

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 at least one single nucleotide polymorphism (SNP), wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647 and 653, ~~737~~, ~~755~~, ~~762~~ and ~~776~~ of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP as follows:

~~[0009b] In another aspect, the present disclosure refers to a method of identifying, partially identifying or classifying at least one bacterium 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 at least one single nucleotide polymorphism (SNP), wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP.~~

~~[0009c] In another aspect, the present disclosure refers to an *in vitro* method of identifying, partially~~

- (A) the at least one bacterium is determined to be a Gram positive bacterium when there is an A at position 273 and a T at position 653; or
- (B) the at least one bacterium is determined to be a Gram positive bacterium when there is a T at position 440; or
- (C) the at least one bacterium is classified as belonging to the *Staphylococcus* genus when there is a T at position 412; or
- (D) the at least one bacterium is classified as belonging to the *Enterococcus* genus when there is a G at position 647; or
- (E) the at least one bacterium is identified as *Enterobacter cloacae* when there is a G at position 653; or
- (F) the at least one bacterium is identified as *Streptococcus pneumoniae* when there is an A at position 378 and a T at position 488 or when there is T at positions 488 and 647; or
- (G) the at least one bacterium is identified as *Streptococcus agalactiae* when there is an A at position 378 and an A at position 488 or position 647; or
- (H) the at least one bacterium is identified as *Streptococcus pyogenes* when there is a G at position 378 and an A at position 488 or position 647; or
- (I) the at least one bacterium is identified as *Acinetobacter calcoaceticus* when there is an A at positions 273, 440 and 647; or
- (J) the at least one bacterium is identified as *Escherichia coli* when there is a T at position 273 and a T at position 653; or
- (K) the at least one bacterium is identified as *Klebsiella pneumoniae* when there is a T at position 273, a C at positions 488 and 647 and an A at position 653; or
- (L) the at least one bacterium is identified as *Proteus mirabilis* when there is a C at positions 440 and 488 and a T at position 647; or
- (M) the at least one bacterium is identified as *Pseudomonas aeruginosa* when there is an A at position 440 and a T at position 647.

~~identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product is used to identify,~~

partially identify, classify or diagnose the bacterial infection in the subject.

[0009d] In another aspect, the present disclosure refers to an *in vitro* method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial infection in the subject.

[0009e] 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 said use comprises: identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject according to the method as disclosed herein, to identify, partially identify, classify or diagnose the bacterial infection in the subject; and that said antibiotic is to be administered to the subject for treating the bacterial infection in the subject.

[0009f] In another aspect, the present disclosure refers to a method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the sample for at least one SNP in a bacterial 16S rRNA gene at a position corresponding to at least one of positions 273, 412, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.

[0009g] In another aspect, the present disclosure refers to a method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the

[0009b] In another aspect, the present disclosure refers to a 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*; *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 at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP as follows:

<u>Bacterial species</u>	<u>SNP position in the 16S rRNA gene as set forth in SEQ ID NO:1</u>										
	<u>273</u>	<u>378</u>	<u>412</u>	<u>440</u>	<u>488</u>	<u>647</u>	<u>653</u>	<u>737</u>	<u>755</u>	<u>762</u>	<u>776</u>
<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	
<i>Neisseria meningitidis</i>	A	G	A	T	T	G	C	T	G	C	
<i>Haemophilus influenzae</i>	A	G	A	C	C	G	A	C	G	C	
<i>Serratia marcescens</i>	T	G	A	C	C	C	A	C	G	T	
<i>Salmonella sp.</i>	A	G	A	C	C	C	T	C	G	T	
<i>Staphylococcus epidermidis</i>	C	G	A	C	T	C	T	T	G	T	

~~sample for at least two SNPs in a bacterial 16S rRNA gene at a position corresponding to at least two of positions 273, 412, 440, 488, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.~~

~~[0009h] In another aspect, the present disclosure refers to an *in vitro* method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at a position corresponding to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacteria in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.~~

~~[0009i] In another aspect, the present disclosure refers to an *in vitro* method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacteria in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.~~

~~[0009j] In yet another aspect, the present disclosure refers to at least one isolated probe, tool or reagent capable of identifying, partially identifying, or classifying at least one bacteria in a sample, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product, wherein the at least one probe, tool or reagent comprises 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 comprises 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~~

[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, or 776 of the 16S rRNA gene as set forth in SEQ ID NO:1, 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 said use comprises: diagnosing a bacterial infection in a subject 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

and 56.

