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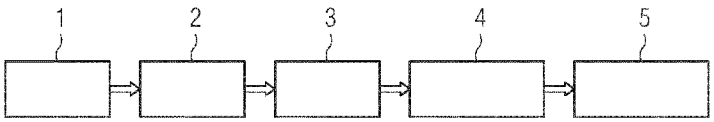
(54) Title: GENETIC TESTING FOR PREDICTING RESISTANCE OF STENOTROPHOMONAS SPECIES AGAINST ANTIMICROBIAL AGENTS

FIG 1



(57) Abstract: The invention relates to a method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, a method of selecting a treatment of a patient suffering from an antibiotic resistant Stenotrophomonas infection, and a method of determining an antibiotic resistance profile for bacterial microorganisms of Stenotrophomonas species, as well as computer program products used in these methods. In an exemplary method, a sample 1, is used for molecular testing 2, and then a molecular fingerprint 3 is taken. The result is then compared to a reference library 4, and the result 5 is reported.

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Description

Genetic testing for predicting resistance of Stenotrophomonas species against antimicrobial agents

The present invention relates to a method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, a method of selecting a treatment of a patient suffering from an infection with a potentially resistant Stenotrophomonas strain, and a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Stenotrophomonas species, as well as computer program products used in these methods.

Antibiotic resistance is a form of drug resistance whereby a sub-population of a microorganism, e.g. a strain of a bacterial species, can survive and multiply despite exposure to an antibiotic drug. It is a serious and health concern for the individual patient as well as a major public health issue. Timely treatment of a bacterial infection requires the analysis of clinical isolates obtained from patients with regard to antibiotic resistance, in order to select an efficacious therapy. Generally, for this purpose an association of the identified resistance with a certain microorganism (i.e. ID) is necessary.

Antibacterial drug resistance (ADR) represents a major health burden. According to the World Health Organization's antimicrobial resistance global report on surveillance, ADR leads to 25,000 deaths per year in Europe and 23,000 deaths per year in the US. In Europe, 2.5 million extra hospital days lead to societal cost of 1.5 billion euro. In the US, the direct cost of 2 million illnesses leads to 20 billion dollar direct cost. The overall cost is estimated to be substantially higher, reducing the gross domestic product (GDP) by up to 1.6%.

Stenotrophomonas maltophilia is a Gram-negative obligate aerobe organism that is rod shaped and motile with a few polar flagella. The organism is an environmental bacterium found in aqueous habitats, including plant rhizospheres, animals, foods, and water sources.

Although not inherently virulent organisms, these environmental Gram negatives can complicate treatment in those who are immunocompromised, critically ill in the intensive care unit and those patients with suppurative lung disease, such as cystic fibrosis.

Infections associated with Stenotrophomonas maltophilia include (most commonly) respiratory tract infections (pneumonia and acute exacerbations of chronic obstructive pulmonary disease [COPD]); bacteremia; biliary sepsis; infections of the bones and joints, urinary tract, and soft tissues; endophthalmitis; eye infections; endocarditis and meningitis.

Stenotrophomonas maltophilia exhibits resistance to a broad array of antibiotics, including TMP-SMX, β-lactam antibiotics, macrolides, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, chloramphenicol, tetracyclines, and polymyxins and is an emerging multidrug-resistant global opportunistic pathogen.

In general the mechanisms for resistance of bacteria against antimicrobial treatments rely to a very substantial part on the organism's genetics. The respective genes or molecular mechanisms are either encoded in the genome of the bacteria or on plasmids that can be interchanged between different bacteria. The most common resistance mechanisms include:

1) Efflux pumps are high-affinity reverse transport systems located in the membrane that transports the antibiotic out of the cell, e.g. resistance to tetracycline.

2) Specific enzymes modify the antibiotic in a way that it loses its activity. In the case of streptomycin, the antibiotic is chemically modified so that it will no longer bind to the ribosome to block protein synthesis.
3) An enzyme is produced that degrades the antibiotic, thereby inactivating it. For example, the penicillinases are a group of beta-lactamase enzymes that cleave the beta lactam ring of the penicillin molecule.

In addition, some pathogens show natural resistance against drugs. For example, an organism can lack a transport system for an antibiotic or the target of the antibiotic molecule is not present in the organism.

Pathogens that are in principle susceptible to drugs can become resistant by modification of existing genetic material (e.g. spontaneous mutations for antibiotic resistance, happening in a frequency of one in about 100 mio bacteria in an infection) or the acquisition of new genetic material from another source. One example is horizontal gene transfer, a process where genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species. Horizontal gene transfer may happen by transduction, transformation or conjugation.

Generally, testing for susceptibility/resistance to antimicrobial agents is performed by culturing organisms in different concentration of these agents.

In brief, agar plates are inoculated with patient sample (e.g. urine, sputum, blood, stool) overnight. On the next day individual colonies are used for identification of organisms, either by culturing or using mass spectroscopy. Based on the identity of organisms new plates containing increasing concentration of drugs used for the treatment of these organisms are inoculated and grown for additional 12 - 24 hours. The

lowest drug concentration which inhibits growth (minimal inhibitory concentration - MIC) is used to determine susceptibility/resistance for tested drugs. The process takes at least 2 to 3 working days during which the patient is treated empirically. A significant reduction of time-to-result is needed especially in patients with life-threatening disease and to overcome the widespread misuse of antibiotics.

Recent developments include PCR based test kits for fast bacterial identification (e.g. Biomerieux Biofire Tests, Curetis Unyvero Tests). With these test the detection of selected resistance loci is possible for a very limited number of drugs, but no correlation to culture based AST is given. Mass spectroscopy is increasingly used for identification of pathogens in clinical samples (e.g. Bruker Biotyper), and research is ongoing to establish methods for the detection of susceptibility/resistance against antibiotics.
For some drugs such it is known that at least two targets are addressed, e.g. in case of Ciprofloxacin (drug bank ID 00537; http://www.drugbank.ca/drugs/DB00537) targets include DNA Topoisomerase IV, DNA Topoisomerase II and DNA Gyrase. It can be expected that this is also the case for other drugs although the respective secondary targets have not been identified yet. In case of a common regulation, both relevant genetic sites would naturally show a co-correlation or redundancy.

It is known that drug resistance can be associated with genetic polymorphisms. This holds for viruses, where resistance testing is established clinical practice (e.g. HIV genotyping). More recently, it has been shown that resistance has also genetic causes in bacteria and even higher organisms, such as humans where tumors resistance against certain cytostatic agents can be linked to genomic mutations.

Wozniak et al. (BMC Genomics 2012, 13(Suppl 7):S23) disclose genetic determinants of drug resistance in Staphylococcus

aureus based on genotype and phenotype data. Stoesser et al. disclose prediction of antimicrobial susceptibilities for Escherichia coli and Klebsiella pneumoniae isolates using whole genomic sequence data (J Antimicrob Chemother 2013; 68: 2234-2244).

Chewapreecha et al (Chewapreecha et al (2014) Comprehensive Identification of single nucleotid polymorphisms associated with beta-lactam resistance within pneumococcal mosaic genes. PLoS Genet 10(8): e1004547) used a comparable approach to identify mutations in gram-positive Streptococcus Pneumonia.

The fast and accurate detection of infections with Stenotrophomonas species and the prediction of response to anti-microbial therapy represent a high unmet clinical need.

This need is addressed by the present invention.

Summary of the Invention

The present inventors addressed this need by carrying out whole genome sequencing of a large cohort of Stenotrophomonas clinical isolates and comparing the genetic mutation profile to classical culture based antimicrobial susceptibility testing with the goal to develop a test which can be used to detect bacterial susceptibility/resistance against antimicrobial drugs using molecular testing.

The inventors performed extensive studies on the genome of bacteria of Stenotrophomonas species either susceptible or resistant to antimicrobial, e.g. antibiotic, drugs. Based on this information, it is now possible to provide a detailed analysis on the resistance pattern of Stenotrophomonas strains based on individual genes or mutations on a nucleotide level. This analysis involves the identification of a resistance against individual antimicrobial, e.g. antibiotic, drugs as well as clusters of them. This allows not only for

the determination of a resistance to a single antimicrobial, e.g. antibiotic, drug, but also to groups of antimicrobial drugs, e.g. antibiotics such as lactam or quinolone antibiotics, or even to all relevant antibiotic drugs.

Therefore, the present invention will considerably facilitate the selection of an appropriate antimicrobial, e.g. antibiotic, drug for the treatment of a Stenotrophomonas infection in a patient and thus will largely improve the quality of diagnosis and treatment.

According to a first aspect, the present invention discloses a diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, which can be also described as a method of determining an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection of a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes listed in Table 1 or Table 2 below, wherein the presence of said at least two mutations is indicative of an infection with an antimicrobial drug resistant, e.g. antibiotic resistant, Stenotrophomonas strain in said patient.

An infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment herein means an infection of a patient with Stenotrophomonas species wherein it is unclear if the Stenotrophomonas species is susceptible to treatment with a specific antimicrobial drug or if it is resistant to the antimicrobial drug.

In step b) above, as well as corresponding steps, at least one mutation in at least two genes is determined, so that in

total at least two mutations are determined, wherein the two mutations are in different genes.

Table 1: List of genes

SMD_3200	SMD_3579	SMD_3610	SMD_4135	SMD_3493
SMD_0995	SMD_2708	cbpD	SMD_2457	SMD_2911
gspL	SMD_2447	SMD_0184	SMD_1120	SMD_4120
SMD_0498	recF	fliK	rtcB	SMD_0069
SMD_1911	SMD_1996	glgX	SMD_4066	SMD_3568
ku	gcd	SMD_3572	SMD_3603	SMD_2199
SMD_2221	thiC	SMD_3992	SMD_3982	SMD_1353
StmPr1	SMD_3983	SMD_2572	tolA	SMD_1351
cyoA2	SMD_1123	SMD_1926	SMD_3570	SMD_2620
SMD_2277	SMD_1205	SMD_1639	SMD_2575	SMD_1320

Table 2: List of genes

SMD_3691	SMD_0155	glnD	SMD_0669	SMD_2553
SMD_3631	aspC	SMD_0301	SMD_2035	SMD_1117
SMD_1654	SMD_3927	nth	SMD_0861	SMD_1105
SMD_3707	fpr	petB	SMD_0692	SMD_3559
ftsW	yceG	SMD_1902	poxB	SMD_3500
SMD_1737	pepA	adi	hmgA	SMD_0173
SMD_4199	SMD_0947	ppk	SMD_3929	sspB
SMD_2800	SMD_3928	fadL	selD	otsA
SMD_2693	xpsD	atpG	SMD_1163	SMD_0676
SMD_1030	mgtE2	engB	uvrA	dsbA2

According to a second aspect, the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant Stenotrophomonas strain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;
b) determining the presence of at least one mutation in at least two genes from the group of genes listed in Table 1 or Table 2 above, wherein the presence of said at least two mutations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;
c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and
d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

A third aspect of the present invention relates to a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Stenotrophomonas species, comprising:
obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Stenotrophomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Stenotrophomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Stenotrophomonas, and/or assembling the gene sequence of the first data set, at least in part;
analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants;
correlating the third data set with the second data set and statistically analyzing the correlation; and
determining the genetic sites in the genome of Stenotrophomonas associated with antimicrobial drug, e.g. antibiotic, resistance.

In addition, the present invention relates in a fourth aspect to a method of determining an antimicrobial drug, e.g. anti-

biotic, resistance profile for a bacterial microorganism belonging to the species Stenotrophomonas comprising the steps of

a) obtaining or providing a sample containing or suspected of containing the bacterial microorganism;

b) determining the presence of a mutation in at least one gene of the bacterial microorganism as determined by the method according to the third aspect of the present invention;

wherein the presence of a mutation is indicative of a resistance to an antimicrobial, e.g. antibiotic, drug.

Furthermore, the present invention discloses in a fifth aspect a diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, which can, like in the first aspect, also be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection of a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Stenotrophomonas from the patient;

b) determining the presence of at least one mutation in at least one gene of the bacterial microorganism belonging to the species Stenotrophomonas as determined by the method according to the third aspect of the present invention, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection in said patient.

Also disclosed is in a sixth aspect a method of selecting a treatment of a patient suffering from an infection with a potentially resistant Stenotrophomonas strain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Stenotrophomonas from the patient;
b) determining the presence of at least one mutation in at least one gene of the bacterial microorganism belonging to the species Stenotrophomonas as determined by the method according to the third aspect of the present invention, wherein the presence of said at least one mutation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;
c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and
d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

A seventh aspect of the present invention relates to a method of acquiring, respectively determining, an antimicrobial drug, e.g. antibiotic, resistance profile for a bacterial microorganism of Stenotrophomonas species, comprising:
obtaining or providing a first data set of gene sequences of a clinical isolate of Stenotrophomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of Stenotrophomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Stenotrophomonas, and/or assembling the gene sequence of the first data set, at least in part;
analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants of the first data set;
correlating the third data set with the second data set and statistically analyzing the correlation; and
determining the genetic sites in the genome of Stenotrophomonas of the first data set associated with antimicrobial drug, e.g. antibiotic, resistance.

According to an eighth aspect, the present invention discloses a computer program product comprising executable instructions which, when executed, perform a method according to the third, fourth, fifth, sixth or seventh aspect of the present invention.

Further aspects and embodiments of the invention are disclosed in the dependent claims and can be taken from the following description, figures and examples, without being limited thereto.

Figures

The enclosed drawings should illustrate embodiments of the present invention and convey a further understanding thereof. In connection with the description they serve as explanation of concepts and principles of the invention. Other embodiments and many of the stated advantages can be derived in relation to the drawings. The elements of the drawings are not necessarily to scale towards each other. Identical, functionally equivalent and acting equal features and components are denoted in the figures of the drawings with the same reference numbers, unless noted otherwise.

Fig. 1 shows schematically a read-out concept for a diagnostic test according to a method of the present invention.

Detailed description of the present invention

Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

An “antimicrobial drug” in the present invention refers to a group of drugs that includes antibiotics, antifungals, antiprotozoals, and antivirals. According to certain embodiments, the antimicrobial drug is an antibiotic.

The term "nucleic acid molecule" refers to a polynucleotide molecule having a defined sequence. It comprises DNA molecules, RNA molecules, nucleotide analog molecules and combinations and derivatives thereof, such as DNA molecules or RNA molecules with incorporated nucleotide analogs or cDNA.

The term “nucleic acid sequence information” relates to information which can be derived from the sequence of a nucleic acid molecule, such as the sequence itself or a variation in the sequence as compared to a reference sequence.

