762 and G at position 776; *Yersinia pestis* when there is a C at position 737, G at position 755, T at position 762 and G at position 776; *Francisella tularensis* when there is a T at position 737, A at position 755, G at position 762 and G at position 776; *Vibrio cholerae* when there is a C at position 737, A at position 755, T at position 762 and G at position 776; and *Burkholderia pseudomallei* when there is a C at position 737, G at position 755, C at position 762 and G at position 776.

[0009d] In another aspect, the present disclosure refers to a use of an antibiotic in the manufacture of a medicament for treating a subject having a bacterial infection, wherein the subject has been diagnosed according to the method as disclosed herein; and wherein said antibiotic is to be administered to the subject for treating the diagnosed bacterial infection in the subject

[0010] In various examples the present disclosure is predicated in part on high conservation of the 16S (Svedberg unit) ribosomal RNA (16S rRNA) gene between prokaryotes, including bacteria, and the high conservation of the 18S ribosomal RNA (18S rRNA) gene between yeast organisms and filamentous fungi, and also in part on the discovery of multiple single nucleotide polymorphisms (SNPs) within the 16S rRNA gene and 18S rRNA gene that may be useful in the identification and classification of microbes, particularly bacterial microbes, and yeast organisms and/or filamentous fungi in a sample.

[0011] Generally speaking, prokaryotes, including bacteria, contain 16S rRNA, which is a component of the 30S small subunit of the prokaryotic ribosome. The 16S rRNA is approximately 1,500 nucleotides in length and encoded by the 16S rRNA gene (also referred to as 16S rDNA), which is generally part of a co-transcribed operon also containing the 23S and 5S rRNA genes. Although the DNA sequence of the 16S rRNA genes (and thus the RNA sequence of the 16S rRNA molecules) is highly conserved between prokaryotes, there are regions of variation (Weisberg W.G., *et al.*, 1991).

[0012] Similarly, the 18S rRNA gene in yeast and filamentous fungi is the homologue of the 16S rRNA gene in prokaryotes. The 18S rRNA is a component of the 40S small eukaryotic ribosomal subunit. The DNA sequence of the 18S rRNA gene (and thus the RNA sequence of the 18S rRNA molecules) is also highly conserved in yeast and filamentous fungi.

[0013] According to a first example of the disclosure, there is provided a method of

Table 2

Bacterial species	SNP position in the 16S rRNA gene as set forth in SEQ ID NO:1										
	273	378	412	440	488	647	653	737	755	762	776
<i>Escherichia coli</i>	T	G	A	C	C	C	T	C	G	T	G
	A	A	A	T	T	T	T	C	G	C	G
	A	A	A	T	A	A	T	C	G	C	G
<i>Streptococcus pneumoniae</i>	A	G	A	T	A	A	T	C	G	T	G
<i>Streptococcus agalactiae</i>	T	G	A	C	C	T	A	C	G	T	G
<i>Streptococcus pyogenes</i>	T	G	A	C	C	C	G	C	G	T	G
<i>Proteus mirabilis</i>	T	G	A	C	C	C	A	C	G	T	G
<i>Enterobacter cloacae</i>	T	G	A	C	C	C	A	C	G	T	G
<i>Klebsiella pneumoniae</i>	T	G	A	C	C	C	A	C	G	T	G
<i>Pseudomonas aeruginosa</i>	A	G	A	A	C	T	A	A	A	T	G
<i>Acinetobacter calcoaceticus</i>	A	G	A	A	C	A	A	A	G	T	A
<i>Enterococcus faecalis</i>	A	A	A	T	T	G	T	C	G	C	G
<i>Listeria monocytogenes</i>	A	A	A	T	T	A	G	C	G	T	G
<i>Staphylococcus aureus</i>	A	G	T	T	C	A	T	T	G	T	G
<i>Clostridium perfringens</i>	A	G	T	C	C	T	A	C	G	C	G
<i>Corynebacterium jeikeium</i>	A	G	T	C	C	C	A	C	G	A	G
<i>Bacteroides fragilis</i>	A	G	T	T	A	C	T	C	A	C	T
<i>Neisseria meningitidis</i>	A	G	A	T	T	G	C	T	G	C	T
<i>Haemophilus influenzae</i>	A	G	A	C	C	G	A	C	G	C	G
<i>Serratia marcescens</i>	T	G	A	C	T	C	A	C	G	T	G
<i>Salmonella</i> sp.	A	G	A	C	C	C	T	C	G	T	G
<i>Staphylococcus epidermidis</i>	A	G	T	T	C	A	T	T	G	T	G

