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(71) Applicant: **CURETIS GMBH** [DE/DE]; Max-Eyth-Strasse 42, 71088 Holzgerlingen (DE).

(72) Inventors: **KELLER, Andreas**; Albert-Schweitzer-Str. 6, 66346 Püttlingen (DE). **SCHMOLKE, Susanne**; Olivierstrasse 15, 81447 München (DE). **STÄHLER, Cord Friedrich**; Muldweg 8, 69493 Hirschberg an der Bergstraße (DE). **BACKES, Christina**; Am Geisenberg 45, 66125 Saarbrücken (DE). **GALATA, Valentina**; Auf der Schlecht 4, 66123 Saarbrücken (DE).

(74) Agent: **SCHNAPPAUF, Georg**; ZSP Patentanwälte PartG mbB, Radlkoferstrasse 2, 81373 München (DE).

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

(57) Abstract: The invention relates to a method of determining an infection of a patient with Pseudomonas species potentially resistant to antimicrobial drug treatment, a method of selecting a treatment of a patient suffering from an antibiotic resistant Pseudomonas infection, and a method of determining an antibiotic resistance profile for bacterial microorganisms of Pseudomonas 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.

Genetic testing for predicting resistance of Pseudomonas species against antimicrobial agents

The present invention relates to a method of determining an infection of a patient with Pseudomonas species potentially resistant to antimicrobial drug treatment, a method of selecting a treatment of a patient suffering from an infection with a potentially resistant Pseudomonas strain, and a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Pseudomonas 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%.

Pseudomonas ssp. are gram-negative, aerobic bacilli belonging to the family of Pseudomonadaceae. Pseudomonas aeruginosa has received the most attention because of the frequency with which it is involved in human disease. Although it seldom causes disease in healthy individuals, it is a major threat to hospitalized patients, particularly those with serious underlying diseases such as cancer and burns. The high mortality associated with these infections is due to a combination of weakened host defenses, bacterial resistance to antibiotics, and the production of extracellular bacterial enzymes and toxins.

Pseudomonas aeruginosa causes various diseases. The pathogen is increasingly recognized as an important etiology of healthcare-associated pneumonia and is consistently identified as the most commonly isolated pathogen causing ventilator-associated pneumonia. Furthermore, Pseudomonas aeruginosa is well known as a cause of chronic infection of the lungs and airways in patients with cystic fibrosis. Localized infection following surgery or burns commonly results in a generalized and frequently fatal bacteremia. Urinary tract infections following introduction of Pseudomonas aeruginosa on catheters or in irrigating solutions are not uncommon. Pseudomonas aeruginosa can cause severe corneal infections following eye surgery or injury. It occasionally causes meningitis following lumbar puncture and endocarditis following cardiac surgery. It has been associated with some diarrheal disease episodes.

Pseudomonas is intrinsically resistant to a multitude of antibiotics presumably as a result of impermeability of the outer membrane combined with active efflux pumps. Besides intrinsic resistance, Pseudomonas easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants.

In a recent report by CDC, titled Antibiotic Resistance Threats in the United States, 2013, multidrug-resistant Pseudomonas aeruginosa was listed among bacteria that pose a serious threat level. Approximately 8% of all healthcare-associated infections reported to CDC's National Healthcare Safety Network are caused by Pseudomonas aeruginosa. About 13% of severe healthcare-associated infections caused by Pseudomonas aeruginosa are multidrug resistant, meaning several classes of antibiotics no longer cure these infections.

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

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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, Pseudomonas strain in said patient.

An infection of a patient with Pseudomonas species potentially resistant to antimicrobial drug treatment herein means an infection of a patient with Pseudomonas species wherein it is unclear if the Pseudomonas 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

SCV20265_1892	SCV20265_5625	SCV20265_1467	SCV20265_5607	SCV20265_3294
SCV20265_1879	SCV20265_5242	SCV20265_2224	SCV20265_0530	SCV20265_3289
SCV20265_1858	SCV20265_2193	SCV20265_6274	SCV20265_2958	SCV20265_3248
SCV20265_1132	SCV20265_1451	SCV20265_6120	SCV20265_4839	SCV20265_2195
SCV20265_0968	SCV20265_2464	SCV20265_2518	SCV20265_2654	SCV20265_3101
SCV20265_3909	SCV20265_2610	SCV20265_1805	SCV20265_4445	SCV20265_2883
SCV20265_2916	SCV20265_1721	SCV20265_3099	SCV20265_1735	SCV20265_6289
SCV20265_2974	SCV20265_2404	SCV20265_6135	SCV20265_3626	SCV20265_1050
SCV20265_0188	SCV20265_5329	SCV20265_2792	SCV20265_1617	SCV20265_2236
SCV20265_0491	SCV20265_2422	SCV20265_5463	SCV20265_5597	SCV20265_0241

Table 2: List of genes

SCV20265_1892	SCV20265_5625	SCV20265_1467	SCV20265_5607	SCV20265_3294
SCV20265_1879	SCV20265_5242	SCV20265_2224	SCV20265_0530	SCV20265_3289
SCV20265_1858	SCV20265_2193	SCV20265_6274	SCV20265_2958	SCV20265_3248
SCV20265_1132	SCV20265_1451	SCV20265_6120	SCV20265_4839	SCV20265_2195
SCV20265_0968	SCV20265_2464	SCV20265_2518	SCV20265_2654	SCV20265_3101
SCV20265_3909	SCV20265_2610	SCV20265_1805	SCV20265_4445	SCV20265_2883
SCV20265_2916	SCV20265_1721	SCV20265_3099	SCV20265_1735	SCV20265_6289
SCV20265_2974	SCV20265_2404	SCV20265_6135	SCV20265_3626	SCV20265_1050
SCV20265_0188	SCV20265_5329	SCV20265_2792	SCV20265_1617	SCV20265_2236
SCV20265_0491	SCV20265_2422	SCV20265_5463	SCV20265_5597	SCV20265_0241

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 Pseudomonas strain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 Pseudomonas 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 Pseudomonas species, comprising:

obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Pseudomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Pseudomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Pseudomonas, 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 Pseudomonas 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. antibiotic, resistance profile for a bacterial microorganism belonging to the species Pseudomonas 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 Pseudomonas species potentially resistant to anti-

microbial drug treatment, which can, like in the first aspect, also be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 Pseudomonas strain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 microorganisms of Pseudomonas species, comprising:
obtaining or providing a first data set of gene sequences of a clinical isolate of Pseudomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of Pseudomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Pseudomonas, 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 Pseudomonas 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 an 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 Pseudomonas species, particularly Pseudomonas aeruginosa.

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 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.