The term “mutation” relates to a variation in the sequence as compared to a reference sequence. Such a reference sequence can be a sequence determined in a predominant wild type organism or a reference organism, e.g. a defined and known bacterial strain or substrain. A mutation is for example a deletion of one or multiple nucleotides, an insertion of one or multiple nucleotides, or substitution of one or multiple nucleotides, duplication of one or a sequence of multiple nucleotides, translocation of one or a sequence of multiple nucleotides, and, in particular, a single nucleotide polymorphism (SNP).

In the context of the present invention a “sample” is a sample which comprises at least one nucleic acid molecule from a bacterial microorganism. Examples for samples are: cells, tissue, body fluids, biopsy specimens, blood, urine, saliva, sputum, plasma, serum, cell culture supernatant, swab sample and others. According to certain embodiments, the sample is a patient sample (clinical isolate).

New and highly efficient methods of sequencing nucleic acids referred to as next generation sequencing have opened the possibility of large scale genomic analysis. The term "next generation sequencing" or "high throughput sequencing" refers to high-throughput sequencing technologies that parallelize the sequencing process, producing thousands or millions of sequences at once. Examples include Massively Parallel Signature Sequencing (MPSS), Polony sequencing, 454 pyrosequencing, Illumina (Solexa) sequencing, SOLiD sequencing, Ion semiconductor sequencing, DNA nanoball sequencing, Helioscope(TM) single molecule sequencing, Single Molecule SMRT(TM) sequencing, Single Molecule real time (RNAP) sequencing, Nanopore DNA sequencing, Sequencing By Hybridization, Amplicon Sequencing, GnuBio.

Within the present description the term "microorganism" comprises the term microbe. The type of microorganism is not particularly restricted, unless noted otherwise or obvious, and, for example, comprises bacteria, viruses, fungi, microscopic algae und protozoa, as well as combinations thereof. According to certain aspects, it refers to one or more Stenotrophomonas species, particularly Stenotrophomonas maltophilia.

A reference to a microorganism or microorganisms in the present description comprises a reference to one microorganism as well a plurality of microorganisms, e.g. two, three, four, five, six or more microorganisms.

A vertebrate within the present invention refers to animals having a vertebrae, which includes mammals - including humans, birds, reptiles, amphibians and fishes. The present invention thus is not only suitable for human medicine, but also for veterinary medicine.

According to certain embodiments, the patient in the present methods is a vertebrate, more preferably a mammal and most preferred a human patient.

Before the invention is described in exemplary detail, it is to be understood that this invention is not limited to the particular component parts of the process steps of the methods described herein as such methods may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include singular and/or plural referents unless the context clearly dictates otherwise. For example, the term "a" as used herein can be understood as one single entity or in the meaning of "one or more" entities. It is also to be understood that plural forms include singular and/or plural referents unless the context clearly dictates otherwise. It is moreover to be understood that, in case parameter ranges are given which are delimited by numeric values, the ranges are deemed to include these limitation values.

Regarding the dosage of the antimicrobial, e.g. antibiotic, drugs, it is referred to the established principles of pharmacology in human and veterinary medicine. For example, Forth, Henschler, Rummel "Allgemeine und spezielle Pharmakologie und Toxikologie", 9th edition, 2005, pp. 781 - 919, might be used as a guideline. Regarding the formulation of a ready-to-use medicament, reference is made to "Remington, The Science and Practice of Pharmacy", 22nd edition, 2013, pp. 777 - 1070.

Assembling of a gene sequence can be carried out by any known method and is not particularly limited.

According to certain embodiments, mutations that were found using alignments can also be compared or matched with align-

ment-free methods, e.g. for detecting single base exchanges, for example based on contigs that were found by assemblies. For example, reads obtained from sequencing can be assembled to contigs and the contigs can be compared to each other.

According to a first aspect, the present invention relates to a diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, which can also be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection of a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, wherein the presence of said at least two mutations is indicative of an infection with an antimicrobial, e.g. antibiotic, resistant Stenotrophomonas strain in said patient.

In this method, as well as the other methods of the invention, the sample can be provided or obtained in any way, preferably non-invasive, and can be e.g. provided as an *in vitro* sample or prepared as *in vitro* sample.

According to certain aspects, mutations in at least two, three, four, five, six, seven, eight, nine or ten genes are determined in any of the methods of the present invention, e.g. in at least two genes or in at least three genes. Instead of testing only single genes or mutants, a combination of several variant positions can improve the prediction accuracy and further reduce false positive findings that are influenced by other factors. Therefore, it is in particular preferred to determine the presence of a mutation in 2, 3, 4, 5, 6, 7, 8 or 9 (or more) genes selected from Table 1 or 2.

For the above genes, i.e. the genes also denoted in Tables 1 and 2, the highest probability of a resistance to at least one antimicrobial drug, e.g. antibiotic, could be observed, with p-values smaller than 10^{-10}, particularly smaller than 10^{-20}, particularly smaller than 10^{-30},indicating the high significance of the values (n= 519; α = 0.05). Details regarding Tables 1 and 2 can be taken from Tables 3 and 4 (4a, 4b, 4c) disclosed in the Examples. Having at least two genes with mutations determined, a high probability of an antimicrobial drug, e.g. antibiotic, resistance could be determined. The genes in Table 1 thereby represent the 50 best genes for which a mutation was observed in the genomes of Stenotrophomonas species, whereas the genes in Table 2 represent the 50 best genes for which a cross-correlation could be observed for the antimicrobial drug, e.g. antibiotic, susceptibility testing for Stenotrophomonas species as described below.

According to certain embodiments, the obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient in this method - as

well as the other methods of the invention - can comprise the following:

A sample of a vertebrate, e.g. a human, e.g. is provided or obtained and nucleic acid sequences, e.g. DNA or RNA sequences, are recorded by a known method for recording nucleic acid, which is not particularly limited. For example, nucleic acid can be recorded by a sequencing method, wherein any sequencing method is appropriate, particularly sequencing methods wherein a multitude of sample components, as e.g. in a blood sample, can be analyzed for nucleic acids and/or nucleic acid fragments and/or parts thereof contained therein in a short period of time, including the nucleic acids and/or nucleic acid fragments and/or parts thereof of at least one microorganism of interest, particularly of at least one Stenotrophomonas species. For example, sequencing can be carried out using polymerase chain reaction (PCR), particularly multiplex PCR, or high throughput sequencing or next generation sequencing, preferably using high-throughput sequencing. For sequencing, preferably an *in vitro* sample is used.

The data obtained by the sequencing can be in any format, and can then be used to identify the nucleic acids, and thus genes, of the microorganism, e.g. of Stenotrophomonas species, to be identified, by known methods, e.g. fingerprinting methods, comparing genomes and/or aligning to at least one, or more, genomes of one or more species of the microorganism of interest, i.e. a reference genome, etc., forming a third data set of aligned genes for a Stenotrophomonas species - discarding additional data from other sources, e.g. the vertebrate. Reference genomes are not particularly limited and can be taken from several databases. Depending on the microorganism, different reference genomes or more than one reference genomes can be used for aligning. Using the reference genome - as well as also the data from the genomes of the other species, e.g. Stenotrophomonas species - mutations in the genes for each species and for the whole multitude of

samples of different species, e.g. Stenotrophomonas species, can be obtained.

For example, it is useful in genome-wide association studies to reference the points of interest, e.g. mutations, to one constant reference for enhanced standardization. In case of the human with a high consistency of the genome and 99% identical sequences among individuals this is easy and represents the standard, as corresponding reference genomes are available in databases. In case of organisms that trigger infectious diseases (e.g. bacteria and viruses) this is much more difficult, though. One possibility is to fall back on a virtual pan genome which contains all sequences of a certain genus. A further possibility is the analysis of all available references, which is much more complex. Therein all *n* references from a database (e.g. RefSeq) are extracted and compared with the newly sequenced bacterial genomes *k*. After this, matrices (% of mapped reads, % of covered genome) are applied to estimate which reference is best suited to all new bacteria. However, *n* x *k* complete alignments are carried out. Having a big number of references, though, stable results can be obtained, as is the case for Stenotrophomonas.

According to certain embodiments, the genomes of Stenotrophomonas species are referenced to one reference genome. However, it is not excluded that for other microorganisms more than one reference genome is used. In the present methods, the reference genome of Stenotrophomonas is NC_017671 as annotated at the NCBI according to certain embodiments. The reference genome is attached to this application as sequence listing with SEQ ID NO 1.

The reference sequence was obtained from Stenotrophomonas strain NC_0176712 (http://www.genome.jp/dbget-bin/www_bget?refseq+NC_017671)
LOCUS NC_017671 4769156 bp DNA circular CON 07-FEB-2015

DEFINITION Stenotrophomonas maltophilia D457 complete genome.
ACCESSION NC_017671
VERSION NC_017671.1 GI:386716467
DBLINK BioProject: PRJNA224116
Assembly: GCF_000284595.1
KEYWORDS RefSeq; complete genome.
SOURCE Stenotrophomonas maltophilia D457
ORGANISM Stenotrophomonas maltophilia D457
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas; Stenotrophomonas maltophilia group.
REFERENCE 1
AUTHORS Lira,F., Hernandez,A., Belda,E., Sanchez,M.B., Moya,A., Silva,F.J. and Martinez,J.L.
TITLE Whole-genome sequence of Stenotrophomonas maltophilia D457, a clinical isolate and a model strain
JOURNAL J. Bacteriol. 194 (13), 3563-3564 (2012)
PUBMED 22689246
REFERENCE 2 (bases 1 to 4769156)
AUTHORS Silva,F.
TITLE Direct Submission
JOURNAL Submitted (28-MAR-2012) Institut Cavanilles de Biodiversitat i
Biol. Evol., University of Valencia, Apartat 22085, Valencia 46071, SPAIN

Alternatively or in addition, the gene sequence of the first data set can be assembled, at least in part, with known methods, e.g. by de-novo assembly or mapping assembly. The sequence assembly is not particularly limited, and any known genome assembler can be used, e.g. based on Sanger, 454, Solexa, Illumina, SOLid technologies, etc., as well as hybrids/mixtures thereof.

According to certain embodiments, the data of nucleic acids of different origin than the microorganism of interest, e.g.

Stenotrophomonas species, can be removed after the nucleic acids of interest are identified, e.g. by filtering the data out. Such data can e.g. include nucleic acids of the patient, e.g. the vertebrate, e.g. human, and/or other microorganisms, etc. This can be done by e.g. computational subtraction, as developed by Meyerson et al. 2002. For this, also aligning to the genome of the vertebrate, etc., is possible. For aligning, several alignment-tools are available. This way the original data amount from the sample can be drastically reduced.

Also after such removal of "excess" data, fingerprinting and/or aligning, and/or assembly, etc. can be carried out, as described above, forming a third data set of aligned and/or assembled genes for a Stenotrophomonas species.

Using these techniques, genes with mutations of the microorganism of interest, e.g. Stenotrophomonas species, can be obtained for various species.

When testing these same species for antimicrobial drug, e.g. antibiotic, susceptibility of a number of antimicrobial drugs, e.g. antibiotics, e.g. using standard culturing methods on dishes with antimicrobial drug, e.g. antibiotic, intake, as e.g. described below, the results of these antimicrobial drug, e.g. antibiotic, susceptibility tests can then be cross-referenced/correlated with the mutations in the genome of the respective microorganism, e.g. Stenotrophomonas. Using several, e.g. 50 or more than 50, 100 or more than 100, 200 or more than 200, 300 or more than 300, 400 or more than 400, or 500 or more than 500 different species of a microorganism, e.g. different Stenotrophomonas species, statistical analysis can be carried out on the obtained cross-referenced data between mutations and antimicrobial drug, e.g. antibiotic, susceptibility for these number of species, using known methods.

Regarding culturing methods, samples can be e.g. cultured overnight. On the next day individual colonies can be used for identification of organisms, either by culturing or using mass spectroscopy. Based on the identity of organisms new plates containing increasing concentration of antibiotics used for the treatment of these organisms are inoculated and grown for additional 12 – 24 hours. The lowest drug concentration which inhibits growth (minimal inhibitory concentration - MIC) can be used to determine susceptibility/resistance for tested antibiotics.

Correlation of the nucleic acid / gene mutations with antimicrobial drug, e.g. antibiotic, resistance can be carried out in a usual way and is not particularly limited. For example, resistances can be correlated to certain genes or certain mutations, e.g. SNPs, in genes. After correlation, statistical analysis can be carried out.

In addition, statistical analysis of the correlation of the gene mutations with antimicrobial drug, e.g. antibiotic, resistance is not particularly limited and can be carried out, depending on e.g. the amount of data, in different ways, for example using analysis of variance (ANOVA) or Student's t-test, for example with a sample size n of 50 or more, 100 or more, 200 or more, 300 or more, 400 or more or 500 or more, and a level of significance (α-error-level) of e.g. 0.05 or smaller, e.g. 0.05, preferably 0.01 or smaller. A statistical value can be obtained for each gene and/or each position in the genome as well as for all antibiotics tested, a group of antibiotics or a single antibiotic. The obtained p-values can also be adapted for statistical errors, if needed.

For statistically sound results a multitude of individuals should be sampled, with n = 50, 100, 200, 300, 400 or 500, and a level of significance (α-error-level) of e.g. 0.05 or smaller, e.g. 0.05, preferably 0.01 or smaller. According to

certain embodiments, particularly significant results can be obtained for n = 200, 300, 400 or 500.

For statistically sound results a multitude of individuals should be sampled, with *n* = 50 or more, 100 or more, 200 or more, 300 or more, 400 or more or 500 or more, and a level of significance (α-error-level) of e.g. 0.05 or smaller, e.g. 0.05, preferably 0.01 or smaller. According to certain embodiments, particularly significant results can be obtained for n = 200 or more, 300 or more, 400 or more or 500 or more.

After the above procedure has been carried out for more than 500, e.g. 519, individual species of Stenotrophomonas, the data disclosed in Tables 1 and 2 were obtained for the statistically best correlations between gene mutations and antimicrobial drug, e.g. antibiotic, resistances. Thus, mutations in these genes were proven as valid markers for antimicrobial drug, e.g. antibiotic, resistance.

According to a further aspect, the present invention relates in a second aspect to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant Stenotrophomonas strain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620,

SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, wherein the presence of said at least two mutations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

In this method, the steps a) of obtaining or providing a sample and b) of determining the presence of at least one mutation are as in the method of the first aspect.

The identification of the at least one or more antimicrobial, e.g. antibiotic, drug in step c) is then based on the results obtained in step b) and corresponds to the antimicrobial, e.g. antibiotic, drug(s) that correlate(s) with the mutations. Once these antimicrobial drugs, e.g. antibiotics, are ruled out, the remaining antimicrobial drugs, e.g. antibiotic drugs/antibiotics, can be selected in step d) as being suitable for treatment.

In the description, references to the first and second aspect also apply to the 14th, 15th, 16th and 17th aspect, referring to the same genes, unless clear from the context that they don't apply.

