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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**; Gebbertstraße 37, 91052 Erlangen (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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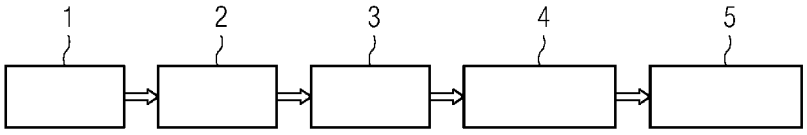
(54) Title: GENETIC TESTING FOR PREDICTING RESISTANCE OF SERRATIA SPECIES AGAINST ANTIMICROBIAL AGENTS



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

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Genetic testing for predicting resistance of Serratia species against antimicrobial agents

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

Serratia is a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria of the Enterobacteriaceae family. Currently 14 species of Serratia are recognized within the genus, eight of which are associated with human infection. Of all Serratia species, Serratia marcescens is the most common clinical isolate and the most important human pathogen.

Serratia marcescens is an opportunistic pathogen whose clinical significance has been appreciated only in the last four decades. While S. marcescens is a rare cause of community-acquired infections, it has emerged as an important nosocomial healthcare-associated pathogen and a frequent source of outbreaks of hospital infection, in both adult and pediatric patients. Results from a recent surveillance program in the US and Europe, indicate that Serratia spp. accounts for an average of 6.5% of all Gram negative infection in Intensive Care Units (ranked 5th amongst Gram negative organisms in ICU) and an average of 3.5% in non-ICU patients. Currently Serratia is the seventh most common cause of pneumonia with an incidence of 4.1% in the US, 3.2% in Europe and 2.4% in Latin America, and the tenth most common cause of bloodstream infection with an incidence of 2.0% amongst hospitalized patients.

Serratia marcescens is rarely associated with primary invasive infection, it operates as a true opportunist producing infection whenever it gains access to a suitably compromised host. Patients most at risk include those with debilitating or immunocompromising disorders, those treated with broad-spectrum antibiotics and patients in ICU who are subjected to invasive instrumentation. The indwelling urinary catheter is a major risk factor for infection. The risk of a catheterized patient becoming infected with S. marcescens has been directly related to the proximity of other catheterized patients colonized or infected with the organism. The respiratory tract is also recognized as a major portal of entry with S. marcescens being isolated from the respiratory tract of up to

80% of post-operative patients developing S. marcescens bacteremia. Not surprisingly, common infections include urinary tract infection in patients with indwelling catheters, respiratory tract infection in intubated patients and bloodstream infection in post-surgical patients, especially in those with intravenous catheters.

In the last two decades Enterobacteriaceae have demonstrated an exceptional ability to acquire, transfer, and modify the expression of multiple antimicrobial resistance genes. As a typical member of the Enterobacteriaceae family Serratia ssp. demonstrates a propensity to express antimicrobial resistance and the emergence and spread of multiresistant strains is becoming a very serious problem over the last decades.

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

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

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

Table 1: List of genes

actP	SMWW4_v1c03050	amiD	SMWW4_v1c38520
selB	SMWW4_v1c13480	bglX	SMWW4_v1c14040
SMWW4_v1c13470	SMWW4_v1c38510	SMWW4_v1c07960	SMWW4_v1c19810
folX	SMWW4_v1c00800	SMWW4_v1c13910	SMWW4_v1c09360
ybiO	SMWW4_v1c25040	znuB	nrdH
lysR	SMWW4_v1c24620	SMWW4_v1c24800	SMWW4_v1c20760
rfaC	SMWW4_v1c21930	SMWW4_v1c12350	galT
alsK	SMWW4_v1c24810	glrK	rihB
yhiN	alx	SMWW4_v1c44490	cnu
SMWW4_v1c30050	vasD	impL	SMWW4_v1c16540