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

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, wherein the presence of said at least two mutations is indicative of an infection

with an antimicrobial, e.g. antibiotic, resistant Pseudomonas 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^{-30}, particularly smaller than 10^{-35}, further particularly smaller than 10^{-40}, indicating the high significance of the values ($n= 1104$; $\alpha = 0.05$). 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 Pseudomonas species, whereas the genes in Table 2 represent the 50 best genes for which a cross-correlation could be ob-

served for the antimicrobial drug, e.g. antibiotic, susceptibility testing for Pseudomonas species as described below.

According to certain embodiments, the obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 Pseudomonas 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. Pseudomonas species - mutations in the genes for each species and for the whole multitude of samples of different species, e.g. Pseudomonas 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 Pseudomonas.

According to certain embodiments, the genomes of Pseudomonas 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 Pseudomonas is NC_023149 as annotated at the NCBI according to certain embodiments. The reference genome is attached to this application as sequence listing.

The reference sequence was obtained from Pseudomonas strain NC_023149 (http://www.genome.jp/dbget-bin/www_bget?refseq+NC_023149)

LOCUS NC_023149 6725183 bp DNA circular CON 07-FEB-2015
DEFINITION Pseudomonas aeruginosa SCV20265, complete genome.
ACCESSION NC_023149
VERSION NC_023149.1 GI:568306739
DBLINK BioProject: PRJNA224116
BioSample: SAMN02415141
Assembly: GCF_000510305.1
KEYWORDS RefSeq.
SOURCE Pseudomonas aeruginosa SCV20265
ORGANISM Pseudomonas aeruginosa SCV20265
Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.
REFERENCE 1 (bases 1 to 6725183)
AUTHORS Eckweiler,D., Bunk,B., Sproer,C., Overmann,J. and Haussler,S.
TITLE Complete Genome Sequence of Highly Adherent Pseudomonas aeruginosa Small-Colony Variant SCV20265
JOURNAL Genome Announc 2 (1) (2014)
PUBMED 24459283
REMARK Publication Status: Online-Only
REFERENCE 2 (bases 1 to 6725183)
AUTHORS Eckweiler,D., Bunk,B., Overmann,J. and Haeussler,S.
TITLE Direct Submission

JOURNAL Submitted (02-DEC-2013) Bioinformatics, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Inhoffenstr. 7B, Braunschweig 38124, Germany

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. Pseudomonas 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 Pseudomonas species.

Using these techniques, genes with mutations of the microorganism of interest, e.g. Pseudomonas 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. Pseudomonas. 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, e.g. 1000 or more than 1000, e.g. 1100 or more than 1100 different species of a microorganism, e.g. different Pseudomonas 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, 100, 200, 300, 400, 500, e.g. 1000 or 1100, 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, e.g. 1000 or 1100, 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, 200, 400 or 500, e.g. 1000 or 1100.

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, e.g. 1000 or more or 1100 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, 200 or more, 400 or more or 500 or more, e.g. 1000 or more or 1100 or more.

After the above procedure has been carried out for more than 1100, e.g. 1104, individual species of Pseudomonas, 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 Pseudomonas strain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, 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 Pseudomonas 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 SCV20265_1892 and/or SCV20265_5625, particularly in positions 1979239 and/or 5987559, respectively, with regard to reference genome NC_023149 as annotated at the NCBI, is determined. For such mutations, a particularly relevant correlation with antimicrobial drug, e.g. antibiotic, resistance could be deter-

mined. In particular, the mutation in position 1979239 with regard to reference genome NC_023149 as annotated at the NCBI is a non-synonymous coding, particularly a codon change aCc/aTc;aCc/aAc, and the mutation in position 5987559 with regard to reference genome NC_023149 as annotated at the NCBI is a non-synonymous coding, particularly a codon change tCg/tTg;tCg/tGg.

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 Pseudomonas to one or more antimicrobial, e.g. antibiotic, drugs can be determined according to certain embodiments.

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 the antimicrobial, e.g. antibiotic, drug is selected from lactam antibiotics, and the presence of a mutation in the following genes is determined: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_1805,

SCV20265_4445, SCV20265_2883, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_5329, SCV20265_2792, and/or SCV20265_2236.

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, e.g. fluoroquinolone antibiotics, and the presence of a mutation in the following genes is determined: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and/or SCV20265_0241.

According to certain embodiments of the first and/or second aspect of the invention the antimicrobial, e.g. antibiotic, drug is selected from aminoglycoside antibiotics, and the presence of a mutation in the following genes is determined: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195,

SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and/or SCV20265_0241.

According to certain embodiments of the first and/or second aspect of the invention the antimicrobial, e.g. antibiotic, drug is selected from other antibiotics ((benzene derived)/sulfonamide), and the presence of a mutation in the following genes is determined: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and/or SCV20265_0241.

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 or 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_023149: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_5329, SCV20265_2792, SCV20265_2236.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 or Table 2, the antibiotic drug is selected from quinolone antibiotics, e.g. fluoroquinolone antibiotics, and a mutation in at least one of the following genes is detected with regard to reference genome NC_023149: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, SCV20265_0241.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 or Table 2, the antibiotic drug is selected from aminoglycoside antibiotics and a mutation in at least one of the following genes is detected with regard to reference genome NC_023149: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974,

SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, SCV20265_0241.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 or Table 2, the antibiotic drug is selected from other antibiotics ((benzene derived)/sulfonamide) and a mutation in at least one of the following genes is detected with regard to reference genome NC_023149: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, SCV20265_0241.

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 Pseudomonas.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 or 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_023149: 1979239, 5987559, 1537406, 5965080, 1967346, 5569783, 2350860, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 3301233, 1899865, 4712288, 3019764, 1805165, 3299685, 1821163, 6702956, 3160788, 6535290, 3881624, 1099519, 5662982, 2903129, 2363393, 2350862, 3507601, 3507667, 6519971.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 or Table 2, the antibiotic drug is selected from quinolone antibiotics, e.g. fluoroquinolone antibiotics, and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5965080, 3513162, 1967346, 5569783, 2350860, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1194383, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 3301233, 4166792, 2709322, 1899865, 4712288, 3019764, 3068671, 1805165, 3299685, 1821163, 6702956, 3160788, 2515627, 6535290, 3881624, 1099519, 208902, 5662982, 2903129, 1701758, 2363393, 525701, 2530203, 5806684, 5954547, 261978, 2350862, 3507601, 3507667, 6519971.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 or Table 2, the antibiotic drug is selected from aminoglycoside antibiotics and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5965080, 3513162, 1967346, 5569783, 2350860, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1194383, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 3301233, 4166792, 2709322, 1899865, 4712288, 3019764, 3068671, 1805165, 3299685, 1821163, 6702956, 3160788, 2515627, 6535290, 3881624, 1099519, 208902, 5662982, 2903129, 1701758, 2363393, 525701, 2530203, 5806684, 5954547, 261978, 2350862, 3507601, 3507667, 6519971.