According to certain embodiments in the method of the first or second aspect, at least a mutation in SMD_3691, particularly in position 4131408, and/or SMD_0155, particularly in position 194882, with regard to reference genome NC_017671 as annotated at the NCBI, is determined. For such mutations, a particularly relevant correlation with antimicrobial drug, e.g. antibiotic, resistance could be determined. In particular, the mutation in position 4131408 with regard to reference genome NC_017671 as annotated at the NCBI is a frame shift mutation or results in a non-synonymous substitution, particularly a codon change -/C;Agc/Cgc;Agc/Tgc, and the mutation in position 194882 with regard to reference genome NC_017671 as annotated at the NCBI is a frame shift mutation or a non-synonymous substitution, particularly a codon change -/-;gCt/gTt.

According to certain embodiments, the antimicrobial drug, e.g. antibiotic, in the method of the first or second aspect, as well as in the other methods of the invention, is at least one selected from the group of β-lactams, β-lactam inhibitors, quinolines and derivatives thereof, aminoglycosides, polyketides, respectively tetracyclines, and folate synthesis inhibitors.

In the methods of the invention the resistance of Stenotrophomonas to one or more antimicrobial, e.g. antibiotic, drugs can be determined according to certain embodiments.

According to certain embodiments of the first and/or second aspect of the invention the antimicrobial, e.g. antibiotic, drug is selected from lactam antibiotics and the presence of a mutation in the following genes is determined: SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572,

SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and/or SMD_1320, or SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, SMD_0301, SMD_2035, SMD_1117, SMD_1654, nth, SMD_0861, SMD_1105, and/or fadL.

According to certain embodiments of the first and/or second aspect of the invention the antimicrobial, e.g. antibiotic, drug is selected from polyketide antibiotics, preferably tetracycline antibiotics, and the presence of a mutation in the following genes is determined: glnD, aspC, SMD_3927, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and/or dsbA2.

According to certain embodiments of the first and/or second aspect of the invention the antimicrobial, e.g. antibiotic, drug is selected from quinolone antibiotics, preferably fluoroquinolone antibiotics, and the presence of a mutation in the following genes is determined: SMD_0947.

According to certain embodiments, the antimicrobial drug is an antibiotic/antibiotic drug.

According to certain embodiments of the first and/or second aspect of the invention, determining the nucleic acid sequence information or the presence of a mutation comprises determining the presence of a single nucleotide at a single position in a gene. Thus the invention comprises methods wherein the presence of a single nucleotide polymorphism or mutation at a single nucleotide position is detected.

According to certain embodiments, the antibiotic drug in the methods of the present invention is selected from the group

consisting of Amoxicillin/K Clavulanate (AUG), Ampicillin (AM), Aztreonam (AZT), Cefazolin (CFZ), Cefepime (CPE), Cefotaxime (CFT), Ceftazidime (CAZ), Ceftriaxone (CAX), Cefuroxime (CRM), Cephalotin (CF), Ciprofloxacin (CP), Ertapenem (ETP), Gentamicin (GM), Imipenem (IMP), Levofloxacin (LVX), Meropenem (MER), Piperacillin/Tazobactam (P/T), Ampicillin/Sulbactam (A/S), Tetracycline (TE), Tobramycin (TO), and Trimethoprim/Sulfamethoxazole (T/S).

The inventors have surprisingly found that mutations in certain genes are indicative not only for a resistance to one single antimicrobial, e.g. antibiotic, drug, but to groups containing several drugs.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1, the antibiotic drug is selected from lactam antibiotics and a mutation in at least one of the following genes is detected with regard to reference genome NC_017671: SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, SMD_1320.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 2, the antibiotic drug is selected from lactam antibiotics and a mutation in at least one of the following genes is detected with regard to reference genome NC_017671: SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, SMD_0301, SMD_2035, SMD_1117, SMD_1654, nth, SMD_0861, SMD_1105, fadL.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 2, the antibiotic drug is selected from polyketide, preferably tetracycline antibiotics and a mutation in at least one of the following genes is detected with regard to reference genome NC_017671: glnD, aspC, SMD_3927, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, dsbA2.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 2, the antibiotic drug is selected from quinolone, preferably fluoroquinolone antibiotics and a mutation in at least one of the following genes is detected with regard to reference genome NC_017671: SMD_0947.

For specific antimicrobial drugs, e.g. antibiotics, specific positions in the above genes can be determined where a high statistical significance is observed. The inventors found that, apart from the above genes indicative of a resistance against antibiotics, also single nucleotide polymorphisms (= SNP's) may have a high significance for the presence of a resistance against defined antibiotic drugs. The analysis of these polymorphisms on a nucleotide level may further improve and accelerate the determination of a drug resistance to antimicrobial drugs, e.g. antibiotics, in Stenotrophomonas.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1, the antibiotic drug is selected from lactam antibiotics and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 3557927, 4007302, 4041091, 4641615, 3901645, 1114873, 3012515, 4561815, 2737238, 2737915, 3237877, 2685196, 2727935, 230900, 1246108, 4624913, 565544, 3743, 2290079, 4624707, 2737932,

4578962, 88895, 2736730, 3901643, 2113221, 2207398, 2692687, 4567013, 3997485, 3997701, 26655, 3020959, 4002023, 4032795, 88791, 2455892, 2470228, 2736735, 2736748, 3923235, 4471203, 3997839, 4001908, 2736904, 4458076, 1514781, 88957, 846345, 3997896, 4458551, 2693885, 2871674, 2737121, 203554, 1512298, 2684693, 4455112, 4624706, 1249159, 2128948, 4000529, 4458520, 2915609, 2540079, 1334488, 4459119, 3997781, 2694515, 1813212, 2128949, 2684936, 2873563, 1475785.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 2, the antibiotic drug is selected from lactam antibiotics and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 4131408, 194882, 1506862, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228, 413850.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 2, the antibiotic drug is selected from polyketide, preferably tetracycline antibiotics and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 1506862, 22076, 4395988, 4146408, 3120515, 1605192, 800868, 3986074, 740620, 1103580, 2105625, 3721056, 3907377, 1911810, 646078, 2875710, 4392432, 217129, 4722389, 966118, 4398229, 1607396, 3115508, 4397270, 413850, 3860405, 3728481, 2995516, 678940, 4141030, 1292846, 782557, 1150463, 4589465, 4019103, 1315236, 4021595.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 2, the antibiotic drug is selected from quinolone, preferably fluoroquinolone antibiotics and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 1058512.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is CAZ and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 4131408, 194882, 1506862, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228, 413850.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is at least one of AZT and CAX and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 4131408, 194882, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is at least one of CFT and CPE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 4131408, 194882, 1506862, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is TE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 1506862, 22076, 4395988, 4146408, 3120515, 1605192, 800868, 3986074, 740620, 1103580, 2105625, 3721056, 3907377, 1911810, 646078, 2875710, 4392432, 217129, 4722389, 966118, 4398229, 1607396, 3115508, 4397270, 413850, 3860405, 3728481, 2995516, 678940, 4141030, 1292846, 782557, 1150463, 4589465, 4019103, 1315236, 4021595.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is at least one of CP and LVX and a mutation in at least one of the following

nucleotide positions is detected with regard to reference genome NC_017671: 1058512.

According to certain embodiments of the first and/or second aspect of the invention, the resistance of a bacterial microorganism belonging to the species Stenotrophomonas against 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 17, 18, 19, 20 or 21 antibiotic drugs is determined.

According to certain embodiments of the first and/or second aspect of the invention, a detected mutation is a mutation leading to an altered amino acid sequence in a polypeptide derived from a respective gene in which the detected mutation is located. According to this aspect, the detected mutation thus leads to a truncated version of the polypeptide (wherein a new stop codon is created by the mutation) or a mutated version of the polypeptide having an amino acid exchange at the respective position.

According to certain embodiments of the first and/or second aspect of the invention, determining the nucleic acid sequence information or the presence of a mutation comprises determining a partial sequence or an entire sequence of the at least two genes.

According to certain embodiments of the first and/or second aspect of the invention, determining the nucleic acid sequence information or the presence of a mutation comprises determining a partial or entire sequence of the genome of the Stenotrophomonas species, wherein said partial or entire sequence of the genome comprises at least a partial sequence of said at least two genes.

According to certain embodiments of the first and/or second aspect of the invention, determining the nucleic acid sequence information or the presence of a mutation comprises using a next generation sequencing or high throughput se-

quencing method. According to preferred embodiments of the first and/or second aspect of the invention, a partial or entire genome sequence of the bacterial organism of Stenotrophomonas species is determined by using a next generation sequencing or high throughput sequencing method.

In a further, third aspect, the present invention relates to a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Stenotrophomonas species, comprising:
obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Stenotrophomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Stenotrophomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Stenotrophomonas, and/or assembling the gene sequence of the first data set, at least in part;
analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants;
correlating the third data set with the second data set and statistically analyzing the correlation; and
determining the genetic sites in the genome of Stenotrophomonas associated with antimicrobial drug, e.g. antibiotic, resistance.

The different steps can be carried out as described with regard to the method of the first aspect of the present invention.

When referring to the second data set, wherein the second data set e.g. comprises, respectively is, a set of antimicrobial drug, e.g. antibiotic, resistances of a plurality of clinical isolates, this can, within the scope of the invention, also refer to a self-learning data base that, whenever a new sample is analyzed, can take this sample into the second data

set and thus expand its data base. The second data set thus does not have to be static and can be expanded, either by external input or by incorporating new data due to self-learning. This is, however, not restricted to the third aspect of the invention, but applies to other aspects of the invention that refer to a second data set, which does not necessarily have to refer to antimicrobial drug resistance. The same applies, where applicable, to the first data set, e.g. in the third aspect.

According to certain embodiments, statistical analysis in the present methods is carried out using Fisher's test with $p < 10^{-6}$, preferably $p < 10^{-9}$, particularly $p < 10^{-10}$, particularly $p < 10^{-11}$.

The method of the third aspect of the present invention, as well as related methods, e.g. according to the 7th and 10th aspect, can, according to certain embodiments, comprise correlating different genetic sites to each other, e.g. in at least two, three, four, five, six, seven, eight, nine or ten genes. This way even higher statistical significance can be achieved.

According to certain embodiments of the method of the third aspect and related methods – as above, the second data set is provided by culturing the clinical isolates of Stenotrophomonas species on agar plates provided with antimicrobial drugs, e.g. antibiotics, at different concentrations and the second data is obtained by taking the minimal concentration of the plates that inhibits growth of the respective Stenotrophomonas species.

According to certain embodiments of the method of the third aspect and related methods, the antibiotic is at least one selected from the group of β-lactams, β-lactam inhibitors, quinolines and derivatives thereof, aminoglycosides, tetracyclines, and folate synthesis inhibitors, preferably

Amoxicillin/K Clavulanate, Ampicillin, Aztreonam, Cefazolin, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Cefuroxime, Cephalothin, Ciprofloxacin, Ertapenem, Gentamicin, Imipenem, Levofloxacin, Meropenem, Piperacillin/Tazobactam, Ampicillin/Sulbactam, Tetracycline, Tobramycin, and Trimethoprim/Sulfamethoxazole.

According to certain embodiments of the method of the third aspect and related methods, the gene sequences in the third data set are comprised in at least one gene from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, or from the genes listed in Table 5.

According to certain embodiments of the method of the third aspect and related methods, the genetic sites in the genome of Stenotrophomonas associated with antimicrobial drug, e.g. antibiotic, resistance are at least comprised in one gene from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA,

SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2.

According to certain embodiments of the method of the third aspect and related methods, the genetic variant has a point mutation, an insertion and or deletion of up to four bases, and/or a frameshift mutation, particularly a frameshift mutation or a non-synonymous substitution in YP_006186368.1 or YP_006182939.1.

A fourth aspect of the present invention relates to a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for a bacterial microorganism belonging to the species Stenotrophomonas comprising the steps of

a) obtaining or providing a sample containing or suspected of containing the bacterial microorganism;

b) determining the presence of a mutation in at least one gene of the bacterial microorganism as determined by the method of the third aspect of the invention;

wherein the presence of a mutation is indicative of a resistance to an antimicrobial drug, e.g. antibiotic, drug.

Steps a) and b) can herein be carried out as described with regard to the first aspect, as well as for the following aspects of the invention.

With this method, any mutations in the genome of Stenotrophomonas species correlated with antimicrobial drug, e.g. antibiotic, resistance can be determined and a thorough antimicrobial drug, e.g. antibiotic, resistance profile can be established.

A simple read out concept for a diagnostic test as described in this aspect is shown schematically in Fig. 1.

According to Fig. 1, a sample 1, e.g. blood from a patient, is used for molecular testing 2, e.g. using next generation sequencing (NGS), and then a molecular fingerprint 3 is taken, e.g. in case of NGS a sequence of selected genomic/plasmid regions or the whole genome is assembled. This is then compared to a reference library 4, i.e. selected sequences or the whole sequence are/is compared to one or more reference sequences, and mutations (SNPs, sequence- gene additions/deletions, etc.) are correlated with susceptibility/ reference profile of reference strains in the reference library. The reference library 4 herein contains many genomes and is different from a reference genome. Then the result 5 is reported comprising ID (pathogen identification), i.e. a list of all (pathogenic) species identified in the sample, and AST (antimicrobial susceptibility testing), i.e. a list including a susceptibility /resistance profile for all species listed

A fifth aspect of the present invention relates to a diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, which also can be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection in a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Stenotrophomonas from the patient;

b) determining the presence of at least one mutation in at least one gene of the bacterial microorganism belonging to the species Stenotrophomonas as determined by the method of the third aspect of the present invention, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection in said patient.

Again, steps a) and b) can herein be carried out as described with regard to the first aspect of the present invention.

According to this aspect, a Stenotrophomonas infection in a patient can be determined using sequencing methods as well as a resistance to antimicrobial drugs, e.g. antibiotics, of the Stenotrophomonas species be determined in a short amount of time compared to the conventional methods.

In a sixth aspect the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant Stenotrophomonas strain, e.g. an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Stenotrophomonas from the patient;

b) determining the presence of at least one mutation in at least one gene of the bacterial microorganism belonging to the species Stenotrophomonas as determined by the method of the third aspect of the invention, wherein the presence of said at least one mutation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

This method can be carried out similarly to the second aspect of the invention and enables a fast was to select a suitable treatment with antibiotics for any infection with an unknown Stenotrophomonas species.

A seventh aspect of the present invention relates to a method of acquiring, respectively determining, an antimicrobial

drug, e.g. antibiotic, resistance profile for a bacterial microorganism of Stenotrophomonas species, comprising:
obtaining or providing a first data set of gene sequences of a clinical isolate of Stenotrophomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of Stenotrophomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Stenotrophomonas, and/or assembling the gene sequence of the first data set, at least in part;
analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants of the first data set;
correlating the third data set with the second data set and statistically analyzing the correlation; and
determining the genetic sites in the genome of Stenotrophomonas of the first data set associated with antimicrobial drug, e.g. antibiotic, resistance.