SMWW4_v1c13350	yeaN	SMWW4_v1c40850	kdpA
dppB	ydaN	cysK	yceA
yhjK	SMWW4_v1c25770		

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 2: List of genes

actP	SMWW4_v1c03050	amiD	SMWW4_v1c38520
selB	SMWW4_v1c13480	bglX	SMWW4_v1c14040
SMWW4_v1c13470	SMWW4_v1c38510	SMWW4_v1c07960	SMWW4_v1c19810
folX	SMWW4_v1c00800	SMWW4_v1c13910	SMWW4_v1c09360
ybiO	SMWW4_v1c25040	znuB	nrdH
lysR	SMWW4_v1c24620	SMWW4_v1c24800	SMWW4_v1c20760
rfaC	SMWW4_v1c21930	SMWW4_v1c12350	galT
alsK	SMWW4_v1c24810	glrK	rihB
yhiN	alx	SMWW4_v1c44490	cnu
SMWW4_v1c30050	vasD	impL	SMWW4_v1c16540
SMWW4_v1c13350	yeaN	SMWW4_v1c40850	kdpA
dppB	ydaN	cysK	yceA
yhjK	SMWW4_v1c25770		

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

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 mu-

tations 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 Serratia 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 Serratia species, comprising:

obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Serratia species;

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

aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Serratia, 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 Serratia 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 Serratia 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 Serratia species potentially resistant to antimicrobial drug treatment, which can, like in the first aspect, also be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Serratia 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 Serratia 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 Serratia 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 Serratia 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 Serratia strain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Serratia infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Serratia 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 Serratia 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 Serratia infection.

A seventh aspect of the present invention relates to a method of acquiring, respectively determining, an antimicrobial drug, e.g. antibiotic, resistance profile for a bacterial microorganism of Serratia species, comprising:

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

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

aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Serratia, 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 Serratia of the first data set associated with antimicrobial drug, e.g. antibiotic, resistance.

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

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

Figures

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

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

Detailed description of the present invention

Definitions

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

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

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

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

The term "mutation" relates to a variation in the sequence as compared to a reference sequence. Such a reference sequence can be a sequence determined in a predominant wild type organism or a reference organism, e.g. a defined and known bacterial strain or substrain. A mutation is for example a deletion of one or multiple nucleotides, an insertion of one or multiple nucleotides, or substitution of one or multiple nucleotides, duplication of one or a sequence of multiple nucleotides, translocation of one or a sequence of multiple nu-

cleotides, 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 Serratia species, particularly Serratia marcescens.

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

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

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

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

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

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

According to certain embodiments, mutations that were found using alignments can also be compared or matched with 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 Serratia species potentially resistant to antimicrobial drug treatment, which can also be described as method of determining an antimicrobial drug, e.g. antibiotic, resistant Serratia infection of a patient, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510,

SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, wherein the presence of said at least two mutations is indicative of an infection with an antimicrobial, e.g. antibiotic, resistant Serratia 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^{-40}, indicating the high significance of the values (n= 438; α = 0.05). Details regarding Tables 1 and 2 can be taken from

Tables 3 and 4 (4a, 4b, 4c) disclosed in the Examples. Having at least two genes with mutations determined, a high probability of an antimicrobial drug, e.g. antibiotic, resistance could be determined. The genes in Table 1 thereby represent the 50 best genes for which a mutation was observed in the genomes of Serratia species, whereas the genes in Table 2 represent the 50 best genes for which a cross-correlation could be observed for the antimicrobial drug, e.g. antibiotic, susceptibility testing for Serratia species as described below.

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

According to certain embodiments, the genomes of Serratia 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 Serratia is NC_020211 as annotated at the NCBI according to certain embodiments. The reference genome is attached to this application as sequence listing with SEQ ID NO 1.

The reference sequence was obtained from Serratia strain NC_020211 (http://www.genome.jp/dbget-bin/www_bget?refseq+NC_020211)

LOCUS NC_020211 5241455 bp DNA circular CON 07-FEB-2015
DEFINITION Serratia marcescens WW4, complete genome.
ACCESSION NC_020211
VERSION NC_020211.1 GI:448239774
DBLINK BioProject: PRJNA224116
BioSample: SAMN02602965
Assembly: GCF_000336425.1
KEYWORDS RefSeq.
SOURCE Serratia marcescens WW4
ORGANISM Serratia marcescens WW4
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Serratia.
REFERENCE 1 (bases 1 to 5241455)
AUTHORS Kuo,P.A., Kuo,C.H., Lai,Y.K., Graumann,P.L. and Tu,J.