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 or Table 2, the antibiotic drug is selected from other antibiotics ((benzene derived)/sulfonamide) and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5965080, 3513162, 1967346, 5569783, 2350860, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1194383, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 3301233, 4166792, 2709322, 1899865, 4712288, 3019764, 3068671, 1805165, 3299685, 1821163, 6702956, 3160788, 2515627, 6535290, 3881624, 1099519, 208902, 5662982, 2903129, 1701758, 2363393, 525701, 2530203, 5806684, 5954547, 261978, 2350862, 3507601, 3507667, 6519971.

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

nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5965080, 3513162, 1967346, 5569783, 2350860, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1194383, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 3301233, 4166792, 2709322, 1899865, 4712288, 3019764, 3068671, 1805165, 3299685, 1821163, 6702956, 3160788, 2515627, 6535290, 3881624, 1099519, 208902, 5662982, 2903129, 1701758, 2363393, 525701, 2530203, 5806684, 5954547, 261978, 2350862, 3507601, 3507667, 6519971.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is CPE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5569783, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 1899865, 4712288, 1805165, 1821163, 6702956, 3160788, 6535290, 3881624, 1099519, 5662982, 2903129, 2363393, 3507601, 3507667, 6519971.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is P/T and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5965080, 1967346, 2350860, 2754829, 3301233, 3019764, 6702956, 3881624, 2350862.

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

is detected with regard to reference genome NC_023149: 1979239, 5987559, 2754829, 3301233, 3299685, 1099519.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is CFT, IMP, MER, CAX, AZT, and/or CAZ and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559.

According to certain embodiments of the first and/or second aspect of the invention, the resistance of a bacterial micro-organism belonging to the species Pseudomonas 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 se-

quence information or the presence of a mutation comprises determining a partial or entire sequence of the genome of the Pseudomonas 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 sequencing 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 Pseudomonas 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 Pseudomonas species, comprising:
obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Pseudomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Pseudomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Pseudomonas, 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 Pseudomonas 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 $p < 10^{-10}$.

The method of the third aspect of the present invention, as well as related methods, e.g. according to the 7^{th} and 10^{th} aspect, can, according to certain embodiments, comprise correlating different genetic sites to each other. 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 Pseudomonas 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 Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883,

SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, or from the genes listed in Table 5.

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 non-synonymous coding in YP_008980900.1 and/or YP_008984625.1.

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 Pseudomonas 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 Pseudomonas 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 Pseudomonas species potentially resistant to antimicrobial drug treatment, which also can be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 Pseudomonas infection in a patient can be determined using sequencing methods as well as a resistance to antimicrobial drugs, e.g. antibiotics, of the Pseudomonas 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 Pseudomonas strain, e.g. an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 microorganisms of Pseudomonas species, comprising:
obtaining or providing a first data set of gene sequences of a clinical isolate of Pseudomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of Pseudomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Pseudomonas, 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 Pseudomonas 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 Pseudomonas can be determined.

According to certain embodiments, the reference genome of Pseudomonas is NC_023149 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 $p < 10^{-10}$. Also, according to certain embodiments, the method further comprises correlating different genetic sites to each other.

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 Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 $p < 10^{-10}$. 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 instructions 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 Pseudomonas species potentially resistant to antimicrobial drug treatment, which can also be described as a method of determining an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas infection of a patient is disclosed, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 Pseudomonas 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 Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 Pseudomonas 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:
SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224,

SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, SCV20265_0241, SCV20265_4334, SCV20265_0891, SCV20265_1756, SCV20265_1113, SCV20265_1895, SCV20265_4827, SCV20265_4159, SCV20265_4562, SCV20265_3569, SCV20265_0041, SCV20265_0017, SCV20265_0008, SCV20265_0032, SCV20265_0033, SCV20265_0007, SCV20265_0028, SCV20265_0014, SCV20265_0016.

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 Pseudomonas is again NC_023149 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 $p < 10^{-10}$. Also, according to certain embodiments, the method further comprises correlating different genetic sites to each other. Al-

so the other aspects of the embodiments of the first and second aspect of the invention apply.

Table 5: List of genes

SCV20265_1892	SCV20265_5625	SCV20265_1467	SCV20265_5607
SCV20265_3294	SCV20265_1879	SCV20265_5242	SCV20265_2224
SCV20265_0530	SCV20265_3289	SCV20265_1858	SCV20265_2193
SCV20265_6274	SCV20265_2958	SCV20265_3248	SCV20265_1132
SCV20265_1451	SCV20265_6120	SCV20265_4839	SCV20265_2195
SCV20265_0968	SCV20265_2464	SCV20265_2518	SCV20265_2654
SCV20265_3101	SCV20265_3909	SCV20265_2610	SCV20265_1805
SCV20265_4445	SCV20265_2883	SCV20265_2916	SCV20265_1721
SCV20265_3099	SCV20265_1735	SCV20265_6289	SCV20265_2974
SCV20265_2404	SCV20265_6135	SCV20265_3626	SCV20265_1050
SCV20265_0188	SCV20265_5329	SCV20265_2792	SCV20265_1617
SCV20265_2236	SCV20265_0491	SCV20265_2422	SCV20265_5463
SCV20265_5597	SCV20265_0241	SCV20265_4334	SCV20265_0891
SCV20265_1756	SCV20265_1113	SCV20265_1895	SCV20265_4827
SCV20265_4159	SCV20265_4562	SCV20265_3569	SCV20265_0041
SCV20265_0017	SCV20265_0008	SCV20265_0032	SCV20265_0033
SCV20265_0007	SCV20265_0028	SCV20265_0014	SCV20265_0016

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 num-**

				ber
SCV20265_1892	1979239	T/S;CP;CFT;LVX;GM;IMP;ETP;MER;CAX;AZT;P/T;CPE;CAZ;TO	1,5814E-141	YP_008980900.1
SCV20265_5625	5987559	T/S;CP;CFT;LVX;GM;IMP;ETP;MER;CAX;AZT;P/T;CPE;CAZ;TO	1,2153E-121	YP_008984625.1
SCV20265_4334	4604211	T/S;CP;LVX;GM;ETP;MER;AZT;P/T;CPE;TO	2,21898E-31	YP_008983338.1
SCV20265_0891	938801	T/S;CP;GM;IMP;ETP;MER;P/T;TO;LVX	2,24131E-27	YP_008979899.1
SCV20265_1756	1846678	T/S;CP;GM;IMP;ETP;MER;P/T;TO;LVX	2,14003E-18	YP_008980764.1
SCV20265_1113	1169401	T/S;CP;GM;IMP;ETP;MER;TO;LVX	2,43872E-22	YP_008980121.1
SCV20265_1895	1984529	T/S;CP;LVX;GM;IMP;P/T;CPE;TO	1,12601E-29	YP_008980903.1
SCV20265_4827	5100757	T/S;CP;GM;IMP;MER;P/T;TO;LVX	2,58406E-24	YP_008983827.1
SCV20265_1467	1537406	T/S;CP;LVX;GM;P/T;TO;CPE	1,53793E-41	YP_008980475.1
SCV20265_2654	2754829	T/S;CP;LVX;GM;ETP;TO;CPE	1,05377E-35	YP_008981662.1
SCV20265_6289	6702956	T/S;CP;LVX;GM;P/T;TO;CPE	1,19545E-34	YP_008985285.1
SCV20265_3626	3881624	T/S;CP;LVX;GM;P/T;TO;CPE	2,22675E-34	YP_008982632.1
SCV20265_1050	1099519	T/S;CP;LVX;GM;ETP;TO;CPE	2,28786E-34	YP_008980058.1
SCV20265_4159	4419978	T/S;CP;LVX;GM;P/T;TO;CPE	1,13138E-33	YP_008983163.1
SCV20265_4562	4832599	MER;ETP	2,71389E-33	YP_008983564.1