With this method, antimicrobial drug, e.g. antibiotic, resistances in an unknown isolate of Stenotrophomonas can be determined.

According to certain embodiments, the reference genome of Stenotrophomonas is NC_017671 as annotated at the NCBI. According to certain embodiments, statistical analysis in the present methods is carried out using Fisher's test with $p < 10^{-6}$, preferably $p < 10^{-9}$, particularly $p < 10^{-10}$, particularly $p < 10^{-11}$. Also, according to certain embodiments, the method further comprises correlating different genetic sites to each other, e.g. in at least two, three, four, five, six, seven, eight, nine or ten genes.

An eighth aspect of the present invention relates to a computer program product comprising computer executable instructions which, when executed, perform a method according to the

third, fourth, fifth, sixth or seventh aspect of the present invention.

In certain embodiments the computer program product is one on which program commands or program codes of a computer program for executing said method are stored. According to certain embodiments the computer program product is a storage medium. The same applies to the computer program products of the aspects mentioned afterwards, i.e. the eleventh aspect of the present invention. As noted above, the computer program products of the present invention can be self-learning, e.g. with respect to the first and second data sets.

In order to obtain the best possible information from the highly complex genetic data and develop an optimum model for diagnostic and therapeutical uses as well as the methods of the present invention - which can be applied stably in clinical routine – a thorough in silico analysis can be necessary. The proposed principle is based on a combination of different approaches, e.g. alignment with at least one, preferably more reference genomes and/or assembly of the genome and correlation of mutations found in every sample, e.g. from each patient, with all references and drugs, e.g. antibiotics, and search for mutations which occur in several drug and several strains.

Using the above steps a list of mutations as well of genes is generated. These can be stored in databases and statistical models can be derived from the databases. The statistical models can be based on at least one or more mutations at least one or more genes. Statistical models that can be trained can be combined from mutations and genes. Examples of algorithms that can produce such models are association Rules, Support Vector Machines, Decision Trees, Decision Forests, Discriminant-Analysis, Cluster-Methods, and many more.

The goal of the training is to allow a reproducible, standardized application during routine procedures.

For this, for example, a genome or parts of the genome of a microorganism can be sequenced from a patient to be diagnosed. Afterwards, core characteristics can be derived from the sequence data which can be used to predict resistance. These are the points in the database used for the final model, i.e. at least one mutation or at least one gene, but also combinations of mutations, etc.

The corresponding characteristics can be used as input for the statistical model and thus enable a prognosis for new patients. Not only the information regarding all resistances of all microorganisms, e.g. of Stenotrophomonas species, against all drugs, e.g. antibiotics, can be integrated in a computer decision support tool, but also corresponding directives (e.g. EUCAST) so that only treatment proposals are made that are in line with the directives.

A ninth aspect of the present invention relates to the use of the computer program product according to the eighth aspect for acquiring an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Stenotrophomonas species or in a method of the third aspect of the invention.

In a tenth aspect a method of selecting a treatment of a patient having an infection with a bacterial microorganism of Stenotrophomonas species, comprising:
obtaining or providing a first data set comprising a gene sequence of at least one clinical isolate of the microorganism from the patient;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of the microorganism;

aligning the gene sequences of the first data set to at least one, preferably one, reference genome of the microorganism, and/or assembling the gene sequence of the first data set, at least in part;
analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants of the first data set;
correlating the third data set with the second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of the microorganism and statistically analyzing the correlation;
determining the genetic sites in the genome of the clinical isolate of the microorganism of the first data set associated with antimicrobial drug, e.g. antibiotic, resistance; and
selecting a treatment of the patient with one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in the determination of the genetic sites associated with antimicrobial drug, e.g. antibiotic, resistance is disclosed.

Again, the steps can be carried out as similar steps before. In this method, as well as similar ones, no aligning is necessary, as the unknown sample can be directly correlated, after the genome or genome sequences are produced, with the second data set and thus mutations and antimicrobial drug, e.g. antibiotic, resistances can be determined. The first data set can be assembled, for example, using known techniques.

According to certain embodiments, statistical analysis in the present method is carried out using Fisher's test with $p < 10^{-6}$, preferably $p < 10^{-9}$, particularly $p < 10^{-10}$, particularly $p < 10^{-11}$. Also, according to certain embodiments, the method further comprises correlating different genetic sites to each other.

An eleventh aspect of the present invention is directed to a computer program product comprising computer executable in-

structions which, when executed, perform a method according to the tenth aspect.

According to a twelfth aspect of the present invention, a diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, which can also be described as a method of determining an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection of a patient is disclosed, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes listed in Table 5, wherein the presence of said at least two mutations is indicative of an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection in said patient.

A thirteenth aspect of the invention discloses a method of selecting a treatment of a patient suffering from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes listed in Table 5, wherein the presence of said at least two mutations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

Again, the steps can be carried out as in similar methods before, e.g. as in the first and second aspect of the invention. In the twelfth and thirteenth aspect of the invention, all classes of antibiotics considered in the present method are covered.

Herein, the genes in Table 5 are the following: SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, SMD_1320, SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, dsbA2, SMD_0829, actP, SMD_0288, SMD_3418, iroE2, SMD_1847, aroG, lctP, fecR, SMD_2692, SMD_3036, SMD_3190, mutY, pilM, SMD_2276, SMD_0999, SMD_1131, SMD_0229, SMD_1797, apbE, SMD_1989, SMD_1113, SMD_4224, SMD_4233, SMD_1290, feoB, SMD_3029, lctD, pip2, and citM.

Table 5: List of genes

SMD_3200	SMD_3579	SMD_3610	SMD_4135	SMD_3493	SMD_0995
SMD_2708	cbpD	SMD_2457	SMD_2911	gspL	SMD_2447
SMD_0184	SMD_1120	SMD_4120	SMD_0498	recF	fliK
rtcB	SMD_0069	SMD_1911	SMD_1996	glgX	SMD_4066
SMD_3568	ku	gcd	SMD_3572	SMD_3603	SMD_2199
SMD_2221	thiC	SMD_3992	SMD_3982	SMD_1353	StmPr1
SMD_3983	SMD_2572	tolA	SMD_1351	cyoA2	SMD_1123
SMD_1926	SMD_3570	SMD_2620	SMD_2277	SMD_1205	SMD_1639

SMD_2575	SMD_1320	SMD_3691	SMD_0155	glnD	SMD_0669
SMD_2553	SMD_3631	aspC	SMD_0301	SMD_2035	SMD_1117
SMD_1654	SMD_3927	nth	SMD_0861	SMD_1105	SMD_3707
fpr	petB	SMD_0692	SMD_3559	ftsW	yceG
SMD_1902	poxB	SMD_3500	SMD_1737	pepA	adi
hmgA	SMD_0173	SMD_4199	SMD_0947	ppk	SMD_3929
sspB	SMD_2800	SMD_3928	fadL	selD	otsA
SMD_2693	xpsD	atpG	SMD_1163	SMD_0676	SMD_1030
mgtE2	engB	uvrA	dsbA2	SMD_0829	actP
SMD_0288	SMD_3418	iroE2	SMD_1847	aroG	lctP
fecR	SMD_2692	SMD_3036	SMD_3190	mutY	pilM
SMD_2276	SMD_0999	SMD_1131	SMD_0229	SMD_1797	apbE
SMD_1989	SMD_1113	SMD_4224	SMD_4233	SMD_1290	feoB
SMD_3029	lctD	pip2	citM		

According to certain embodiments, mutations in at least two, three, four, five, six, seven, eight, nine or ten genes are determined in any of the methods of the present invention, e.g. in at least two genes or in at least three genes. Instead of testing only single genes or mutants, a combination of several variant positions can improve the prediction accuracy and further reduce false positive findings that are influenced by other factors. Therefore, it is in particular preferred to determine the presence of a mutation in 2, 3, 4, 5, 6, 7, 8 or 9 (or more) genes selected from Table 5.

Further, according to certain embodiments, the reference genome of Stenotrophomonas is again NC_017671 as annotated at the NCBI. According to certain embodiments, statistical analysis in the present methods is carried out using Fisher's test with $p < 10^{-6}$, preferably $p < 10^{-9}$, particularly $p < 10^{-10}$, particularly $p < 10^{-11}$. Also, according to certain embodiments, the method further comprises correlating different genetic sites to each other. Also the other aspects of the embodiments of the first and second aspect of the invention apply.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antimicrobial drug is an antibiotic. According to certain embodiments, the antibiotic is a lactam antibiotic and a mutation in at least one of the genes listed in Table 6 is detected, or a mutation in at least one of the positions (denoted POS in the tables) listed in Table 6.

Table 6: List for lactam antibiotics

gene name	**POS**	**antibiotic**	**p-value (FDR)**	**genbank protein accession number**
SMD_3691	4131408	CAZ;AZT;CFT;CPE;CAX	3,06509E-33	YP_006186368.1
SMD_0155	194882	CAZ;AZT;CFT;CPE;CAX	2,15692E-32	YP_006182939.1
SMD_0669	775975	CAZ;AZT;CFT;CPE;CAX	1,67621E-19	YP_006183435.1
SMD_2553	2849025	CAZ;AZT;CFT;CPE;CAX	3,65007E-18	YP_006185264.1
SMD_3631	4065975	CAZ;AZT;CFT;CPE;CAX	8,30956E-18	YP_006186308.1
SMD_0301	355423	CAZ;AZT;CFT;CPE;CAX	2,21492E-16	YP_006183083.1
SMD_2035	2263396	CAZ;AZT;CFT;CPE;CAX	1,24688E-15	YP_006184753.1
SMD_1117	1242591	CAZ;AZT;CFT;CPE;CAX	4,92147E-15	YP_006183859.1
SMD_1654	1827864	CAZ;AZT;CFT;CPE;CAX	1,43089E-14	YP_006184380.1
nth	1556268	CAZ;AZT;CFT;CPE;CAX	1,91684E-14	YP_006184125.1
SMD_0861	976566	CAZ;AZT;CFT;CPE;CAX	2,34963E-14	YP_006183620.1
SMD_1105	1227228	CAZ;AZT;CFT;CPE;CAX	1,79895E-13	YP_006183847.1
SMD_0829	938473	CAZ;CFT;CPE;AUG	6,68359E-34	YP_006183588.1
actP	2163735	CAZ;CFT;CPE;AUG	1,16516E-33	YP_006184678.1
SMD_0288	339720	CAZ;CFT;CPE;AUG	3,87865E-32	YP_006183070.1

FDR: determined according to FDR (Benjamini Hochberg) method (Benjamini Hochberg, 1995)

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is at least one of CAZ, CFT and CPE and a mutation

in at least one of the genes of SMD_3691, SMD_0155, SMD_0669, SMD_2553, SMD_3631, SMD_0301, SMD_2035, SMD_1117, SMD_1654, nth, SMD_0861, SMD_1105, SMD_0829, actP, SMD_0288 is detected, or a mutation in at least one of the positions of 4131408, 194882, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228, 938473, 2163735, 339720.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is at least one of AZT and CAX and a mutation in at least one of the genes of SMD_3691, SMD_0155, SMD_0669, SMD_2553, SMD_3631, SMD_0301, SMD_2035, SMD_1117, SMD_1654, nth, SMD_0861, SMD_1105 is detected, or a mutation in at least one of the positions of 4131408, 194882, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is AUG and a mutation in at least one of the genes of SMD_0829, actP, SMD_0288 is detected, or a mutation in at least one of the positions of 938473, 2163735, 339720.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is a quinolone antibiotic, particularly a fluoroquinolone antibiotic, and a mutation in at least one of the genes listed in Table 7 is detected, or a mutation in at least one of the positions (denoted POS in the tables) listed in Table 7.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is LVX and a mutation in at least one of the genes

of SMD_0947, SMD_3418, iroE2, mutY, pilM is detected, or a mutation in at least one of the positions of 1058512, 3811947, 3636526, 1915147, 3823047.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is CP and a mutation in at least one of the genes of SMD_1847, aroG, lctP, fecR, SMD_2692, SMD_3036, SMD_3190, SMD_2276, SMD_0999, SMD_2447 is detected, or a mutation in at least one of the positions of 2043177, 4271025, 2841007, 2688820, 2994845, 3371856, 3543489, 2539872, 1119269, 2727679.

Table 7: List for quinolone antibiotics

gene name	**POS**	**antibiotic**	**p-value (FDR)**	**genbank protein accession number**
SMD_0947	1058512	CP;LVX	1,58337E-11	YP_006183692.1
SMD_3418	3811947	CAZ;CFT;LVX	3,83769E-19	YP_006186100.1
iroE2	3636526	CAZ;CFT;LVX	6,67677E-18	YP_006185948.1
SMD_1847	2043177	CP	6,13124E-12	YP_006184567.1
aroG	4271025	CP	6,13124E-12	YP_006186474.1
lctP	2841007	CP	6,5579E-12	YP_006185257.1
fecR	2688820	CP	3,85329E-11	YP_006185128.1
SMD_2692	2994845	CP	3,8591E-11	YP_006185393.1
SMD_3036	3371856	CP	5,43297E-11	YP_006185727.1
SMD_3190	3543489	CP	5,61682E-11	YP_006185878.1
mutY	1915147	LVX	7,38899E-11	YP_006184463.1
pilM	3823047	LVX	1,02111E-10	YP_006186110.1
SMD_2276	2539872	CP	1,6946E-10	YP_006184990.1
SMD_0999	1119269	CP	1,82051E-10	YP_006183741.1
SMD_2447	2727679	CP	1,87673E-10	YP_006185158.1

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is an aminoglycoside antibiotic and a mutation in at least one of the genes listed in Table 8 is detected, or a

mutation in at least one of the positions (denoted POS in the tables) listed in Table 8.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is TO and a mutation in at least one of the genes of SMD_1131, SMD_0229, SMD_1797, apbE, SMD_1989, SMD_1113, SMD_4224, SMD_4233, SMD_1290, feoB, SMD_3029, lctD, pip2, citM, cgb is detected, or a mutation in at least one of the positions of 1255957, 283252, 1981236, 1178000, 2202346, 1238595, 4751740, 4760878, 1255958, 1439001, 2204390, 3363588, 2839288, 2583118, 3974203, 2850340.