~~[0009k] In yet another aspect, the present disclosure refers to a method of identifying, partially identifying, or classifying at least one bacterium in a sample, said method comprising: combining with the sample the at least one isolated probe, tool or reagent as disclosed herein; and identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product, wherein the at least one SNP in~~

~~the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~{0009l} In yet another aspect, the present disclosure refers to a method of identifying, partially identifying, or classifying at least one bacterium in a sample, said method comprising: combining with the sample the at least one isolated probe, tool or reagent as disclosed herein; and identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product, wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~{0009m} In yet another aspect, the present disclosure refers to an array comprising more than one said isolated probe, tool or reagent as disclosed herein.~~

~~{0009n} In yet another aspect, the present disclosure refers to a biochip comprising a solid substrate and at least one isolated probe, tool or reagent as disclosed herein.~~

~~{0009o} In yet another aspect, the present disclosure refers to a kit or assay for classifying or identifying at least one bacterium in a sample, said kit or assay comprising: the at least one probe, tool or reagent as disclosed herein; the array as disclosed herein; and/or the biochip as disclosed herein.~~

~~{0009p} In yet another aspect, the present disclosure refers to a use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding~~

to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

~~[0009q] In yet another aspect, the present disclosure refers to a use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~[0009r] In yet another aspect, the present disclosure refers to a use of the at least one single nucleotide polymorphism (SNP) as disclosed herein, or the at least one isolated probe, tool or reagent as disclosed herein in identifying, partially identifying or classifying at least one bacterium in a sample, wherein said at least one bacterium is identified, partially identified or classified based on the presence or absence of at least one SNP in at least a portion of the 16S rRNA gene or gene product from the sample.~~

[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

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 at least one single nucleotide polymorphism (SNP),

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647 and 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP as follows:-

- (A) the at least one bacterium is determined to be a Gram positive bacterium when there is an A at position 273 and a T at position 653; or
- (B) the at least one bacterium is determined to be a Gram positive bacterium when there is a T at position 440; or
- (C) the at least one bacterium is classified as belonging to the *Staphylococcus* genus when there is a T at position 412; or
- (D) the at least one bacterium is classified as belonging to the *Enterococcus* genus when there is a G at position 647; or
- (E) the at least one bacterium is identified as *Enterobacter cloacae* when there is a G at position 653; or
- (F) the at least one bacterium is identified as *Streptococcus pneumoniae* when there is an A at position 378 and a T at position 488 or when there is T at positions 488 and 647; or
- (G) the at least one bacterium is identified as *Streptococcus agalactiae* when there is an A at position 378 and an A at position 488 or position 647; or
- (H) the at least one bacterium is identified as *Streptococcus pyogenes* when there is a G at position 378 and an A at position 488 or position 647; or
- (I) the at least one bacterium is identified as *Acinetobacter calcoaceticus* when there is an A at positions 273, 440 and 647; or

(J) the at least one bacterium is identified as *Escherichia coli* when there is a T at position 273 and a T at position 653; or

(K) the at least one bacterium is identified as *Klebsiella pneumoniae* when there is a T at position 273, a C at positions 488 and 647 and an A at position 653; or

(L) the at least one bacterium is identified as *Proteus mirabilis* when there is a C at positions 440 and 488 and a T at position 647; or

(M) the at least one bacterium is identified as *Pseudomonas aeruginosa* when there is an A at position 440 and a T at position 647.