TITLE Phosphate limitation induces the intergeneric inhibition of Pseudomonas aeruginosa by Serratia marcescens isolated from paper machines
JOURNAL FEMS Microbiol. Ecol. 84 (3), 577-587 (2013)
PUBMED 23398522
REFERENCE 2 (bases 1 to 5241455)
AUTHORS Chung,W.C., Chen,L.L., Lo,W.S., Kuo,P.A., Tu,J. and Kuo,C.H.
TITLE Complete Genome Sequence of Serratia marcescens WW4
JOURNAL Genome Announc 1 (2), E0012613 (2013)
PUBMED 23558532
REMARK Publication Status: Online-Only
REFERENCE 3 (bases 1 to 5241455)
AUTHORS Chung,W.-C., Chen,L.-L., Lo,W.-S., Kuo,P.-A., Tu,J. and Kuo,C.-H.
TITLE Direct Submission
JOURNAL Submitted (26-NOV-2012) Institute of Plant and Microbial Biology, Academia Sinica, 128 Sec. 2, Academia Rd., Taipei 115, Taiwan

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. Serratia 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 Serratia species.

Using these techniques, genes with mutations of the microorganism of interest, e.g. Serratia 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. Serratia. Using several, e.g. 50 or more than 50, 100 or more than 100, 200 or more than 200, 300 or more than 300, or 400 or more than 400 different species of a microorganism, e.g. different Serratia species, statistical analysis can be carried out on the obtained cross-referenced data between mutations and antimicrobial drug, e.g. antibiotic, susceptibility for these number of species, using known methods.

Regarding culturing methods, samples can be e.g. cultured overnight. On the next day individual colonies can be used

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

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

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

For statistically sound results a multitude of individuals should be sampled, with n = 50, 100, 200, 300 or 400, 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 cer-

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

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 or 400 or more, and a level of significance (α-error-level) of e.g. 0.05 or smaller, e.g. 0.05, preferably 0.01 or smaller. According to certain embodiments, particularly significant results can be obtained for n = 200 or more, 300 or more or 400 or more.

After the above procedure has been carried out for more than 400, e.g. 438, individual species of Serratia, 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 Serratia stain, e.g. from an antimicrobial drug, e.g. antibiotic, resistant Serratia infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB,

nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, 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 Serratia 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 embodiment, referring to the same genes, unless clear from the context that they don't apply.

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

According to certain embodiments of the first and/or second aspect of the invention the antimicrobial, e.g. antibiotic, drug is selected from lactam antibiotics and the presence of a mutation in the following genes is determined: SMWW4_v1c13480.

According to certain embodiments of the first and/or second aspect of the invention the antimicrobial, e.g. antibiotic, drug is selected from polyketide antibiotics, preferably tetracycline antibiotics, and the presence of a mutation in the following genes is determined: actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and/or SMWW4_v1c25770.

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

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

According to certain embodiments, the antibiotic drug in the methods of the present invention is selected from the group consisting of Amoxicillin/K Clavulanate (AUG), Ampicillin (AM), Aztreonam (AZT), Cefazolin (CFZ), Cefepime (CPE), Cefotaxime (CFT), Ceftazidime (CAZ), Ceftriaxone (CAX), Cefuroxime (CRM), Cephalotin (CF), Ciprofloxacin (CP), Ertapenem (ETP), Gentamicin (GM), Imipenem (IMP), Levofloxacin (LVX), Meropenem (MER), Piperacillin/Tazobactam (P/T), Ampicillin/Sulbactam (A/S), Tetracycline (TE), Tobramycin (TO), and Trimethoprim/Sulfamethoxazole (T/S).

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

According to certain embodiments of the first and/or second aspect of the invention, the gene is from Table 1 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_020211:SMWW4_v1c13480.

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 polyketide, preferably tetracycline antibiotics and a mutation in at least one of the following genes is detected with regard to reference genome NC_020211: actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, SMWW4_v1c25770.

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 Serratia.

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 posi-

tions is detected with regard to reference genome NC_020211: 1489693.