Table 8: List of aminoglycoside antibiotics

gene name	**POS**	**antibiotic**	**p-value (FDR)**	**genbank protein accession number**
SMD_1131	1255957	CAZ;TO;CFT;CPE	2,21444E-24	YP_006183873.1
SMD_0229	283252	CAZ;CFT;TO	5,93789E-21	YP_006183013.1
SMD_1797	1981236	CAZ;TO;CFT;CPE	9,88705E-21	YP_006184517.1
apbE	1178000	CAZ;CFT;TO	1,07697E-20	YP_006183804.1
SMD_1989	2202346	CAZ;TO;CFT;CPE	4,45284E-18	YP_006184707.1
SMD_1113	1238595	CAZ;TO	2,00468E-12	YP_006183855.1
SMD_4224	4751740	TO	8,13873E-12	YP_006186880.1
SMD_4233	4760878	TO	3,79537E-11	YP_006186888.1
SMD_1131	1255958	TO	3,80594E-11	YP_006183873.1
SMD_1290	1439001	CAZ;TO	1,37843E-10	YP_006184031.1
feoB	2204390	TO	1,57625E-10	YP_006184710.1
SMD_3029	3363588	TO;CPE	2,41009E-10	YP_006185720.1
lctD	2839288	TO	2,7338E-10	YP_006185255.1
pip2	2583118	TO	3,16958E-10	YP_006185037.1
citM	3974203	TO	4,29747E-10	YP_006186228.1
cgb	2850340	TO	5,92497E-10	YP_006185265.1

According to certain embodiments of the method of the seventeenth and/or eighteenth aspect of the present invention, the antibiotic is an polyketide antibiotic and a mutation in at

least one of the genes listed in Table 9 is detected, or a mutation in at least one of the positions (denoted POS in the tables) listed in Table 9.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is TE and a mutation in at least one of the genes of glnD, aspC, SMD_3927, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, SMD_0692, poxB, SMD_3500, SMD_1737 is detected, or a mutation in at least one of the positions of 1506862, 22076, 4395988, 4146408, 3120515, 3120516, 1605192, 800868, 3986074, 740620, 1103580, 2105625, 800870, 3721056, 3907377, 1911810.

Table 9: List of polyketides, preferably tetracycline

gene name	**POS**	**antibiotic**	**p-value (FDR)**	**genbank protein accession number**
glnD	1506862	CAZ;TE;CFT;CPE	2,21197E-24	YP_006184087.1
aspC	22076	TE	2,23696E-17	YP_006182811.1
SMD_3927	4395988	TE	1,75663E-14	YP_006186596.1
SMD_3707	4146408	TE	2,94678E-13	YP_006186384.1
fpr	3120515	TE	3,69853E-13	YP_006185505.1
fpr	3120516	TE	3,69853E-13	YP_006185505.1
petB	1605192	TE	5,7886E-13	YP_006184169.1
SMD_0692	800868	TE	5,79169E-13	YP_006183458.1
SMD_3559	3986074	TE	5,82217E-13	YP_006186237.1
ftsW	740620	TE	6,17456E-13	YP_006183408.1
yceG	1103580	TE	2,20615E-12	YP_006183727.1
SMD_1902	2105625	TE	2,22987E-12	YP_006184621.1
SMD_0692	800870	TE	2,58518E-12	YP_006183458.1
poxB	3721056	TE	2,64985E-12	YP_006186024.1
SMD_3500	3907377	TE	2,79605E-12	YP_006186178.1
SMD_1737	1911810	TE	4,0785E-12	YP_006184458.1

A fourteenth aspect of the present invention is directed to a diagnostic method of determining an infection of a patient

with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, which can also be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection of a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least one gene from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, preferably from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320,
or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, preferably from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035,

SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, SMD_1902, poxB, SMD_3500, SMD_1737, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, SMD_3929, sspB, SMD_2800, SMD_3928, selD, SMD_2693, xpsD, SMD_1163, SMD_0676, SMD_1030, mgtE2, and engB, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection in said patient.

A fifteenth aspect of the present invention is directed to a method of selecting a treatment of a patient suffering from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least one gene from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, preferably from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320,
or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035,

SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, preferably from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, SMD_1902, poxB, SMD_3500, SMD_1737, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, SMD_3929, sspB, SMD_2800, SMD_3928, selD, SMD_2693, xpsD, SMD_1163, SMD_0676, SMD_1030, mgtE2, and engB, wherein the presence of said at least one mutation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

Again, in the fourteenth and the fifteenth aspect the steps correspond to those in the first or second aspect, although only a mutation in at least one gene is determined.

A sixteenth aspect of the present invention is directed to a method of treating a patient suffering from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least one gene from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184,

SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, preferably from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320,

or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, preferably from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, SMD_1902, poxB, SMD_3500, SMD_1737, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, SMD_3929, sspB, SMD_2800, SMD_3928, selD, SMD_2693, xpsD, SMD_1163, SMD_0676, SMD_1030, mgtE2, and engB, wherein the presence of said at least one mutation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs;

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being

suitable for the treatment of a Stenotrophomonas infection; and

e) treating the patient with said one or more antimicrobial, e.g. antibiotic, drugs.

A seventeenth aspect of the present invention is directed to a method of treating a patient suffering from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, wherein the presence of said at least two mutations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs;

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being

suitable for the treatment of a Stenotrophomonas infection; and

e) treating the patient with said one or more antimicrobial, e.g. antibiotic, drugs.

An eighteenth aspect of the present invention is directed to a method of treating a patient suffering from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes listed in Table 5, wherein the presence of said at least two mutations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs;

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection; and

e) treating the patient with said one or more antimicrobial, e.g. antibiotic, drugs.

A nineteenth aspect of the present invention is directed to a method of treating a patient suffering from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least one gene from the group of genes listed in Table 5, preferably from the group of genes listed in Table 10, wherein the presence of said at least one mutation is indicative

of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs;

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection; and

e) treating the patient with said one or more antimicrobial, e.g. antibiotic, drugs.

Table 10: List of genes

SMD_3200	SMD_3579	SMD_3610	SMD_4135	SMD_3493	SMD_0995
SMD_2708	cbpD	SMD_2457	SMD_2911	gspL	SMD_2447
SMD_0184	SMD_1120	SMD_4120	SMD_0498	citM	fliK
rtcB	SMD_0069	SMD_1911	SMD_1996	glgX	SMD_4066
SMD_3568	pip2	gcd	SMD_3572	SMD_3603	SMD_2199
SMD_2221	lctD	SMD_3992	SMD_3982	SMD_1353	StmPr1
SMD_3983	SMD_2572	SMD_3029	SMD_1351	cyoA2	SMD_1123
SMD_1926	SMD_3570	SMD_2620	SMD_2277	SMD_1205	SMD_1639
SMD_2575	SMD_1320	SMD_3691	SMD_0155	glnD	SMD_0669
SMD_2553	SMD_3631	aspC	SMD_0301	SMD_2035	SMD_1117
SMD_1654	SMD_3927	nth	SMD_0861	SMD_1105	SMD_3707
fpr	petB	SMD_0692	SMD_3559	ftsW	SMD_1290
SMD_1902	poxB	SMD_3500	SMD_1737	SMD_4233	adi
hmgA	SMD_0173	SMD_4199	SMD_0947	SMD_4224	SMD_3929
sspB	SMD_2800	SMD_3928	SMD_1113	selD	SMD_1989
SMD_2693	xpsD	apbE	SMD_1163	SMD_0676	SMD_1030
mgtE2	engB	SMD_1797	SMD_0229	SMD_0829	actP
SMD_0288	SMD_3418	iroE2	SMD_1847	aroG	lctP
fecR	SMD_2692	SMD_3036	SMD_3190	SMD_1131	pilM
SMD_2276	SMD_0999				

Also in the sixteenth to nineteenth aspect of the invention, steps a) to d) are analogous to the steps in the method of the second aspect of the present invention. Step e) can be

sufficiently carried out without being restricted and can be done e.g. non-invasively.

A twentieth aspect of the present invention is directed to a diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, which can also be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection of a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least one gene from the group of genes listed in Table 5, preferably from the group of genes listed in Table 10, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection in said patient.

A twenty-first aspect of the present invention is directed to a method of selecting a treatment of a patient suffering from an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least one gene from the group of genes listed in Table 5, preferably from the group of genes listed in Table 10, wherein the presence of said at least one mutation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

Again, in the twentieth and the twenty-first aspect the steps correspond to those in the first or second aspect, although only a mutation in at least one gene is determined.

Examples

The present invention will now be described in detail with reference to several examples thereof. However, these examples are illustrative and do not limit the scope of the invention.

Example 1

Whole genome sequencing was carried out in addition to classical antimicrobial susceptibility testing of the same isolates for a cohort of 519 specimens. This allowed performing genome wide correlation studies to find genetic variants (e.g. point mutations, small insertions and deletion, larger structural variants, plasmid copy number gains, gene dosage effects) in the genome and plasmids that are significantly correlated to the resistance against one or several drugs. The approach also allows for comparing the relevant sites in the genome to each other.

In the approach the different sources of genetic resistance as well as the different ways of how bacteria can become resistant were covered. By measuring clinical isolates collected in a broad geographical area and across a broad time span of three decades a complete picture going far beyond the rather artificial step of laboratory generated resistance mechanisms was tried to be generated.

To this end, a set of 21 clinically relevant antimicrobial agents with 5 different modes of action was put together, and

the minimally inhibitory concentration (MIC) of the 21 drugs for the Stenotrophomonas isolates was measured.

The detailed procedure is given in the following:

Bacterial Strains

The inventors selected 519 Stenotrophomonas strains from the microbiology strain collection at Siemens Healthcare Diagnostics (West Sacramento, CA) for susceptibility testing and whole genome sequencing.

Antimicrobial Susceptibility Testing (AST) Panels

Frozen reference AST panels were prepared following Clinical Laboratory Standards Institute (CLSI) recommendations. The following antimicrobial agents (with µg/ml concentrations shown in parentheses) were included in the panels: Amoxicillin/K Clavulanate (0.5/0.25-64/32), Ampicillin (0.25-128), Ampicillin/Sulbactam (0.5/0.25-64/32), Aztreonam (0.25-64), Cefazolin (0.5-32), Cefepime (0.25-64), Cefotaxime (0.25-128), Ceftazidime (0.25-64), Ceftriaxone (0.25-128), Cefuroxime (1-64), Cephalothin (1-64), Ciprofloxacin (0.015-8), Ertepenem (0.12-32), Gentamicin (0.12-32), Imipenem (0.25-32), Levofloxacin (0.25-16), Meropenem (0.12-32), Piperacillin/Tazobactam (0.25/4-256/4), Tetracycline (0.5-64), Tobramycin (0.12-32), and Trimethoprim/Sulfamethoxazole (0.25/4.7-32/608). Prior to use with clinical isolates, AST panels were tested with QC strains. AST panels were considered acceptable for testing with clinical isolates when the QC results met QC ranges described by CLSI16.

Inoculum Preparation

Isolates were cultured on trypticase soy agar with 5% sheep blood (BBL, Cockeysville, Md.) and incubated in ambient air at 35±1°C for 18-24 h. Isolated colonies (4-5 large colonies or 5-10 small colonies) were transferred to a 3 ml Sterile Inoculum Water (Siemens) and emulsified to a final turbidity of a 0.5 McFarland standard. 2 ml of this suspension was add-

ed to 25 ml Inoculum Water with Pluronic-F (Siemens). Using the Inoculator (Siemens) specific for frozen AST panels, 5 µl of the cell suspension was transferred to each well of the AST panel. The inoculated AST panels were incubated in ambient air at 35±1°C for 16-20 h. Panel results were read visually, and minimal inhibitory concentrations (MIC) were determined.

DNA extraction

Four streaks of each Gram-negative bacterial isolate cultured on trypticase soy agar containing 5% sheep blood and cell suspensions were made in sterile 1.5 ml collection tubes containing 50 µl Nuclease-Free Water (AM9930, Life Technologies). Bacterial isolate samples were stored at -20 °C until nucleic acid extraction. The Tissue Preparation System (TPS) (096D0382-02_01_B, Siemens) and the VERSANT® Tissue Preparation Reagents (TPR) kit (10632404B, Siemens) were used to extract DNA from these bacterial isolates. Prior to extraction, the bacterial isolates were thawed at room temperature and were pelleted at 2000 G for 5 seconds. The DNA extraction protocol DNAext was used for complete total nucleic acid extraction of 48 isolate samples and eluates, 50 µl each, in 4 hours. The total nucleic acid eluates were then transferred into 96-Well qPCR Detection Plates (401341, Agilent Technologies) for RNase A digestion, DNA quantitation, and plate DNA concentration standardization processes. RNase A (AM2271, Life Technologies) which was diluted in nuclease-free water following manufacturer's instructions was added to 50 µl of the total nucleic acid eluate for a final working concentration of 20 µg/ml. Digestion enzyme and eluate mixture were incubated at 37°C for 30 minutes using Siemens VERSANT® Amplification and Detection instrument. DNA from the RNase digested eluate was quantitated using the Quant-iT™ PicoGreen dsDNA Assay (P11496, Life Technologies) following the assay kit instruction, and fluorescence was determined on the Siemens VERSANT® Amplification and Detection instrument. Data analysis was performed using Microsoft® Excel 2007. 25 µl of

the quantitated DNA eluates were transferred into a new 96-Well PCR plate for plate DNA concentration standardization prior to library preparation. Elution buffer from the TPR kit was used to adjust DNA concentration. The standardized DNA eluate plate was then stored at -80°C until library preparation.

Next Generation Sequencing

Prior to library preparation, quality control of isolated bacterial DNA was conducted using a Qubit 2.0 Fluorometer (Qubit dsDNA BR Assay Kit, Life Technologies) and an Agilent 2200 TapeStation (Genomic DNA ScreenTape, Agilent Technologies). NGS libraries were prepared in 96 well format using NexteraXT DNA Sample Preparation Kit and NexteraXT Index Kit for 96 Indexes (Illumina) according to the manufacturer's protocol. The resulting sequencing libraries were quantified in a qPCR-based approach using the KAPA SYBR FAST qPCR MasterMix Kit (Peqlab) on a ViiA 7 real time PCR system (Life Technologies). 96 samples were pooled per lane for paired-end sequencing (2x 100bp) on Illumina Hiseq2000 or Hiseq2500 sequencers using TruSeq PE Cluster v3 and TruSeq SBS v3 sequncing chemistry (Illumina). Basic sequencing quality parameters were determined using the FastQC quality control tool for high throughput sequence data (Babraham Bioinformatics Institute).