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 IMP and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_0891, SCV20265_1756, SCV20265_1113, SCV20265_1895, SCV20265_4827 is detected, or a mutation in at least one of the positions of 1979239, 5987559, 938801, 1846678, 1169401, 1984529, 5100757.

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 MER and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_4334, SCV20265_0891, SCV20265_1756, SCV20265_1113, SCV20265_4827, SCV20265_4562 is detected, or a mutation in at least one of

the positions of 1979239, 5987559, 4604211, 938801, 1846678, 1169401, 5100757, 4832599.

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 ETP and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_4334, SCV20265_0891, SCV20265_1756, SCV20265_1113, SCV20265_1467, SCV20265_2654, SCV20265_1050, SCV20265_4562 is detected, or a mutation in at least one of the positions of 1979239, 5987559, 4604211, 938801, 1846678, 1169401, 1537406, 2754829, 1099519, 4832599.

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 P/T and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_4334, SCV20265_0891, SCV20265_1756, SCV20265_1895, SCV20265_4827, SCV20265_6289, SCV20265_3626, SCV20265_4159 is detected, or a mutation in at least one of the positions of 1979239, 5987559, 4604211, 938801, 1846678, 1984529, 5100757, 6702956, 3881624, 4419978.

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 CPE and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_4334, SCV20265_1895, SCV20265_1467, SCV20265_2654, SCV20265_6289, SCV20265_3626, SCV20265_1050, SCV20265_4159 is detected, or a mutation in at least one of the positions of 1979239,

5987559, 4604211, 1984529, 1537406, 2754829, 6702956, 3881624, 1099519, 4419978.

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 AZT and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_4334 is detected, or a mutation in at least one of the positions of 1979239, 5987559, 4604211.

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 CFT, CAX and CAZ and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625 is detected, or a mutation in at least one of the positions of 1979239, 5987559.

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 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.

Table 7: List for quinolone antibiotics

gene name	**POS**	**antibiotic**	**p-value (FDR)**	**genbank protein accession number**
SCV20265_1892	1979239	T/S;CP;CFT;LVX;GM;IMP; ETP;MER;CAX;AZT;P/T;CPE; CAZ;TO	1,5814E-141	YP_008980900.1
SCV20265_5625	5987559	T/S;CP;CFT;LVX;GM;IMP; ETP;MER;CAX;AZT;P/T;CPE; CAZ;TO	1,2153E-121	YP_008984625.1

SCV20265_1467	1537406	T/S;CP;LVX;GM;P/T;TO;CPE	1,53793E-41	YP_008980475.1
SCV20265_5607	5965080	T/S;CP;GM;P/T;LVX;TO	4,52474E-39	YP_008984607.1
SCV20265_3294	3513162	T/S;LVX;CP;TO;GM	1,34708E-37	YP_008982302.1
SCV20265_1879	1967346	T/S;CP;GM;P/T;LVX;TO	1,53005E-37	YP_008980887.1
SCV20265_5242	5569783	T/S;CP;LVX;GM;TO;CPE	2,19789E-37	YP_008984242.1
SCV20265_2224	2350860	T/S;CP;GM;P/T;LVX;TO	2,41127E-37	YP_008981232.1
SCV20265_2224	2350862	T/S;CP;GM;P/T;LVX;TO	2,41127E-37	YP_008981232.1
SCV20265_0530	562872	T/S;CP;LVX;GM;TO;CPE	3,4301E-37	YP_008979538.1
SCV20265_3289	3507580	T/S;CP;LVX;GM;TO;CPE	6,35793E-37	YP_008982297.1
SCV20265_1858	1947689	T/S;CP;LVX;GM;TO;CPE	6,77112E-37	YP_008980866.1
SCV20265_2193	2316386	T/S;CP;LVX;GM;TO;CPE	6,77112E-37	YP_008981201.1
SCV20265_6274	6685845	T/S;CP;LVX;GM;TO;CPE	7,04031E-37	YP_008985270.1
SCV20265_2958	3142437	T/S;CP;LVX;GM;TO;CPE	9,40137E-37	YP_008981966.1
SCV20265_3248	3468647	T/S;CP;LVX;GM;TO;CPE	1,00069E-36	YP_008982256.1

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 at least one of CP and LVX and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248 is detected, or a mutation in at least one of the positions of 1979239, 5987559, 1537406, 5965080, 3513162, 1967346, 5569783, 2350860, 2350862, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647.

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.

Table 8: List of aminoglycoside antibiotics

gene name	POS	antibiotic	p-value (FDR)	genbank protein accession number
SCV20265_1892	1979239	T/S;CP;CFT;LVX;GM;IMP; ETP;MER;CAX;AZT;P/T;CPE; CAZ;TO	1,5814E-141	YP_008980900.1
SCV20265_5625	5987559	T/S;CP;CFT;LVX;GM;IMP; ETP;MER;CAX;AZT;P/T;CPE; CAZ;TO	1,2153E-121	YP_008984625.1
SCV20265_1467	1537406	T/S;CP;LVX;GM;P/T;TO;CPE	1,53793E-41	YP_008980475.1
SCV20265_5607	5965080	T/S;CP;GM;P/T;LVX;TO	4,52474E-39	YP_008984607.1
SCV20265_3294	3513162	T/S;LVX;CP;TO;GM	1,34708E-37	YP_008982302.1
SCV20265_1879	1967346	T/S;CP;GM;P/T;LVX;TO	1,53005E-37	YP_008980887.1
SCV20265_5242	5569783	T/S;CP;LVX;GM;TO;CPE	2,19789E-37	YP_008984242.1
SCV20265_2224	2350860	T/S;CP;GM;P/T;LVX;TO	2,41127E-37	YP_008981232.1
SCV20265_2224	2350862	T/S;CP;GM;P/T;LVX;TO	2,41127E-37	YP_008981232.1
SCV20265_0530	562872	T/S;CP;LVX;GM;TO;CPE	3,4301E-37	YP_008979538.1
SCV20265_3289	3507580	T/S;CP;LVX;GM;TO;CPE	6,35793E-37	YP_008982297.1
SCV20265_1858	1947689	T/S;CP;LVX;GM;TO;CPE	6,77112E-37	YP_008980866.1
SCV20265_2193	2316386	T/S;CP;LVX;GM;TO;CPE	6,77112E-37	YP_008981201.1
SCV20265_6274	6685845	T/S;CP;LVX;GM;TO;CPE	7,04031E-37	YP_008985270.1
SCV20265_2958	3142437	T/S;CP;LVX;GM;TO;CPE	9,40137E-37	YP_008981966.1
SCV20265_3248	3468647	T/S;CP;LVX;GM;TO;CPE	1,00069E-36	YP_008982256.1

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 at least one of GM and TO and a mutation in at least one of the genes of SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248 is detected, or a mutation in at least one of the positions of 1979239, 5987559, 1537406, 5965080, 3513162, 1967346, 5569783, 2350860, 2350862, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647.