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 of the DNA surrounding the SNP at position 412; *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 of the DNA surrounding the SNP at position 647; *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 of the DNA surrounding the SNPs at any one of positions 440, 488 and 647; or *Enterococcus faecalis*; *Enterococcus faecium*; *Streptococcus agalactiae*; and *Streptococcus pyogenes* 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 of the DNA surrounding the SNP at position 378.

4. 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*; *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 at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP as follows:

<u>Bacterial species</u>	<u>SNP position in the 16S rRNA gene as set forth in SEQ ID NO:1</u>										
	<u>273</u>	<u>378</u>	<u>412</u>	<u>440</u>	<u>488</u>	<u>647</u>	<u>653</u>	<u>737</u>	<u>755</u>	<u>762</u>	<u>776</u>
<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	
<i>Neisseria meningitidis</i>	A	G	A	T	T	G	C	T	G	C	
<i>Haemophilus influenzae</i>	A	G	A	C	C	G	A	C	G	C	
<i>Serratia marcescens</i>	T	G	A	C	C	C	A	C	G	T	

<u>Salmonella sp.</u>	<u>A</u>	<u>G</u>	<u>A</u>	<u>C</u>	<u>C</u>	<u>C</u>	<u>T</u>	<u>C</u>	<u>G</u>	<u>T</u>	
<u>Staphylococcus epidermidis</u>	<u>C</u>	<u>G</u>	<u>A</u>	<u>C</u>	<u>T</u>	<u>C</u>	<u>T</u>	<u>T</u>	<u>G</u>	<u>T</u>	

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 of the DNA surrounding the SNP at position 412; *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 of the DNA surrounding the SNP at position 647; *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 of the DNA surrounding the SNPs at any one of positions 440, 488 and 647.
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, or 776 of the 16S rRNA gene as set forth in SEQ ID NO:1, 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 taken from a subject and a diagnosis of a bacterial infection is made by identifying at least one causative bacterium.

~~2. A method of identifying, partially identifying or classifying at least one bacterium 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 at least one single nucleotide polymorphism (SNP);~~

~~wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;~~

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

~~3. An *in vitro* method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject;~~

~~wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;~~

~~wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial infection in the subject.~~

~~4. An *in vitro* method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject;~~

~~wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273,~~

~~412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;~~

~~wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product o is used to identify, partially identify, classify or diagnose the bacterial infection in the subject.~~

~~5.10. _____ Use of an antibiotic in the manufacture of a medicament for treating a subject having a bacterial infection, wherein said use comprises:~~

~~identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject according to the method of claim 93 or claim 4, to identify, partially identify, classify or diagnose the bacterial infection in the subject; and~~

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

~~6. The method of any one of claims 1 to 4 or the use of claim 5, wherein said method comprises 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 in the at least a portion of the bacterial 16S rRNA gene or gene product at a position corresponding to at least four of 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.~~

~~7. A method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the sample for at least one SNP in a bacterial 16S rRNA gene at a position corresponding to at least one of positions 273, 412 and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.~~

~~8. A method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the sample for at least two SNPs in a bacterial 16S rRNA gene at a position corresponding to at least two of positions 273, 412, 440, 488, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.~~

~~9. The method of any one of claims 1 to 4 or 6 to 8 or the use of claims 5 or 6, wherein the bacterium is or the bacterial infection is caused by at least one of 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*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Listeria monocytogenes*;~~

~~*Clostridium perfringens*; *Corynebacterium jeikeium*; *Bacteroides fragilis*; *Neisseria meningitidis*; *Haemophilus influenzae*; *Salmonella sp.*; and *Staphylococcus epidermidis*.~~

~~10. The method of any one of claims 1 to 4, 6 to 9 or the use of any one of claims 5, 6 or 9, wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~11. The method or use of claim 10, wherein the bacterium is or the bacterial infection is caused by at least one of the group consisting of: *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae*, and *Burkholderia pseudomallei*.~~