Data analysis

Raw paired-end sequencing data for the 519 Stenotrophomonas samples were mapped against the Stenotrophomonas reference (NC_017671) with BWA 0.6.1.20. The resulting SAM files were sorted, converted to BAM files, and PCR duplicates were marked using the Picard tools package 1.104 (http://picard.sourceforge.net/). The Genome Analysis Toolkit 3.1.1 (GATK)21 was used to call SNPs and indels for blocks of 200 Stenotrophomonas samples (parameters: -ploidy 1 -glm BOTH -stand_call_conf 30 -stand_emit_conf 10). VCF files were combined into a single file and quality filtering for SNPs was

carried out (QD < 2.0 || FS > 60.0 || MQ < 40.0) and indels (QD < 2.0 || FS > 200.0). Detected variants were annotated with SnpEff22 to predict coding effects. For each annotated position, genotypes of all Stenotrophomonas samples were considered. Stenotrophomonas samples were split into two groups, low resistance group (having lower MIC concentration for the considered drug), and high resistance group (having higher MIC concentrations) with respect to a certain MIC concentration (breakpoint). To find the best breakpoint all thresholds were evaluated and p-values were computed with Fisher's exact test relying on a 2x2 contingency table (number of Stenotrophomonas samples having the reference or variant genotype vs. number of samples belonging to the low and high resistance group). The best computed breakpoint was the threshold yielding the lowest p-value for a certain genomic position and drug. For further analyses positions with non-synonymous alterations and p-value < 10^{-10} were considered.

Since a potential reason for drug resistance is gene duplication, gene dose dependency was evaluated. For each sample the genomic coverage for each position was determined using BED Tools. Gene ranges were extracted from the reference assembly NC_017671.gff and the normalized median coverage per gene was calculated. To compare low- and high-resistance isolates the best area under the curve (AUC) value was computed. Groups of at least 20% of all samples having a median coverage larger than zero for that gene and containing more than 15 samples per group were considered in order to exclude artifacts and cases with AUC > 0.75 were further evaluated.

To include data on the different ways how resistance mechanisms are acquired Stenotrophomonas isolates collected over more than three decades were analyzed such that also horizontal gene transfer could potentially be discovered.

In detail, the following steps were carried out:

Stenotrophomonas strains to be tested were seeded on agar plates and incubated under growth conditions for 24 hours. Then, colonies were picked and incubated in growth medium in the presence of a given antibiotic drug in dilution series under growth conditions for 16-20 hours. Bacterial growth was determined by observing turbidity.

Next mutations were searched that are highly correlated with the results of the phenotypic resistance test.

For sequencing, samples were prepared using a Nextera library preparation, followed by multiplexed sequencing using the Illuminat HiSeq 2500 system, paired end sequencing. Data were mapped with BWA (Li H. and Durbin R. (2010) Fast and accurate long-read alignment with Burrows-Wheeler Transform. Bioinformatics, Epub. [PMID: 20080505])and SNP were called using samtools (Li H.*, Handsaker B.*, Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R. and 1000 Genome Project Data Processing Subgroup (2009) The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics, 25, 2078-9. [PMID: 19505943]).

As reference genome, NC_017671 as annotated at the NCBI was determined as best suited.

The mutations were matched to the genes and the amino acid changes were calculated. Using different algorithms (SVM, homology modeling) mutations leading to amino acid changes with likely pathogenicity / resistance were calculated.

In total, whole genomes and plasmids of 519 different clinical isolates of Stenotrophomonas species, particularly Stenotrophomonas maltophilia, were sequenced, and classical antimicrobial susceptibility testing (AST) against 21 therapy forms as described above was performed for all organisms. From the classical AST a table with 519 rows (isolates) and 21 columns (MIC values for 21 drugs) was obtained. Each table

entry contained the MIC for the respective isolate and the respective drug. The genetic data were mapped to different reference genomes of Stenotrophomonas that have been annotated at the NCBI (http://www.ncbi.nlm.nih.gov/), and the best reference was chosen as template for the alignment - NC_017671 as annotated at the NCBI. Additionally, assemblies were carried out and it was verified that the sequenced genomes fulfil all quality criteria to become reference genomes.

Next, genetic variants were evaluated. This approach resulted in a table with the genetic sites in columns and the same isolates in 519 rows. Each table entry contained the genetic determinant at the respective site (A, C, T, G, small insertions and deletions, ...) for the respective isolate.

In a next step different statistical tests were carried out

1) For comparing resistance / susceptibility to genetic sites we calculated contingency tables and determined the significance using Fishers test
2) For comparing different sites to each other we calculated the correlation between different genetic sites
3) For detecting gene dosage effects, e.g. loss or gain of genes (in the genome or on plasmids) we calculated the coverage (i.e. how many read map to the current position) at each site for resistant and not resistant isolates.

From the data, first the 50 genes with the best p-value were chosen for the list of mutations as well as the list of correlated antibiotic resistance, representing Tables 1 and 2. As can be seen from Tables 1 and 2, differences between the tables can be observed, showing the necessity to carry out both steps for determining statistical significant data for antimicrobial drug, e.g. antibiotic, resistance profiles.

A full list of all genetic sites, drugs, drug classes, affected genes etc. is provided in Tables 3 and 4a, 4b and 4c, wherein Table 3 corresponds to Table 1 and represents the genes having the lowest p-values after determining mutations in the genes, and Table 4, respectively Tables 4a, 4b and 4c correspond to Table 2 and represent the genes having the lowest p-values after correlating the mutations with antibiotic resistance for the respective antibiotics.

In addition, the data with the best p-values for each antibiotic class with the most antibiotic drugs as well as each antibiotic, respectively, were evaluated, being disclosed in Tables 5 – 9.

Table 3: Detailed results for the genes in Example 1 (corresponding to Table 1)

POS	drug class	#drug classes	p-value	gene name	genbank protein accession number
3557927	Lactams	1	2,14765E-48	SMD_3200	YP_006185888.1
4007302	Lactams	1	7,46484E-48	SMD_3579	YP_006186257.1
4041091	Lactams	1	1,51961E-47	SMD_3610	YP_006186287.1
4641615	Lactams	1	1,63788E-46	SMD_4135	YP_006186801.1
3901645	Lactams	1	2,55789E-46	SMD_3493	YP_006186171.1
1114873	Lactams	1	5,46428E-46	SMD_0995	YP_006183737.1
3012515	Lactams	1	9,21174E-46	SMD_2708	YP_006185409.1
4561815	Lactams	1	9,21174E-46	cbpD	YP_006186728.1
2737238	Lactams	1	1,85929E-45	SMD_2457	YP_006185168.1
2737915	Lactams	1	2,51269E-45	SMD_2457	YP_006185168.1
3237877	Lactams	1	5,35805E-45	SMD_2911	YP_006185609.1
2685196	Lactams	1	5,89678E-45	gspL	YP_006185123.1
2727935	Lactams	1	8,89622E-45	SMD_2447	YP_006185158.1
230900	Lactams	1	9,68687E-45	SMD_0184	YP_006182968.1
1246108	Lactams	1	1,30996E-44	SMD_1120	YP_006183862.1
4624913	Lactams	1	1,30996E-44	SMD_4120	YP_006186787.1
565544	Lactams	1	1,64493E-44	SMD_0498	YP_006183268.1
3743	Lactams	1	1,68869E-44	recF	YP_006182796.1
2290079	Lactams	1	1,81899E-44	fliK	YP_006184777.1
4624707	Lactams	1	1,81899E-44	SMD_4120	YP_006186787.1
2737932	Lactams	1	2,65421E-44	SMD_2457	YP_006185168.1
4578962	Lactams	1	2,65421E-44	rtcB	YP_006186743.1
88895	Lactams	1	3,14858E-44	SMD_0069	YP_006182861.1
2736730	Lactams	1	3,46838E-44	SMD_2457	YP_006185168.1
3901643	Lactams	1	3,96522E-44	SMD_3493	YP_006186171.1

2113221	Lactams	1	4,33501E-44	SMD_1911	YP_006184630.1
2207398	Lactams	1	4,93094E-44	SMD_1996	YP_006184714.1
2692687	Lactams	1	4,93094E-44	glgX	YP_006185134.1
4567013	Lactams	1	4,93094E-44	SMD_4066	YP_006186733.1
3997485	Lactams	1	5,19768E-44	SMD_3568	YP_006186246.1
3997701	Lactams	1	5,83521E-44	SMD_3568	YP_006186246.1
26655	Lactams	1	5,87613E-44	ku	YP_006182815.1
3020959	Lactams	1	5,91129E-44	gcd	YP_006185417.1
4002023	Lactams	1	6,46057E-44	SMD_3572	YP_006186250.1
4032795	Lactams	1	7,43995E-44	SMD_3603	YP_006186281.1
88791	Lactams	1	8,68254E-44	SMD_0069	YP_006182861.1
2455892	Lactams	1	9,80716E-44	SMD_2199	YP_006184913.1
2470228	Lactams	1	9,80716E-44	SMD_2221	YP_006184935.1
2736735	Lactams	1	9,80716E-44	SMD_2457	YP_006185168.1
2736748	Lactams	1	9,80716E-44	SMD_2457	YP_006185168.1
3923235	Lactams	1	9,80716E-44	thiC	YP_006186190.1
4471203	Lactams	1	1,02941E-43	SMD_3992	YP_006186660.1
3997839	Lactams	1	1,11851E-43	SMD_3568	YP_006186246.1
4001908	Lactams	1	1,11851E-43	SMD_3572	YP_006186250.1
2736904	Lactams	1	1,12553E-43	SMD_2457	YP_006185168.1
4458076	Lactams	1	1,17957E-43	SMD_3982	YP_006186650.1
1514781	Lactams	1	1,2511E-43	SMD_1353	YP_006184094.1
88957	Lactams	1	1,33057E-43	SMD_0069	YP_006182861.1
846345	Lactams	1	1,3384E-43	StmPr1	YP_006183505.1
3997896	Lactams	1	1,44624E-43	SMD_3568	YP_006186246.1
4458551	Lactams	1	1,47305E-43	SMD_3983	YP_006186651.1
2693885	Lactams	1	1,4851E-43	glgX	YP_006185134.1
2871674	Lactams	1	1,4851E-43	SMD_2572	YP_006185283.1

2737121	Lactams	1	1,52073E-43	SMD_2457	YP_006185168.1
203554	Lactams	1	1,58812E-43	tolA	YP_006182946.1
1512298	Lactams	1	1,59074E-43	SMD_1351	YP_006184092.1
2684693	Lactams	1	1,59074E-43	gspL	YP_006185123.1
4455112	Lactams	1	1,74416E-43	cyoA2	YP_006186647.1
4624706	Lactams	1	1,74416E-43	SMD_4120	YP_006186787.1
1249159	Lactams	1	1,90388E-43	SMD_1123	YP_006183865.1
2128948	Lactams	1	1,98496E-43	SMD_1926	YP_006184644.1
4000529	Lactams	1	2,16411E-43	SMD_3570	YP_006186248.1
4458520	Lactams	1	2,16411E-43	SMD_3983	YP_006186651.1
2915609	Lactams	1	2,21777E-43	SMD_2620	YP_006185324.1
2540079	Lactams	1	2,23049E-43	SMD_2277	YP_006184991.1
1334488	Lactams	1	2,99366E-43	SMD_1205	YP_006183947.1
4459119	Lactams	1	3,21977E-43	SMD_3983	YP_006186651.1
3997781	Lactams	1	3,2945E-43	SMD_3568	YP_006186246.1
2694515	Lactams	1	3,3261E-43	glgX	YP_006185134.1
1813212	Lactams	1	3,36285E-43	SMD_1639	YP_006184365.1
2128949	Lactams	1	3,6619E-43	SMD_1926	YP_006184644.1
2684936	Lactams	1	3,6619E-43	gspL	YP_006185123.1
2873563	Lactams	1	3,6619E-43	SMD_2575	YP_006185286.1
1475785	Lactams	1	3,70175E-43	SMD_1320	YP_006184061.1

Table 4a: Detailed results for the genes in Example 1 (corresponding to Table 2)

POS	**drug**	**#drugs**	**drug class**	**#drug classes**
4131408	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
194882	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1

1506862	CAZ;TE;CFT;CPE	4	Polyketide*;Lactams	2
775975	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
2849025	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
4065975	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
22076	TE	1	Polyketide*	1
355423	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
2263396	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
1242591	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
1827864	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
4395988	TE	1	Polyketide*	1
1556268	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
976566	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
1227228	CAZ;AZT;CFT;CPE;CAX	5	Lactams	1
4146408	TE	1	polyketide*	1
3120515	TE	1	polyketide*	1
1605192	TE	1	polyketide*	1
800868	TE	1	polyketide*	1
3986074	TE	1	polyketide*	1
740620	TE	1	polyketide*	1
1103580	TE	1	polyketide*	1
2105625	TE	1	polyketide*	1
3721056	TE	1	polyketide*	1
3907377	TE	1	polyketide*	1

1911810	TE	1	polyketide*	1	
646078	TE	1	polyketide*	1	
2875710	TE	1	polyketide*	1	
4392432	TE	1	polyketide*	1	
217129	TE	1	polyketide*	1	
4722389	TE	1	polyketide*	1	
1058512	CP;LVX	2	fluoroquinolone	1	
966118	TE	1	polyketide*	1	
4398229	TE	1	polyketide*	1	
1607396	TE	1	polyketide*	1	
3115508	TE	1	polyketide*	1	
4397270	TE	1	polyketide*	1	
413850	CAZ;TE	2	polyketide*;Lactams	2	
3860405	TE	1	polyketide*	1	
3728481	TE	1	polyketide*	1	
2995516	TE	1	polyketide*	1	
678940	TE	1	polyketide*	1	
4141030	TE	1	polyketide*	1	
1292846	TE	1	polyketide*	1	
782557	TE	1	polyketide*	1	
1150463	TE	1	polyketide*	1	
4589465	TE	1	polyketide*	1	
4019103	TE	1	polyketide*	1	

1315236	TE	1	polyketide*	1
4021595	TE	1	polyketide*	1

*: (tetracycline)

Table 4b: Detailed results for the genes in Example 1 (corresponding to Table 2, continued)

POS	**best drug**	**#significant Lactams**	**#significant fluoroquinolones**	**#significant aminoglycosides**	**#significant polyketide (tetracycline)**	**#significant other (benzene derived)/ sulfonamide**
4131408	CAZ	5	0	0	0	0
194882	CAZ	5	0	0	0	0
1506862	CAZ	3	0	0	1	0
775975	CAZ	5	0	0	0	0
2849025	CAZ	5	0	0	0	0
4065975	CAZ	5	0	0	0	0
22076	TE	0	0	0	1	0
355423	CAZ	5	0	0	0	0
2263396	CAX	5	0	0	0	0
1242591	CAX	5	0	0	0	0
1827864	CAX	5	0	0	0	0
4395988	TE	0	0	0	1	0
1556268	CAX	5	0	0	0	0
976566	CAX	5	0	0	0	0
1227228	CAX	5	0	0	0	0