According to certain embodiments of the method of the seventeenth and/or eighteenth aspect of the present invention, the antibiotic is T/S 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 12.

Table 9: List of others antibiotics ((benzene derived)/sulfonamide)

gene name	POS	antibiotic	p-value (FDR)	genbank protein accession number
SCV20265_1892	1979239	T/S;CP;CFT;LVX;GM;IMP;ETP;MER;CAX;AZT;P/T;CPE;CAZ;TO	1,5814E-141	YP_008980900.1
SCV20265_5625	5987559	T/S;CP;CFT;LVX;GM;IMP;ETP;MER;CAX;AZT;P/T;CPE;CAZ;TO	1,2153E-121	YP_008984625.1
SCV20265_1467	1537406	T/S;CP;LVX;GM;P/T;TO;CPE	1,53793E-41	YP_008980475.1
SCV20265_5607	5965080	T/S;CP;GM;P/T;LVX;TO	4,52474E-39	YP_008984607.1
SCV20265_3294	3513162	T/S;LVX;CP;TO;GM	1,34708E-37	YP_008982302.1
SCV20265_1879	1967346	T/S;CP;GM;P/T;LVX;TO	1,53005E-37	YP_008980887.1
SCV20265_5242	5569783	T/S;CP;LVX;GM;TO;CPE	2,19789E-37	YP_008984242.1
SCV20265_2224	2350860	T/S;CP;GM;P/T;LVX;TO	2,41127E-37	YP_008981232.1
SCV20265_2224	2350862	T/S;CP;GM;P/T;LVX;TO	2,41127E-37	YP_008981232.1
SCV20265_0530	562872	T/S;CP;LVX;GM;TO;CPE	3,4301E-37	YP_008979538.1
SCV20265_3289	3507580	T/S;CP;LVX;GM;TO;CPE	6,35793E-37	YP_008982297.1
SCV20265_1858	1947689	T/S;CP;LVX;GM;TO;CPE	6,77112E-37	YP_008980866.1
SCV20265_2193	2316386	T/S;CP;LVX;GM;TO;CPE	6,77112E-37	YP_008981201.1
SCV20265_6274	6685845	T/S;CP;LVX;GM;TO;CPE	7,04031E-37	YP_008985270.1
SCV20265_0041	46892	T/S;CP;ETP;TO;GM	6,66003E-27	YP_008979049.1
SCV20265_0017	19938	T/S;CP;TO;GM	5,91498E-19	YP_008980900.1

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

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607,

SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas 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 Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721,

SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, 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 Pseudomonas 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 Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805,

SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, 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 Pseudomonas 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 Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721,

SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, 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 Pseudomonas 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 Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 Pseudomonas 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 Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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, 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 Pseudomonas infection; and

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

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 Pseudomonas species potentially resistant to antimicrobial drug treatment, which can also be described as method of

determining an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas infection of a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas 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 Pseudomonas infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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, 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 Pseudomonas 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 1104 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 Pseudomonas isolates was measured.

The detailed procedure is given in the following:

Bacterial Strains

The inventors selected 1104 Pseudomonas strains, particularly Pseudomonas aeruginosa, 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 1104 Pseudomonas samples were mapped against the Pseudomonas reference

(NC_023149) 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 Pseudomonas 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 Pseudomonas samples were considered. Pseudomonas 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 Pseudomonas 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_023149.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 Pseudomonas 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:
Pseudomonas 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_0123149 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 1104 different clinical isolates of Pseudomonas species 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 1104 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 Pseudomonas 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_023149 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 1104 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.

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.

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

In Tables 3 - 9 the columns are designated as follows:
Gene name: affected gene;
POS: genomic position of the SNP / variant in the Pseudomonas 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.

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
1979239	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,5814E-141	SCV20265_1892	YP_008980900.1
5987559	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,2153E-121	SCV20265_5625	YP_008984625.1
1537406	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,53793E-41	SCV20265_1467	YP_008980475.1
5965080	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4	4,52474E-39	SCV20265_5607	YP_008984607.1
3513162	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	1,34708E-37	SCV20265_3294	YP_008982302.1
1967346	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4	1,53005E-37	SCV20265_1879	YP_008980887.1
5569783	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,19789E-37	SCV20265_5242	YP_008984242.1
2350860	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4	2,41127E-37	SCV20265_2224	YP_008981232.1
562872	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	3,4301E-37	SCV20265_0530	YP_008979538.1
3507580	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	6,35793E-37	SCV20265_3289	YP_008982297.1
1947689	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	6,77112E-37	SCV20265_1858	YP_008980866.1
2316386	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	6,77112E-37	SCV20265_2193	YP_008981201.1
6685845	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	7,04031E-37	SCV20265_6274	YP_008985270.1
3142437	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	9,40137E-37	SCV20265_2958	YP_008981966.1
3468647	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,00069E-36	SCV20265_3248	YP_008982256.1
1194383	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	1,4119E-36	SCV20265_1132	YP_008980140.1
1521674	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,1786E-36	SCV20265_1451	YP_008980459.1

6520799	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,1786E-36	SCV20265_6120	YP_008985116.1
5124971	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,77592E-36	SCV20265_4839	YP_008983839.1
2317909	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	3,14019E-36	SCV20265_2195	YP_008981203.1
1009933	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	6,88119E-36	SCV20265_0968	YP_008979976.1
2567532	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	6,88119E-36	SCV20265_2464	YP_008981472.1
2611669	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	8,15723E-36	SCV20265_2518	YP_008981526.1
2754829	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,05377E-35	SCV20265_2654	YP_008981662.1
3301233	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,16268E-35	SCV20265_3101	YP_008982109.1
4166792	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	1,22608E-35	SCV20265_3909	YP_008982913.1
2709322	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	1,42715E-35	SCV20265_2610	YP_008981618.1
1899865	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,06037E-35	SCV20265_1805	YP_008980813.1
4712288	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	3,00478E-35	SCV20265_4445	YP_008983447.1
3019764	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4	4,72183E-35	SCV20265_2883	YP_008981891.1
3068671	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	4,72183E-35	SCV20265_2916	YP_008981924.1
1805165	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	6,32018E-35	SCV20265_1721	YP_008980729.1
3299685	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	7,83399E-35	SCV20265_3099	YP_008982107.1
1821163	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	8,13473E-35	SCV20265_1735	YP_008980743.1
6702956	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,19545E-34	SCV20265_6289	YP_008985285.1