CLAIMS

1. A method of identifying, partially identifying or classifying at least one bacterium selected from the group consisting of *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae* and *Streptococcus pyogenes* in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of single nucleotide polymorphisms (SNPs),

wherein the SNPs in the at least a portion of the bacterial 16S rRNA gene or gene product are at positions corresponding to position 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the sample is a biological sample comprising sputum, blood, cerebrospinal fluid or urine; and

wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the SNPs.

2. The method of claim 1, wherein a melt curve analysis is used to differentiate species.

3. The method of claim 2, wherein *Staphylococcus aureus* and *Staphylococcus epidermidis* are identified when there is a T at position 412 and then distinguished from one another based on high-resolution melt curve analysis; *Enterococcus faecalis* and *Enterococcus faecium* are identified when there is a G at position 647 and then distinguished from one another based on high-resolution melt curve analysis; *Serratia marcescens* and *Enterobacter aerogenes* are identified when there is a C at positions 440 and 647 and a T at position 488 and then distinguished from one another based on high-resolution melt curve analysis; or *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae* are identified in a sample based when there is an A at position 378 and then distinguished from one another based on high-resolution melt curve analysis.

4. A method of identifying at least one bacterium selected from the group consisting of *Acinetobacter calcoaceticus*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Listeria monocytogenes*; *Clostridium perfringens*; *Corynebacterium jeikeium*; *Bacteroides fragilis*; *Neisseria meningitidis*; *Haemophilus*

influenzae and *Salmonella* sp. in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of nucleotide polymorphisms (SNPs) at positions corresponding to positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the sample is a biological sample comprising sputum, blood, cerebrospinal fluid or urine, and wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the SNPs as follows:

Bacterial species	SNP position in the 16S rRNA gene as set forth in SEQ ID NO:1										
	273	378	412	440	488	647	653	737	755	762	776
<i>Escherichia coli</i>	T	G	A	C	C	C	T	C	G	T	G
<i>Streptococcus pneumoniae</i>	A	A	A	T	T	T	T	C	G	C	G
<i>Streptococcus agalactiae</i>	A	A	A	T	A	A	T	C	G	C	G
<i>Streptococcus pyogenes</i>	A	G	A	T	A	A	T	C	G	T	G
<i>Proteus mirabilis</i>	T	G	A	C	C	T	A	C	G	T	G
<i>Enterobacter cloacae</i>	T	G	A	C	C	C	G	C	G	T	G
<i>Klebsiella pneumoniae</i>	T	G	A	C	C	C	A	C	G	T	G
<i>Pseudomonas aeruginosa</i>	A	G	A	A	C	T	A	A	A	T	G
<i>Acinetobacter calcoaceticus</i>	A	G	A	A	C	A	A	A	G	T	A
<i>Enterococcus faecalis</i>	A	A	A	T	T	G	T	C	G	C	G
<i>Listeria monocytogenes</i>	A	A	A	T	T	A	G	C	G	T	G
<i>Staphylococcus aureus</i>	A	G	T	T	C	A	T	T	G	T	G
<i>Clostridium perfringens</i>	A	G	T	C	C	T	A	C	G	C	G
<i>Corynebacterium jeikeium</i>	A	G	T	C	C	C	A	C	G	A	G
<i>Bacteroides fragilis</i>	A	G	T	T	A	C	T	C	A	C	T
<i>Neisseria meningitidis</i>	A	G	A	T	T	G	C	T	G	C	T
<i>Haemophilus influenzae</i>	A	G	A	C	C	G	A	C	G	C	G
<i>Serratia marcescens</i>	T	G	A	C	T	C	A	C	G	T	G
<i>Salmonella</i> sp.	A	G	A	C	C	C	T	C	G	T	G
<i>Staphylococcus epidermidis</i>	A	G	T	T	C	A	T	T	G	T	G

5. The method of claim 4, wherein a melt curve analysis is used to differentiate species.
6. The method of claim 5, wherein *Staphylococcus aureus* and *Staphylococcus epidermidis* are identified when there is a T at position 412 and then distinguished from one another based on high-resolution melt curve analysis; *Enterococcus faecalis* and *Enterococcus faecium* are

identified when there is a G at position 647 and then distinguished from one another based on high-resolution melt curve analysis; *Serratia marcescens* and *Enterobacter aerogenes* are identified when there is a C at positions 440 and 647 and a T at position 488 and then distinguished from one another based on high-resolution melt curve analysis.