~~12.11. The method of any one of claims 1 to 4, 6 to 9, or the use of any one of claims 5, 6 or 9 to 11, 10, wherein the sample is a biological sample comprising sputum, blood, cerebrospinal fluid or urine.~~

~~13.12. The method of any one of claims 1 to 9, or the use of claim 10 to 4 or 6 to 12 or the use of any one of claims 5, 6 or 9 to 12, wherein the step of analysing comprises determining the presence or the absence of the at least one SNP 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.~~

~~14.13. The method of any one of claims 1 to 9, or the use of claim 10 to 4 or 6 to 9 or the use of any one of claims 5, 6 or 9 to 13, wherein said method further comprises the step of determining whether the at least one ~~bacteria~~ bacterium is resistant to a therapeutic agent.~~

~~15. An *in vitro* method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof,~~

~~wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at a position corresponding to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;~~

~~wherein the at least one bacteria in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.~~

~~16. An *in vitro* method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained~~

~~from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof;~~

~~wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;~~

~~wherein the at least one bacteria in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.~~

~~17. At least one isolated probe, tool or reagent capable of identifying, partially identifying, or classifying at least one bacteria in a sample, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product;~~

~~wherein the at least one probe, tool or reagent comprises 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 comprises 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.~~

~~18. The at least one isolated probe, tool or reagent of claim 17, wherein the at least one probe, tool or reagent comprises an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 22, 26-28, 33-35 and 57.~~

~~19.14. A method of identifying, partially identifying, or classifying at least one bacterium in a sample, said method as claimed in claim 1, 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 at least one of SEQ ID NOs: 16-35, of claim 17 or claim 18; and~~

~~identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product;~~

~~wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

15. A method as claimed in claim 14, 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.

16. 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 and 53-57.

17. A method as claimed in claim 16, 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.

18. 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: 53-57.

19. A method as claimed in claim 16, 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

20. A method as claimed in any one of claims 14 to 19, comprising combining the sample with an array or biochip comprising more than one said isolated probe, tool or reagent.

~~20. A method of identifying, partially identifying, or classifying at least one bacterium in a sample, said method comprising:~~

~~combining with the sample the at least one isolated probe, tool or reagent of claim 17 or claim 18; and~~

~~identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product,~~

~~wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~21. An array comprising more than one said isolated probe, tool or reagent of claim 17 or~~

~~claim 18.~~

~~22. A biochip comprising a solid substrate and at least one isolated probe, tool or reagent of claim 17 or claim 18.~~

~~23. A kit or assay for classifying or identifying at least one bacterium in a sample, said kit or assay comprising: the at least one probe, tool or reagent of claim 17 or claim 18; the array of claim 21; and/or the biochip of claim 22.~~

~~24. Use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product for use as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample;~~

~~wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 412, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~25. Use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product for use as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample;~~

~~wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 412, 440, 488, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~26. Use of the at least one single nucleotide polymorphism (SNP) of claim 24 or 25, or the at least one isolated probe, tool or reagent of claim 17 or claim 18 in identifying, partially identifying or classifying at least one bacterium in a sample, wherein said at least one bacterium is identified, partially identified or classified based on the presence or absence of at least one SNP in at least a portion of the 16S rRNA gene or gene product from the sample.~~

~~27. The method of any one of claims 1, 3, 15 and 19, wherein at least a portion of a bacterial 16S rRNA gene or gene product is analysed, and wherein the at least one SNP is at a position corresponding to at least one of positions 273, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~28. The method of any one of claims 2, 4, 16 and 20, wherein at least a portion of a bacterial 16S rRNA gene or gene product is analysed, and wherein the at least one SNP is at least five SNPs at a position corresponding to at least five of 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.~~

~~29. The method of claim 7, wherein the at least one SNP is at a position corresponding to at least one of positions 273 or 653 of the 16S rRNA gene set forth in SEQ ID NO: 1.~~

~~30. The method of claim 8, wherein the at least two SNPs are at least five SNPs at a~~

position corresponding to at least five of positions 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1.