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3160788	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,22139E-34	SCV20265_2974	YP_008981982.1
2515627	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	1,4692E-34	SCV20265_2404	YP_008981412.1
6535290	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,91732E-34	SCV20265_6135	YP_008985131.1
3881624	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,22675E-34	SCV20265_3626	YP_008982632.1
1099519	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,28786E-34	SCV20265_1050	YP_008980058.1
208902	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	2,57554E-34	SCV20265_0188	YP_008979196.1
5662982	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	2,57554E-34	SCV20265_5329	YP_008984329.1
2903129	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	3,70304E-34	SCV20265_2792	YP_008981800.1
1701758	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	4,18431E-34	SCV20265_1617	YP_008980625.1
2363393	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	4,29029E-34	SCV20265_2236	YP_008981244.1
525701	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	5,23384E-34	SCV20265_0491	YP_008979499.1
2530203	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	8,9244E-34	SCV20265_2422	YP_008981430.1
5806684	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	8,9244E-34	SCV20265_5463	YP_008984463.1
5954547	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	8,9244E-34	SCV20265_5597	YP_008984597.1
261978	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3	9,16531E-34	SCV20265_0241	YP_008979249.1
2350862	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4	2,41127E-37	SCV20265_2224	YP_008981232.1
3507601	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,78947E-35	SCV20265_3289	YP_008982297.1
3507667	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	1,87651E-35	SCV20265_3289	YP_008982297.1

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6519971	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4	5,23384E-34	SCV20265_6120	YP_008985116.1

*: fluoroquinolone

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

POS	drug	#drugs	drug class	#drug classes
1979239	T/S;CP;CFT;LVX;GM;IMP;ETP;MER;CAX;AZT;P/T;CPE;CAZ;TO	14	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
5987559	T/S;CP;CFT;LVX;GM;IMP;ETP;MER;CAX;AZT;P/T;CPE;CAZ;TO	14	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
1537406	T/S;CP;LVX;GM;P/T;TO;CPE	7	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
5965080	T/S;CP;GM;P/T;LVX;TO	6	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4
3513162	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3
1967346	T/S;CP;GM;P/T;LVX;TO	6	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4
5569783	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
2350860	T/S;CP;GM;P/T;LVX;TO	6	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4
562872	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
3507580	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
1947689	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
2316386	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
6685845	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4

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3142437	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
3468647	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
1194383	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3
1521674	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
6520799	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
5124971	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
2317909	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
1009933	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
2567532	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
2611669	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
2754829	T/S;CP;LVX;GM;ETP;TO;CPE	7	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
3301233	T/S;CP;GM;ETP;LVX;TO	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
4166792	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3
2709322	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3
1899865	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
4712288	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
3019764	T/S;CP;GM;P/T;LVX;TO	6	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4
3068671	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3

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1805165	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
3299685	T/S;CP;GM;ETP;LVX;TO	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
1821163	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
6702956	T/S;CP;LVX;GM;P/T;TO;CPE	7	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	
3160788	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
2515627	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	4
6535290	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	3
3881624	T/S;CP;LVX;GM;P/T;TO;CPE	7	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
1099519	T/S;CP;LVX;GM;ETP;TO;CPE	7	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
208902	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	4
5662982	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	3
2903129	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
1701758	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	4
2363393	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	3
525701	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	4
2530203	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3
5806684	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3
5954547	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3

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261978	T/S;LVX;CP;TO;GM	5	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*	3
2350862	T/S;CP;GM;P/T;LVX;TO	6	other (benzene derived)/sulfonamide; aminoglycoside;Lactams;quinolone*	4
3507601	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
3507667	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4
6519971	T/S;CP;LVX;GM;TO;CPE	6	other (benzene derived)/sulfonamide; aminoglycoside;quinolone*;Lactams	4

*: fluoroquinolone

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**
1979239	CP	9	2	2	0	1
5987559	CP	9	2	2	0	1
1537406	T/S	2	2	2	0	1
5965080	T/S	1	2	2	0	1
3513162	T/S	0	2	2	0	1
1967346	T/S	1	2	2	0	1
5569783	T/S	1	2	2	0	1
2350860	T/S	1	2	2	0	1
562872	T/S	1	2	2	0	1
3507580	T/S	1	2	2	0	1
1947689	T/S	1	2	2	0	1
2316386	T/S	1	2	2	0	1
6685845	T/S	1	2	2	0	1
3142437	T/S	1	2	2	0	1

3468647	T/S	1	2	2	0	1
1194383	T/S	0	2	2	0	1
1521674	T/S	1	2	2	0	1
6520799	T/S	1	2	2	0	1
5124971	T/S	1	2	2	0	1
2317909	T/S	1	2	2	0	1
1009933	T/S	1	2	2	0	1
2567532	T/S	1	2	2	0	1
2611669	T/S	1	2	2	0	1
2754829	T/S	2	2	2	0	1
3301233	T/S	1	2	2	0	1
4166792	T/S	0	2	2	0	1
2709322	T/S	0	2	2	0	1
1899865	T/S	1	2	2	0	1
4712288	T/S	1	2	2	0	1
3019764	T/S	1	2	2	0	1
3068671	T/S	0	2	2	0	1
1805165	T/S	1	2	2	0	1
3299685	T/S	1	2	2	0	1
1821163	T/S	1	2	2	0	1
6702956	T/S	2	2	2	0	1
3160788	T/S	1	2	2	0	1
2515627	T/S	0	2	2	0	1
6535290	T/S	1	2	2	0	1
3881624	T/S	2	2	2	0	1
1099519	T/S	2	2	2	0	1
208902	T/S	0	2	2	0	1
5662982	T/S	1	2	2	0	1

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2903129	T/S	1	2	2	0	1
1701758	T/S	0	2	2	0	1
2363393	T/S	1	2	2	0	1
525701	T/S	0	2	2	0	1
2530203	T/S	0	2	2	0	1
5806684	T/S	0	2	2	0	1
5954547	T/S	0	2	2	0	1
261978	T/S	0	2	2	0	1
2350862	T/S	1	2	2	0	1
3507601	T/S	1	2	2	0	1
3507667	T/S	1	2	2	0	1
6519971	T/S	1	2	2	0	1