4146408	TE	0	0	0	1	0	
3120515	TE	0	0	0	1	0	
1605192	TE	0	0	0	1	0	
800868	TE	0	0	0	1	0	
3986074	TE	0	0	0	1	0	
740620	TE	0	0	0	1	0	
1103580	TE	0	0	0	1	0	
2105625	TE	0	0	0	1	0	
3721056	TE	0	0	0	1	0	
3907377	TE	0	0	0	1	0	
1911810	TE	0	0	0	1	0	
646078	TE	0	0	0	1	0	
2875710	TE	0	0	0	1	0	
4392432	TE	0	0	0	1	0	
217129	TE	0	0	0	1	0	
4722389	TE	0	0	0	1	0	
1058512	CP	0	2	0	0	0	
966118	TE	0	0	0	1	0	
4398229	TE	0	0	0	1	0	
1607396	TE	0	0	0	1	0	
3115508	TE	0	0	0	1	0	
4397270	TE	0	0	0	1	0	
413850	CAZ	1	0	0	1	0	

3860405	TE	0	0	0	1	0
3728481	TE	0	0	0	1	0
2995516	TE	0	0	0	1	0
678940	TE	0	0	0	1	0
4141030	TE	0	0	0	1	0
1292846	TE	0	0	0	1	0
782557	TE	0	0	0	1	0
1150463	TE	0	0	0	1	0
4589465	TE	0	0	0	1	0
4019103	TE	0	0	0	1	0
1315236	TE	0	0	0	1	0
4021595	TE	0	0	0	1	0

Table 4c: Detailed results for the genes in Example 1 (corresponding to Table 2, continued)

POS	**p-value**	**gene name**	**genbank protein accession number**
4131408	3,06509E-33	SMD_3691	YP_006186368.1
194882	2,15692E-32	SMD_0155	YP_006182939.1
1506862	2,21197E-24	glnD	YP_006184087.1
775975	1,67621E-19	SMD_0669	YP_006183435.1
2849025	3,65007E-18	SMD_2553	YP_006185264.1
4065975	8,30956E-18	SMD_3631	YP_006186308.1
22076	2,23696E-17	aspC	YP_006182811.1
355423	2,21492E-16	SMD_0301	YP_006183083.1

2263396	1,24688E-15	SMD 2035	YP 006184753.1
1242591	4,92147E-15	SMD 1117	YP 006183859.1
1827864	1,43089E-14	SMD 1654	YP 006184380.1
4395988	1,75663E-14	SMD 3927	YP 006186596.1
1556268	1,91684E-14	nth	YP 006184125.1
976566	2,34963E-14	SMD 0861	YP 006183620.1
1227228	1,79895E-13	SMD 1105	YP 006183847.1
4146408	2,94678E-13	SMD 3707	YP 006186384.1
3120515	3,69853E-13	fpr	YP 006185505.1
1605192	5,7886E-13	petB	YP 006184169.1
800868	5,79169E-13	SMD 0692	YP 006183458.1
3986074	5,82217E-13	SMD 3559	YP 006186237.1
740620	6,17456E-13	ftsW	YP 006183408.1
1103580	2,20615E-12	yceG	YP 006183727.1
2105625	2,22987E-12	SMD 1902	YP 006184621.1
3721056	2,64985E-12	poxB	YP 006186024.1
3907377	2,79605E-12	SMD 3500	YP 006186178.1
1911810	4,0785E-12	SMD 1737	YP 006184458.1
646078	4,83567E-12	pepA	YP 006183337.1
2875710	1,00049E-11	adi	YP 006185288.1
4392432	1,15168E-11	hmgA	YP 006186592.1
217129	1,34077E-11	SMD 0173	YP 006182957.1
4722389	1,4285E-11	SMD 4199	YP 006186855.1

1058512	1,58337E-11	SMD_0947	YP_006183692.1
966118	1,62533E-11	ppk	YP_006183609.1
4398229	1,67748E-11	SMD_3929	YP_006186598.1
1607396	1,86958E-11	sspB	YP_006184172.1
3115508	2,06785E-11	SMD_2800	YP_006185501.1
4397270	2,17107E-11	SMD_3928	YP_006186597.1
413850	2,51517E-11	fadL	YP_006183124.1
3860405	3,0546E-11	selD	YP_006186132.1
3728481	4,22737E-11	otsA	YP_006186030.1
2995516	4,55742E-11	SMD_2693	YP_006185394.1
678940	4,61779E-11	xpsD	YP_006183358.1
4141030	5,5179E-11	atpG	YP_006186376.1
1292846	5,98952E-11	SMD_1163	YP_006183905.1
782557	6,7015E-11	SMD_0676	YP_006183442.1
1150463	7,02925E-11	SMD_1030	YP_006183772.1
4589465	8,05304E-11	mgtE2	YP_006186751.1
4019103	8,17204E-11	engB	YP_006186269.1
1315236	8,97531E-11	uvrA	YP_006183932.1
4021595	9,46823E-11	dsbA2	YP_006186272.1

In Tables 3 - 9 the columns are designated as follows:
Gene name: affected gene;
POS: genomic position of the SNP / variant in the Stenotrophomonas reference genome (see above);
p-value: significance value calculated using Fishers exact test (determined according to FDR (Benjamini Hochberg) method (Benjamini Hochberg, 1995))
genbank protein accession number: (NCBI) Accession number of the corresponding protein of the genes

Also the antibiotic/drug classes, the number of significant antibiotics correlated to the mutations (over all antibiotics or over certain classes), as well as the correlated antibiotics are denoted in the Tables.

The p-value was calculated using the Fisher exact test based on contingency table with 4 fields: #samples Resistant / wild type; #samples Resistant / mutant; #samples not Resistant / wild type; #samples not Resistant / mutant

The test is based on the distribution of the samples in the 4 fields. Even distribution indicates no significance, while clustering into two fields indicates significance.

The following results were obtained
- A total of 70.108 different correlations between genetic sites and anti-microbial agents were detected (p-value < 10^{-11}).
- The biggest part of these were point mutations (i.e. single base exchanges)
- The highest significance reached was 10^{-48}, respectively 10^{-33} after correlation. The highest significances were reached for mutations in YP_006186368.1 and YP_006182939.1, respectively, particularly in positions 4131408 and 194882, respectively, with regard to reference genome NC_017671 as annotated at the NCBI. They both are particularly a frame shift mutation or a non-synonymous substitution, particularly a codon change -/C;Agc/Cgc;Agc/Tgc, respectively -/-;gCt/gTt.

- Besides these, insertions or deletions of up to four bases were discovered
- Further, potential genetic tests for four different drug classes relating to resistances were discovered
  - β-lactams (includes Penicillins, Cephalosporins, Carbapenems, Monobactams)
  - Quinolones, particularly Fluoroquinolones
  - Aminoglycosides
  - Polyketides, particularly Tetracyclines
- Potential genetic tests for the tested drugs/drug combinations were discovered:
Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Aztreonam, Cefazolin, Cefepime, Ceftazidime,Cefuroxime, Cephalothin, Imipenem, Piperacillin/Tazobactam, Ciprofloxacin, Levofloxacin, Gentamycin, Tobramycin, Tetracycline, Trimethoprim/Sulfamethoxazol
- Mutations were observed in 3.626 different genes

While in the tables only the best mutations in each gene are represented, a manifold of different SNPs has been found for each gene. Examples for multiple SNPs for two of the genes given in Table 3 are shown in the following Tables 11 and 12.

Table 11: Statistically significant SNPs in gene recF (genbank protein accession number YP_006182796.1) (headers as in Tables 3 and 4, respectively)

POS	drug	#drugs	drug class	best drug	p-value
3753	CAZ;CFT;CPE	3	Lactams	CAZ	8.8846E-043
3743	CAZ;CFT;CPE	3	Lactams	CAZ	1.6887E-044
4362	CAZ;AUG	2	Lactams	CAZ	7.5937E-014
4682	CAZ;CFT	2	Lactams	CAZ	9.8620E-024
4404	CAZ;CFT;CPE	3	Lactams	CAZ	2.9897E-030
3704	AUG	1	Lactams	AUG	4.8768E-018
4657	CAZ	1	Lactams	CAZ	1.3475E-012
4733	CAZ;CFT	2	Lactams	CAZ	1.4737E-017
4669	CAZ;CFT;CPE	3	Lactams	CAZ	2.1126E-035

4737	CAZ	1	Lactams	CAZ	3.1691E-012
4631	CAZ;CFT	2	Lactams	CAZ	5.6460E-021
3937	CAZ;CFT	2	Lactams	CAZ	6.6504E-021
4399	CAZ;CFT	2	Lactams	CAZ	7.6947E-024
4333	CAZ;CFT;CPE	3	Lactams	CAZ	3.8058E-036
4371	AUG	1	Lactams	AUG	1.1852E-010
4364	CAZ;CFT;CPE	3	Lactams	CAZ	2.8799E-030
4681	CAZ;CFT	2	Lactams	CAZ	1.3393E-029

Table 12: Statistically significant SNPs in gene ku (genbank protein accession number YP_006182815.1) (headers as in Tables 3 and 4, respectively)

POS	drug	#drugs	drug class	best drug	p-value
26621	CAZ	1	Lactams	CAZ	1.2518E-013
26530	CAZ	1	Lactams	CAZ	7.1276E-010
26759	CAZ;CFT;CPE	3	Lactams	CAZ	4.8218E-038
26709	CAZ;AUG	2	Lactams	CAZ	1.1176E-012
26643	CAZ;CFT;CPE	3	Lactams	CAZ	5.8203E-039
26545	CAZ;CFT	2	Lactams	CAZ	8.2832E-020
26450	CAZ;CFT;CPE	3	Lactams	CAZ	6.3199E-038
26786	AUG	1	Lactams	AUG	8.1410E-011
26757	AUG	1	Lactams	AUG	7.4899E-014
26626	CAZ;CFT;CPE	3	Lactams	CAZ	5.1434E-032
26746	AUG	1	Lactams	AUG	9.2584E-017
26379	CAZ;CFT;CPE	3	Lactams	CAZ	2.9376E-033
26799	CAZ;AUG	2	Lactams	AUG	2.1465E-013
26814	CAZ;CFT;CPE	3	Lactams	CAZ	2.1352E-027
26655	CAZ;CFT;CPE	3	Lactams	CAZ	5.8761E-044
26747	AUG	1	Lactams	AUG	1.3257E-011
26815	AUG	1	Lactams	AUG	2.0795E-015
26807	CAZ;CFT	2	Lactams	CAZ	2.3932E-035
26750	CAZ	1	Lactams	CAZ	1.9720E-011
26308	CAZ;CFT	2	Lactams	CAZ	3.9544E-019
26763	AUG	1	Lactams	AUG	6.4641E-010

Similar results were obtained for other genes, also in Table 2, but are omitted for the sake of brevity.

Further, a synergistic effect of individual SNPs was demonstrated by exhaustively comparing significance levels for association of single SNPs with antibiotic susceptibility/resistance and significance levels for association of combinations of SNPs with antibiotic susceptibility/resistance. For a representative example of 2 SNPs the significance level for synergistic association of two SNPs was improved with the values given in Tables 13 (for combinations of genes in Table 1) and 14 (for combinations of genes in Table 2) compared to the association of either SNP alone, given for exemplary different antibiotics.

Table 13: Synergistic increase for association of two SNPs of Table 1

drug	POS 1	Ref	Alt	POS 2	Ref	Alt	Improv [%]
T/S	1246108	G	A	2470228	T	C	369.4
T/S	1475785	C	G	2470228	T	C	449.3
T/S	1512298	G	C,T	2873563	C	T	2700.6
T/S	1512298	G	C,T	2470228	T	C	5457.7
T/S	1512298	G	C,T	203554	G	C	109.
T/S	1512298	G	C,T	4458076	T	A,G	135.4
T/S	1512298	G	C,T	4455112	A	C,T	119.8
T/S	1514781	A	C,T	2915609	A	C	286.9
T/S	1514781	A	C,T	2470228	T	C	741.6
T/S	2873563	C	T	2915609	A	C	188.3
T/S	2873563	C	T	2470228	T	C	15622.4
T/S	2915609	A	C	2470228	T	C	6929.8
T/S	2915609	A	C	3901645	G	C	271.3
T/S	2915609	A	C	355423	G	A,T;GCAGGTC	335.0
T/S	3012515	T	C	2470228	T	C	493.0
T/S	2470228	T	C	203554	G	C	574.0

T/S	2470228	T	C	4567013	T	A,G	881.9
T/S	2470228	T	C	4458551	G	A	275.5
T/S	2470228	T	C	4458076	T	A,G	273.9
T/S	2470228	T	C	4041091	A	C,T	867.7
T/S	2470228	T	C	4000529	C	T	853.0
T/S	2470228	T	C	2128948	ATGC	A	876.7
T/S	2470228	T	C	3743	A	G	997.5
T/S	2470228	T	C	3557927	G	A,C,T	1078.7
T/S	2470228	T	C	4398229	C	A	367.4
T/S	2470228	T	C	3923235	C	T	464.5
T/S	2470228	T	C	1813212	G	A,C	388.2
T/S	2470228	T	C	846345	G	A	386.4
T/S	2470228	T	C	3020959	C	T	556.4
T/S	2470228	T	C	4455112	A	C,T	331.3

POS 1, 2 = position 1, 2 used for combination; Ref = reference base; Alt = alternated base in samples; improv = improvement compared to minimum p-value of single SNP

For example, the improvement of 331.3 % in the last example in Table 13 with positions 2470228 and 4455112 for T/S results from a p-value change from 0.000200735 to 6.05883e-05.

Table 14: Synergistic increase for association of two SNPs of Table 2

drug	POS 1	Ref	Alt	POS 2	Ref	Alt	Improv [%]
T/S	1607396	G	C,T	4392432	G	A,T	616.7
T/S	1607396	G	C,T	4395988	C	T	113.4
T/S	4392432	G	A,T	4397270	G	T,A	5640.7
T/S	4395988	C	T	4397270	G	T,A	223.4

POS 1, 2 = position 1, 2 used for combination; Ref = reference base; Alt = alternated base in samples; improv = improvement compared to minimum p-value of single SNP

For example, the improvement of 223.4 % in the last example of Table 14 with positions 4395988 and 4397270 for T/S results from a p-value change from 0.000200735 to 8.98703e-05.

Again, similar results were obtained for other SNPs in respective genes.

A genetic test for the combined pathogen identification and antimicrobial susceptibility testing direct from the patient sample can reduce the time-to actionable result significantly from several days to hours, thereby enabling targeted treatment. Furthermore, this approach will not be restricted to central labs, but point of care devices can be developed that allow for respective tests. Such technology along with the present methods and computer program products could revolutionize the care, e.g. in intense care units or for admissions to hospitals in general. Furthermore, even applications like real time outbreak monitoring can be achieved using the present methods.

Instead of using only single variants, a combination of several variant positions can improve the prediction accuracy and further reduce false positive findings that are influenced by other factors.