7. A method of identifying at least one bacterium selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum* type A, *Clostridium botulinum* type B, *Clostridium botulinum* type C, *Clostridium botulinum* type D, *Clostridium botulinum* type G, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of nucleotide polymorphisms (SNPs) at positions corresponding to positions 737, 755, 762 and 776 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the sample is a biological sample comprising sputum, blood, cerebrospinal fluid or urine, and wherein the at least one bacterium in the sample is identified based on the presence or absence of the SNPs, wherein the bacterium is: *Bacillus anthracis* when there is a T at position 737, A at position 755, C at position 762 and G at position 776; *Clostridium botulinum* type A or *Clostridium botulinum* type B when there is a T at position 737, G at position 755, C at position 762 and T at position 776; *Clostridium botulinum* type C when there is a T at position 737, A at position 755, T at position 762 and T at position 776; *Clostridium botulinum* type D when there is a C at position 737, A at position 755, T at position 762 and T at position 776; *Clostridium botulinum* type G when there is a T at position 737, G at position 755, C at position 762 and G at position 776; *Yersinia pestis* when there is a C at position 737, G at position 755, T at position 762 and G at position 776; *Francisella tularensis* when there is a T at position 737, A at position 755, G at position 762 and G at position 776; *Vibrio cholerae* when there is a C at position 737, A at position 755, T at position 762 and G at position 776; and *Burkholderia pseudomallei* when there is a C at position 737, G at position 755, C at position 762 and G at position 776.

8. The method of claim 7, wherein a melt curve analysis is used to differentiate species.

9. The method of any one of claims 1 to 8 wherein the sample is from a subject and a diagnosis of a bacterial infection in the subject is made by identifying at least one causative bacterium.

10. Use of an antibiotic in the manufacture of a medicament for treating a subject having a bacterial infection,

wherein the subject has been diagnosed according to the method of claim 9; and

wherein said antibiotic is to be administered to the subject for treating the diagnosed bacterial infection in the subject.

11. The method of any one of claims 1 to 9, or the use of claim 10, wherein the step of analysing comprises determining the presence or the absence of the SNPs using high resolution melt analysis, 5' nuclease digestion, molecular beacons, oligonucleotide ligation, microarray, restriction fragment length polymorphism, antibody detection methods, direct sequencing or any combination thereof.
12. The method of any one of claims 1 to 9, or the use of claim 10, wherein said method further comprises the step of determining whether the at least one bacterium is resistant to a therapeutic agent.
13. A method as claimed in claim 1, comprising:
combining with the sample at least one isolated probe, tool or reagent comprising an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 16-35.
14. A method as claimed in claim 13, comprising:
combining with the sample at least one isolated probe, tool or reagent comprising an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 22, 26-28 and 33-35, or comprising an oligonucleotide consisting of a nucleotide sequence as set forth in at least one of SEQ ID NOs: 16, 17, 21, 23, 24, 25, 29 and 30-32.
15. A method as claimed in claim 4, comprising:
combining with the sample at least one isolated probe, tool or reagent comprising an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 16-35, 56 and 57.
16. A method as claimed in claim 15, comprising:
combining with the sample at least one isolated probe, tool or reagent comprising an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 22, 26-28, 33-35 and 57, or comprising an oligonucleotide consisting of a nucleotide sequence as set forth in at least one of SEQ ID NOs: 16, 17, 21, 23, 24, 25, 29, 30-32 and 56.
17. A method as claimed in claim 7, comprising:
combining with the sample at least one isolated probe, tool or reagent comprising an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 56 or 57.
18. A method as claimed in claim 15, comprising:
combining with the sample the at least one isolated probe, tool or reagent comprising an oligonucleotide comprising a nucleotide sequence as set forth in SEQ ID NO: 57, or comprising an oligonucleotide consisting of a nucleotide sequence as set forth in SEQ ID NO: 56

19. A method as claimed in any one of claims 13 to 18, comprising combining the sample with an array or biochip comprising more than one said isolated probe, tool or reagent.