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

POS	**p-value**	**gene name**	**genbank protein accession number**
1979239	1,5814E-141	SCV20265_1892	YP_008980900.1
5987559	1,2153E-121	SCV20265_5625	YP_008984625.1
1537406	1,53793E-41	SCV20265_1467	YP_008980475.1
5965080	4,52474E-39	SCV20265_5607	YP_008984607.1
3513162	1,34708E-37	SCV20265_3294	YP_008982302.1
1967346	1,53005E-37	SCV20265_1879	YP_008980887.1
5569783	2,19789E-37	SCV20265_5242	YP_008984242.1
2350860	2,41127E-37	SCV20265_2224	YP_008981232.1
562872	3,4301E-37	SCV20265_0530	YP_008979538.1
3507580	6,35793E-37	SCV20265_3289	YP_008982297.1
1947689	6,77112E-37	SCV20265_1858	YP_008980866.1
2316386	6,77112E-37	SCV20265_2193	YP_008981201.1

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6685845	7,04031E-37	SCV20265_6274	YP_008985270.1
3142437	9,40137E-37	SCV20265_2958	YP_008981966.1
3468647	1,00069E-36	SCV20265_3248	YP_008982256.1
1194383	1,4119E-36	SCV20265_1132	YP_008980140.1
1521674	2,1786E-36	SCV20265_1451	YP_008980459.1
6520799	2,1786E-36	SCV20265_6120	YP_008985116.1
5124971	2,77592E-36	SCV20265_4839	YP_008983839.1
2317909	3,14019E-36	SCV20265_2195	YP_008981203.1
1009933	6,88119E-36	SCV20265_0968	YP_008979976.1
2567532	6,88119E-36	SCV20265_2464	YP_008981472.1
2611669	8,15723E-36	SCV20265_2518	YP_008981526.1
2754829	1,05377E-35	SCV20265_2654	YP_008981662.1
3301233	1,16268E-35	SCV20265_3101	YP_008982109.1
4166792	1,22608E-35	SCV20265_3909	YP_008982913.1
2709322	1,42715E-35	SCV20265_2610	YP_008981618.1
1899865	2,06037E-35	SCV20265_1805	YP_008980813.1
4712288	3,00478E-35	SCV20265_4445	YP_008983447.1
3019764	4,72183E-35	SCV20265_2883	YP_008981891.1
3068671	4,72183E-35	SCV20265_2916	YP_008981924.1
1805165	6,32018E-35	SCV20265_1721	YP_008980729.1
3299685	7,83399E-35	SCV20265_3099	YP_008982107.1
1821163	8,13473E-35	SCV20265_1735	YP_008980743.1
6702956	1,19545E-34	SCV20265_6289	YP_008985285.1
3160788	1,22139E-34	SCV20265_2974	YP_008981982.1
2515627	1,4692E-34	SCV20265_2404	YP_008981412.1
6535290	1,91732E-34	SCV20265_6135	YP_008985131.1
3881624	2,22675E-34	SCV20265_3626	YP_008982632.1
1099519	2,28786E-34	SCV20265_1050	YP_008980058.1

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208902	2,57554E-34	SCV20265_0188	YP_008979196.1
5662982	2,57554E-34	SCV20265_5329	YP_008984329.1
2903129	3,70304E-34	SCV20265_2792	YP_008981800.1
1701758	4,18431E-34	SCV20265_1617	YP_008980625.1
2363393	4,29029E-34	SCV20265_2236	YP_008981244.1
525701	5,23384E-34	SCV20265_0491	YP_008979499.1
2530203	8,9244E-34	SCV20265_2422	YP_008981430.1
5806684	8,9244E-34	SCV20265_5463	YP_008984463.1
5954547	8,9244E-34	SCV20265_5597	YP_008984597.1
261978	9,16531E-34	SCV20265_0241	YP_008979249.1
2350862	2,41127E-37	SCV20265_2224	YP_008981232.1
3507601	1,78947E-35	SCV20265_3289	YP_008982297.1
3507667	1,87651E-35	SCV20265_3289	YP_008982297.1
6519971	5,23384E-34	SCV20265_6120	YP_008985116.1

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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 6,734 different correlations between genetic sites and anti-microbial agents were detected (p-value < 10^{-10}).
- The biggest part of these were point mutations (i.e. single base exchanges)
- The highest significances (10^{-141} and 10^{-121}) were reached for non-synonymous codings in YP_008980900.1 and YP_008984625.1, respectively, particular in positions 1979239 and/or 5987559, respectively, with regard to reference genome NC_023149 as annotated at the NCBI; the mutation in position 1979239 with regard to reference genome NC_023149 as annotated at the NCBI is a non-synonymous coding, particularly a codon change aCc/aTc;aCc/aAc, and the mutation in position 5987559 with regard to reference genome NC_023149 as annotated at the NCBI is a non-synonymous coding, particularly a codon change tCg/tTg;tCg/tGg

- 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
- Folate synthesis inhibitors

- 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 2,757 different 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 defined 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 Pseudomonas 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 Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, wherein the presence of said at least two mutations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant Pseudomonas strain in said patient.

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

a) obtaining or providing a sample containing or suspected of containing at least one Pseudomonas 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 SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and SCV20265_0241, 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 Pseudomonas infection.

3. The method of one or more of the preceding claims, wherein at least a mutation in SCV20265_1892 and/or SCV20265_5625, particularly in positions 1979239 and/or 5987559, respectively, with regard to reference genome NC_023149 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 Pseudomonas 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: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_5329, SCV20265_2792, and/or SCV20265_2236; and/or
the antimicrobial, e.g. antibiotic, drug is selected from quinolone antibiotics, e.g. fluoroquinolone antibiotics, and the presence of a mutation in the following genes is

determined: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and/or SCV20265_0241; and/or

the antimicrobial, e.g. antibiotic, drug is selected from aminoglycoside antibiotics, and the presence of a mutation in the following genes is determined: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050,

SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and/or SCV20265_0241; and/or

the antimicrobial, e.g. antibiotic, drug is selected from benzene derived/sulfonamide antibiotics, and the presence of a mutation in the following genes is determined: SCV20265_1892, SCV20265_5625, SCV20265_1467, SCV20265_5607, SCV20265_3294, SCV20265_1879, SCV20265_5242, SCV20265_2224, SCV20265_0530, SCV20265_3289, SCV20265_1858, SCV20265_2193, SCV20265_6274, SCV20265_2958, SCV20265_3248, SCV20265_1132, SCV20265_1451, SCV20265_6120, SCV20265_4839, SCV20265_2195, SCV20265_0968, SCV20265_2464, SCV20265_2518, SCV20265_2654, SCV20265_3101, SCV20265_3909, SCV20265_2610, SCV20265_1805, SCV20265_4445, SCV20265_2883, SCV20265_2916, SCV20265_1721, SCV20265_3099, SCV20265_1735, SCV20265_6289, SCV20265_2974, SCV20265_2404, SCV20265_6135, SCV20265_3626, SCV20265_1050, SCV20265_0188, SCV20265_5329, SCV20265_2792, SCV20265_1617, SCV20265_2236, SCV20265_0491, SCV20265_2422, SCV20265_5463, SCV20265_5597, and/or SCV20265_0241.