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 polyketide, preferably tetracycline antibiotics and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_020211: 342947, 352212, 1816830, 352221, 1817267, 4149382, 86770, 86742, 86744, 1489672, 1489673, 1489681, 1490996, 1545409, 1487651, 1489693, 4148368, 897774, 2154027, 2154042, 2154044, 3716584, 87742, 1532249, 4148381, 1049796, 1601495, 4148825, 2715811, 3025014, 4143093, 4284592, 2154037, 1489972, 2662382, 2687128, 2250726, 4148361, 5161374, 5161396, 2371667, 1371641, 1398352, 4339539, 2687789, 4057459, 2716368, 4712441, 5025276, 4636300, 4812879, 3231402, 3243004, 3244657, 3249370, 3249507, 2716411, 1814748, 1476885, 1049699, 4296135, 4419488, 1347521, 1347533, 156541, 2816076, 3844397, 2018803, 176654, 176722, 176784, 2796043, 2796045.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is AM and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_020211: 1489693.

According to certain embodiments of the first and/or second aspect of the invention, the antibiotic drug is TE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_020211: 342947, 352212, 1816830, 352221, 1817267, 4149382, 86770, 86742, 86744, 1489672, 1489673, 1489681, 1490996, 1545409,

1487651, 1489693, 4148368, 897774, 2154027, 2154042, 2154044, 3716584, 87742, 1532249, 4148381, 1049796, 1601495, 4148825, 2715811, 3025014, 4143093, 4284592, 2154037, 1489972, 2662382, 2687128, 2250726, 4148361, 5161374, 5161396, 2371667, 1371641, 1398352, 4339539, 2687789, 4057459, 2716368, 4712441, 5025276, 4636300, 4812879, 3231402, 3243004, 3244657, 3249370, 3249507, 2716411, 1814748, 1476885, 1049699, 4296135, 4419488, 1347521, 1347533, 156541, 2816076, 3844397, 2018803, 176654, 176722, 176784, 2796043, 2796045.

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 Serratia against 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 17, 18, 19, 20 or 21 antibiotic drugs is determined.

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

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

According to certain embodiments of the first and/or second aspect of the invention, determining the nucleic acid sequence information or the presence of a mutation comprises determining a partial or entire sequence of the genome of the Serratia 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 Serratia 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 Serratia species, comprising:
obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Serratia species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Serratia species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Serratia, 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 Serratia 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, e.g. in at

least two, three, four, five, six, seven, eight, nine or ten genes. This way even higher statistical significance can be achieved.

According to certain embodiments of the method of the third aspect and related methods – as above, the second data set is provided by culturing the clinical isolates of Serratia 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 Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC,

SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, 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.

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

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

According to certain embodiments, the reference genome of Serratia is NC_020211 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, e.g. in at least two, three, four, five, six, seven, eight, nine or ten genes.

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

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

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

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

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

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 Serratia 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:
actP, alsK, alx, amiD, bglX, cnu, cysK, dppB, folX, galT, glrK, impL, kdpA, lysR, nrdH, rfaC, rihB, selB, SMWW4_v1c00800, SMWW4_v1c03050, SMWW4_v1c07960, SMWW4_v1c09360, SMWW4_v1c12350, SMWW4_v1c13350, SMWW4_v1c13470, SMWW4_v1c13480, SMWW4_v1c13910, SMWW4_v1c14040, SMWW4_v1c16540, SMWW4_v1c19810,

SMWW4_v1c20760, SMWW4_v1c21930, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c24810, SMWW4_v1c25040, SMWW4_v1c25770, SMWW4_v1c30050, SMWW4_v1c38510, SMWW4_v1c38520, SMWW4_v1c40850, SMWW4_v1c44490, vasD, ybiO, yceA, ydaN, yeaN, yhiN, yhjK, znuB, gyrA, csiE, mnmC, bioD, rlmG, SMWW4_v1c08980, SMWW4_v1c01000, SMWW4_v1c22750, SMWW4_v1c00940, recD, SMWW4_v1c09000, dhaR, rluC, SMWW4_v1c25060, SMWW4_v1c28700, nuoM, SMWW4_v1c31130, SMWW4_v1c11380, SMWW4_v1c21000, ybcJ, SMWW4_v1c01360, SMWW4_v1c24150, tmcA, SMWW4_v1c31090, yjjX, yafE, SMWW4_v1c42330, SMWW4_v1c34690, SMWW4_v1c06040.