Compared to approaches using MALDI-TOF MS, the present approach has the advantage that it covers almost the complete genome and thus enables us to identify the potential genomic sites that might be related to resistance. While MALDI-TOF MS can also be used to identify point mutations in bacterial proteins, this technology only detects a subset of proteins and of these not all are equally well covered. In addition, the identification and differentiation of certain related strains is not always feasible.

The present method allows computing a best breakpoint for the separation of isolates into resistant and susceptible groups. The inventors designed a flexible software tool that allows to consider - besides the best breakpoints - also values de-

fined by different guidelines (e.g. European and US guidelines), preparing for an application of the GAST in different countries.

The inventors demonstrate that the present approach is capable of identifying mutations in genes that are already known as drug targets, as well as detecting potential new target sites.

The current approach enables

a. Identification and validation of markers for genetic identification and susceptibility/resistance testing within one diagnostic test
b. validation of known drug targets and modes of action
c. detection of potentially novel resistance mechanisms leading to putative novel target / secondary target genes for new therapies

Claims

1. A diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug, e.g. antibiotic, treatment, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, wherein the presence of said at least two mutations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant Stenotrophomonas strain in said patient.

2. A method of selecting a treatment of a patient suffering from an infection with a potentially resistant Stenotrophomonas strain, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Stenotrophomonas species from the patient;

b) determining the presence of at least one mutation in at least two genes from the group of genes consisting of SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and SMD_1320, or from the group of genes consisting of SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, aspC, SMD_0301, SMD_2035, SMD_1117, SMD_1654, SMD_3927, nth, SMD_0861, SMD_1105, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, SMD_0947, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and dsbA2, wherein the presence of said at least two mutations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs;

c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and

d) selecting one or more antimicrobial, e.g. antibiotic, drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

3. The method of one or more of the preceding claims, wherein at least a mutation in SMD_3691, particularly in position 4131408, and/or SMD_0155, particularly in position 194882, with regard to reference genome NC_017671 as annotated at the NCBI, is determined.

4. The method of one or more of the preceding claims, wherein the method involves determining the resistance of Stenotrophomonas to one or more antimicrobial, e.g. antibiotic, drugs.

5. The method of any one of claims 1 to 4, wherein the antimicrobial, e.g. antibiotic, drug is selected from lactam antibiotics and the presence of a mutation in the following genes is determined: SMD_3200, SMD_3579, SMD_3610, SMD_4135, SMD_3493, SMD_0995, SMD_2708, cbpD, SMD_2457, SMD_2911, gspL, SMD_2447, SMD_0184, SMD_1120, SMD_4120, SMD_0498, recF, fliK, rtcB, SMD_0069, SMD_1911, SMD_1996, glgX, SMD_4066, SMD_3568, ku, gcd, SMD_3572, SMD_3603, SMD_2199, SMD_2221, thiC, SMD_3992, SMD_3982, SMD_1353, StmPr1, SMD_3983, SMD_2572, tolA, SMD_1351, cyoA2, SMD_1123, SMD_1926, SMD_3570, SMD_2620, SMD_2277, SMD_1205, SMD_1639, SMD_2575, and/or SMD_1320, or SMD_3691, SMD_0155, glnD, SMD_0669, SMD_2553, SMD_3631, SMD_0301, SMD_2035, SMD_1117, SMD_1654, nth, SMD_0861, SMD_1105, and/or fadL; and/or
wherein the antimicrobial, e.g. antibiotic, drug is selected from polyketide antibiotics, preferably tetracycline antibiotics, and the presence of a mutation in the following genes is determined: glnD, aspC, SMD_3927, SMD_3707, fpr, petB, SMD_0692, SMD_3559, ftsW, yceG, SMD_1902, poxB, SMD_3500, SMD_1737, pepA, adi, hmgA, SMD_0173, SMD_4199, ppk, SMD_3929, sspB, SMD_2800, SMD_3928, fadL, selD, otsA, SMD_2693, xpsD, atpG, SMD_1163, SMD_0676, SMD_1030, mgtE2, engB, uvrA, and/or dsbA2; and/or
wherein the antimicrobial, e.g. antibiotic, drug is selected from quinolone antibiotics, preferably fluoroquinolone antibiotics, and the presence of a mutation in the following genes is determined: SMD_0947.

6. The method of one or more of the preceding claims, wherein the antimicrobial drug, e.g. antibiotic drug, is selected from the group consisting of Amoxicillin/K

Clavulanate (AUG), Ampicillin (AM), Aztreonam (AZT), Cefazolin (CFZ), Cefepime (CPE), Cefotaxime (CFT), Ceftazidime (CAZ), Ceftriaxone (CAX), Cefuroxime (CRM), Cephalotin (CF), Ciprofloxacin (CP), Ertapenem (ETP), Gentamicin (GM), Imipenem (IMP), Levofloxacin (LVX), Meropenem (MER), Piperacillin/Tazobactam (P/T), Ampicillin/Sulbactam (A/S), Tetracycline (TE), Tobramycin (TO), and Trimethoprim/Sulfamethoxazole (T/S).

7. The method of any one of claims 1 to 6, wherein the antibiotic drug is CAZ and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671 as annotated at the NCBI: 4131408, 194882, 1506862, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228, 413850; and/or
wherein the antibiotic drug is at least one of AZT and CAX and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 4131408, 194882, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228; and/or
wherein the antibiotic drug is at least one of CFT and CPE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 4131408, 194882, 1506862, 775975, 2849025, 4065975, 355423, 2263396, 1242591, 1827864, 1556268, 976566, 1227228; and/or
wherein the antibiotic drug is TE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 1506862, 22076, 4395988, 4146408, 3120515, 1605192, 800868, 3986074, 740620, 1103580, 2105625, 3721056, 3907377, 1911810, 646078, 2875710, 4392432, 217129, 4722389, 966118, 4398229, 1607396, 3115508, 4397270, 413850, 3860405, 3728481, 2995516, 678940, 4141030, 1292846, 782557, 1150463, 4589465, 4019103, 1315236, 4021595; and/or

wherein the antibiotic drug is at least one of CP and LVX and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_017671: 1058512.

8. The method of any one of claims 1 to 7, wherein the resistance of a bacterial microorganism belonging to the species Stenotrophomonas against 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 17, 18, 19, 20 or 21 antibiotic drugs is determined.

9. The method of one or more of the preceding claims, wherein determining the nucleic acid sequence information or the presence of a mutation comprises determining a partial sequence or an entire sequence of the at least two genes.

10. The method of one or more of the preceding claims, wherein determining the nucleic acid sequence information or the presence of a mutation comprises determining a partial or entire sequence of the genome of the Stenotrophomonas species, wherein said partial or entire sequence of the genome comprises at least a partial sequence of said at least two genes.

11. The method of one or more of the preceding claims, wherein determining the nucleic acid sequence information or the presence of a mutation comprises using a next generation sequencing or high throughput sequencing method, preferably wherein a partial or entire genome sequence of the bacterial organism of Stenotrophomonas species is determined by using a next generation sequencing or high throughput sequencing method.

12. A method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Stenotrophomonas species, comprising:

obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Stenotrophomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Stenotrophomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Stenotrophomonas, and/or assembling the gene sequence of the first data set, at least in part;
analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants;
correlating the third data set with the second data set and statistically analyzing the correlation; and
determining the genetic sites in the genome of Stenotrophomonas associated with antimicrobial drug, e.g. antibiotic, resistance.

13. A diagnostic method of determining an infection of a patient with Stenotrophomonas species potentially resistant to antimicrobial drug treatment, comprising the steps of:
a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Stenotrophomonas from the patient;
b) determining the presence of at least one mutation in at least one gene of the bacterial microorganism belonging to the species Stenotrophomonas as determined by the method of claim 12, wherein the presence of said at least one mutation is indicative of an infection with an antimicrobial drug resistant Stenotrophomonas strain in said patient.

14. A method of selecting a treatment of a patient suffering from an infection with a potentially resistant Stenotrophomonas strain, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Stenotrophomonas from the patient;

b) determining the presence of at least one mutation in at least one gene of the bacterial microorganism belonging to the species Stenotrophomonas as determined by the method of claim 12, wherein the presence of said at least one mutation is indicative of a resistance to one or more antimicrobial drugs;

c) identifying said at least one or more antimicrobial drugs; and

d) selecting one or more antimicrobial drugs different from the ones identified in step c) and being suitable for the treatment of a Stenotrophomonas infection.

15. A method of acquiring an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Stenotrophomonas species, comprising:

obtaining or providing a first data set of gene sequences of a clinical isolate of Stenotrophomonas species;

providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of Stenotrophomonas species;

aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Stenotrophomonas, and/or assembling the gene sequence of the first data set, at least in part;

analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants of the first data set;

correlating the third data set with the second data set and statistically analyzing the correlation; and

determining the genetic sites in the genome of Stenotrophomonas of the first data set associated with antimicrobial drug, e.g. antibiotic, resistance.

16. Computer program product comprising computer executable instructions which, when executed, perform a method according to any one of claims 12 to 15.

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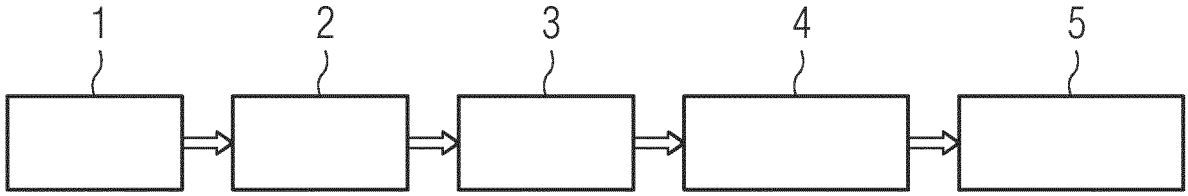
FIG 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/067611

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P.G. VIDIGAL ET AL: "Adaptation of Stenotrophomonas maltophilia in cystic fibrosis: Molecular diversity, mutation frequency and antibiotic resistance", INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, vol. 304, no. 5-6, 1 July 2014 (2014-07-01), pages 613-619, XP055266763, DE ISSN: 1438-4221, DOI: 10.1016/j.ijmm.2014.04.002 the whole document ----- -/--	1-11

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2016	13/01/2017

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GARCÍA-LEÓN G ET AL: "High-level quinolone resistance is associated with the overexpression of smeVWX in Stenotrophomonas maltophilia clinical isolates.", CLINICAL MICROBIOLOGY AND INFECTION : THE OFFICIAL PUBLICATION OF THE EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES MAY 2015, vol. 21, no. 5, May 2015 (2015-05), pages 464-467, XP009189608, ISSN: 1469-0691 the whole document	1-11
X	GOULD VIRGINIA C ET AL: "SmeDEF-mediated antimicrobial drug resistance in Stenotrophomonas maltophilia clinical isolates having defined phylogenetic relationships", JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol. 57, no. 6, June 2006 (2006-06), pages 1070-1076, XP002756703, ISSN: 0305-7453 the whole document	1-11
X	SANCHEZ PATRICIA ET AL: "Regulatory regions of smeDEF in Stenotrophomonas maltophilia strains expressing different amounts of the multidrug efflux pump SmeDEF", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 48, no. 6, June 2004 (2004-06), pages 2274-2276, XP002756704, ISSN: 0066-4804 the whole document	1-11
X	GARCIA-LEON GUILLERMO ET AL: "Interplay between intrinsic and acquired resistance to quinolones in Stenotrophomonas maltophilia", ENVIRONMENTAL MICROBIOLOGY, vol. 16, no. 5, May 2014 (2014-05), pages 1282-1296, XP002756705, the whole document	1-11

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CROSSMAN LISA C ET AL: "The complete genome, comparative and functional analysis of Stenotrophomonas maltophilia reveals an organism heavily shielded by drug resistance determinants", GENOME BIOLOGY, BIOMED CENTRAL LTD., LONDON, GB, vol. 9, no. 4, 17 April 2008 (2008-04-17), page R74, XP021041618, ISSN: 1465-6906 the whole document	1-11

A	JIA W ET AL: "Resistance of Stenotrophomonas maltophilia to fluoroquinolones: Prevalence in a university hospital and possible mechanisms", INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH AND PUBLIC HEALTH 2015 MDPI AG CHE, vol. 12, no. 5, 13 May 2015 (2015-05-13), pages 5177-5195, XP002756706, ISSN: 1661-7827 the whole document	1-11

A	WO 01/79540 A2 (VIRCO NV [BE]; LARDER BRENDAN [GB]; BLOOR STUART [GB]; HERTOGS KURT [B) 25 October 2001 (2001-10-25) abstract; claims 1-33	1-11

A	WO 2012/106432 A2 (BAYLOR COLLEGE MEDICINE [US]; ZECHIEDRICH E LYNN [US]; SWICK MICHELLE) 9 August 2012 (2012-08-09) abstract; claims 1-9	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/067611

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-11(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-11(partially)

Methods for diagnosis an antimicrobial drug resistant Stenotrophomonas species and methods for selecting a treatment by determining the presence of at least one mutation in at least two marker genes, wherein the first marker gene is the first gene listed in the first group of genes listed in step b) of claim 1 (i.e. SMD_3200) in combination with at least one other gene as listed in the first group of genes as listed in step b) of claim 1.

2-50. claims: 1-11(partially)

For the second invention the same as for invention 1, however, wherein the first marker gene is the second gene listed in the first group of genes of step b) of claim 1 (i.e. SMD_3579 gene) in combination with at least one other gene as listed in the first group of genes as listed in step b) of claim 1. The same applies subsequently for inventions 3 to 50.

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51. claims: 1-11(partially)

Methods for diagnosis an antimicrobial drug resistant Stenotrophomonas species and methods for selecting a treatment by determining the presence of at least one mutation in at least two marker genes, wherein the first marker gene is the first gene listed in the second group of genes listed in step b) of claim 1 (i.e. SMD_3691) in combination with at least one other gene as listed in the second group of genes as listed in step b) of claim 1.

52-100. claims: 1-11(partially)

For inventions 52 to 100 applies the same as for invention 51, i.e. for invention 52 wherein the first marker gene is the second gene listed in the second group of genes listed in step b) of claim 1 (i.e. SMD_0155) in combination with at least one other gene as listed in the second group of genes as listed in step b) of claim 1; i.e. for invention 53 wherein the first marker gene is the third gene listed in the second group of genes listed in step b) of claim 1 (i.e. gnl_D) in combination with at least one other gene as listed in the second group of genes as listed in step b) of claim 1.

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101. claims: 12-16

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Methods for determining genetic sites associated with antimicrobial drug resistance in Stenotrophomonas species by sequence comparison analysis. Computer program product for performing the method.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/067611

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0179540 A2	25-10-2001	AT 404698 T	15-08-2008
		AT 526423 T	15-10-2011
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