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 T/S, CP, LVX, GM, and/or TO and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5965080, 3513162, 1967346, 5569783, 2350860, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1194383, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 3301233, 4166792, 2709322, 1899865, 4712288, 3019764, 3068671, 1805165, 3299685, 1821163, 6702956, 3160788, 2515627, 6535290, 3881624, 1099519, 208902, 5662982, 2903129, 1701758, 2363393, 525701, 2530203, 5806684, 5954547, 261978, 2350862, 3507601, 3507667, 6519971; and/or
wherein the antibiotic drug is CPE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5569783, 562872, 3507580, 1947689, 2316386, 6685845, 3142437, 3468647, 1521674, 6520799, 5124971, 2317909, 1009933, 2567532, 2611669, 2754829, 1899865, 4712288, 1805165, 1821163, 6702956, 3160788, 6535290, 3881624, 1099519, 5662982, 2903129, 2363393, 3507601, 3507667, 6519971; and/or
wherein the antibiotic drug is P/T and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 1537406, 5965080, 1967346, 2350860, 2754829, 3301233, 3019764, 6702956, 3881624; and/or

wherein the antibiotic drug is ETP and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559, 2754829, 3301233, 3299685, 1099519, 2350862; and/or
wherein the antibiotic drug is CFT, IMP, MER, CAX, AZT, and/or CAZ and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_023149: 1979239, 5987559.

8. The method of any one of claims 1 to 7, wherein the resistance of a bacterial microorganism belonging to the species Pseudomonas 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 Pseudomonas 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 genera-

tion sequencing or high throughput sequencing method, preferably wherein a partial or entire genome sequence of the bacterial organism of Pseudomonas 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 Pseudomonas species, comprising:
obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Pseudomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Pseudomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Pseudomonas, 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 Pseudomonas associated with antimicrobial drug, e.g. antibiotic, resistance.

13. A diagnostic method of determining an infection of a patient with Pseudomonas 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 Pseudomonas 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 Pseudomonas 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 Pseudomonas strain in said patient.

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

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

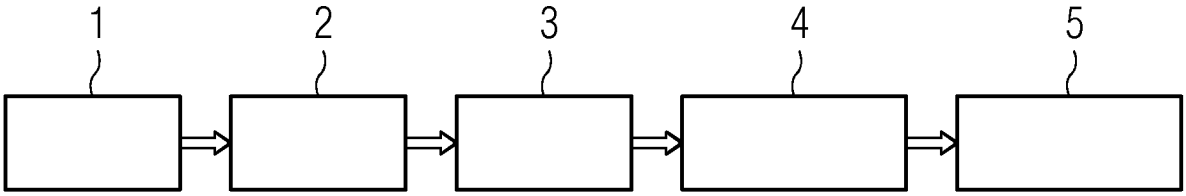
c) identifying said at least one or more antimicrobial drugs; and

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

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

obtaining or providing a first data set of gene sequences of a clinical isolate of Pseudomonas species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of a plurality of clinical isolates of Pseudomonas species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Pseudomonas, 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 Pseudomonas 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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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/066773

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/066773

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.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/066773

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MICHAL WOZNIAK ET AL: "An approach to identifying drug resistance associated mutations in bacterial strains", BMC GENOMICS, BIOMED CENTRAL LTD, LONDON, UK, vol. 13, no. Suppl 7, 7 December 2012 (2012-12-07), page S23, XP021127984, ISSN: 1471-2164, DOI: 10.1186/1471-2164-13-S7-S23 cited in the application whole document and s24-s25 "materials and methods", p. S 35 " conclusion " and tab. 2, 3 and Fig. 2	1-11

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[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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2015	16/12/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lapopin, Laurence

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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.
A	N. STOESSER ET AL: "Predicting antimicrobial susceptibilities for Escherichia coli and Klebsiella pneumoniae isolates using whole genomic sequence data", JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol. 4, 30 May 2013 (2013-05-30), XP055157002, ISSN: 0305-7453, DOI: 10.1093/jac/dkt180 cited in the application the whole document	1-11
A	CLAIRE CHEWAPREECHA ET AL: "Comprehensive Identification of Single Nucleotide Polymorphisms Associated with Beta-lactam Resistance within Pneumococcal Mosaic Genes", PLOS GENETICS, vol. 10, no. 8, 7 August 2014 (2014-08-07) , page e1004547, XP055204428, DOI: 10.1371/journal.pgen.1004547 cited in the application the whole document	1-11
X	WO 2006/097232 A2 (EPPENDORF AG [DE]; WEILE JAN [DE]; SUSA MILORAD [DE]; KNABBE CORNELIUS) 21 September 2006 (2006-09-21) whole document and p. 5, l. 1-5, tab. 1, and 3-7, and claims 20	1-11
X	WO 2012/027302 A2 (UNIV CALIFORNIA [US]; TESSARAE INC [US]; LYNCH SUSAN V [US]; BRODIE EO) 1 March 2012 (2012-03-01) whole document and [0011], [0012], [0023], [0030], [00116]-[00119] and claim 3	1-11
A	US 2012/071336 A1 (BALASHOV SERGEY [US] ET AL) 22 March 2012 (2012-03-22) claims 1-16	1-11
A	WO 2012/106432 A2 (BAYLOR COLLEGE MEDICINE [US]; ZECHIEDRICH E LYNN [US]; SWICK MICHELLE) 9 August 2012 (2012-08-09) claims 1-9	1-11

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/066773

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006097232 A2	21-09-2006	CA 2601047 A1	21-09-2006
		EP 1859052 A2	28-11-2007
		US 2006211002 A1	21-09-2006
		US 2009215638 A1	27-08-2009
		WO 2006097232 A2	21-09-2006
WO 2012027302 A2	01-03-2012	US 2013157876 A1	20-06-2013
		WO 2012027302 A2	01-03-2012
US 2012071336 A1	22-03-2012	NONE	
WO 2012106432 A2	09-08-2012	US 2014030712 A1	30-01-2014
		WO 2012106432 A2	09-08-2012

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)

concerns a method for diagnosing an antimicrobial resistant Pseudomonas and a method for detecting a treatment by determining the presence of at least one mutation in at least two genes marker, wherein the first gene marker is SCV20265_1892 in combination with at least one other genes listed in claim 1.

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

concern a method for diagnosing an antimicrobial resistant Pseudomonas and a method for delecting a treatment by determining the presence of at least one mutation in at least two genes marker, wherein the first gene marker is SCV20265_5625 to SCV20265_0241, respectively in combination with at least one other of the genes listed in claim 1.

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51. claims: 12-16

a method for determining genetic sites associated with antimicrobial resistance in Pseudomonas (resistance profile) by sequence comparison analysis.