Table 5: List of genes

actP	alsK	alx	amiD
bglX	cnu	cysK	dppB
folX	galT	glrK	impL
kdpA	lysR	nrdH	rfaC
rihB	selB	SMWW4_v1c00800	SMWW4_v1c03050
SMWW4_v1c07960	SMWW4_v1c09360	SMWW4_v1c12350	SMWW4_v1c13350
SMWW4_v1c13470	SMWW4_v1c13480	SMWW4_v1c13910	SMWW4_v1c14040
SMWW4_v1c16540	SMWW4_v1c19810	SMWW4_v1c20760	SMWW4_v1c21930
SMWW4_v1c24620	SMWW4_v1c24800	SMWW4_v1c24810	SMWW4_v1c25040
SMWW4_v1c25770	SMWW4_v1c30050	SMWW4_v1c38510	SMWW4_v1c38520
SMWW4_v1c40850	SMWW4_v1c44490	vasD	ybiO
yceA	ydaN	yeaN	yhiN
yhjK	znuB	gyrA	csiE
mnmC	bioD	rlmG	SMWW4_v1c08980
SMWW4_v1c01000	SMWW4_v1c22750	SMWW4_v1c00940	recD
SMWW4_v1c09000	dhaR	rluC	SMWW4_v1c25060
SMWW4_v1c28700	nuoM	SMWW4_v1c31130	SMWW4_v1c11380
SMWW4_v1c21000	ybcJ	SMWW4_v1c01360	SMWW4_v1c24150
tmcA	SMWW4_v1c31090	yjjX	yafE
SMWW4_v1c42330	SMWW4_v1c34690	SMWW4_v1c06040	

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 Serratia is again NC_020211 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. Also the other aspects of the embodiments of the first and second aspect of the invention apply.

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

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 CAX and a mutation in at least one of the genes of gyrA, csiE, mnmC, bioD, rlmG, SMWW4_v1c22750, recD is detected, or a mutation in at least one of the positions of

3652928, 4037047, 3757631, 1423417, 4631898, 2454764, 2454405, 4253544.

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 gyrA, csiE, mnmC is detected, or a mutation in at least one of the positions of 3652928, 4037047, 3757631.

Table 6: List for lactam antibiotics

gene name	POS	antibiotic	p-value (FDR)	genbank protein accession number
gyrA	3652928	T/S;CP;CAX;AZT;P/T;CPE; CAZ;LVX	2,71641E-31	YP_007407188.1
csiE	4037047	TE;CFT;CAX;AZT;P/T;CAZ	4,08141E-15	YP_007407543.1
mnmC	3757631	CAZ;AZT;CFT;P/T;CAX	2,47485E-12	YP_007407285.1
bioD	1423417	CFT;CPE;P/T;CAX	6,87138E-13	YP_007405106.1
rlmG	4631898	TE;CFT;CPE;CAX	1,89355E-16	YP_007408080.1
SMWW4_v1c08980	1008174	IMP;MER;ETP	9,88166E-13	YP_007404721.1
SMWW4_v1c01000	106274	IMP;MER;ETP	9,65084E-12	YP_007403927.1
SMWW4_v1c22750	2454764	CFT;P/T;CAX	2,25878E-11	YP_007406095.1
SMWW4_v1c00940	101412	IMP;MER;ETP	3,11418E-11	YP_007403921.1
SMWW4_v1c22750	2454405	CFT;CPE;CAX	3,15149E-11	YP_007406095.1
recD	4253544	CAZ;CFT;CAX	7,84012E-11	YP_007407740.1
SMWW4_v1c09000	1009779	IMP;MER;ETP	2,08854E-10	YP_007404723.1
dhaR	4554545	A/S;TE;AM	6,48952E-36	YP_007408008.1
rluC	2047091	A/S;TE;AM	1,18283E-34	YP_007405678.1
SMWW4_v1c25060	2719311	A/S;TE;AM	1,08791E-31	YP_007406322.1
SMWW4_v1c25060	2719308	A/S;TE;AM	2,76029E-31	YP_007406322.1
SMWW4_v1c08620	971081	A/S;TE;AM	5,43808E-29	YP_007404686.1

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 P/T and a mutation in at least one of the genes of gyrA, csiE, mnmC, bioD, SMWW4_v1c22750 is detected, or a mutation in at least one of the positions of 3652928, 4037047, 3757631, 1423417, 2454764.

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 gyrA, bioD, rlmG, SMWW4_v1c22750 is detected, or a mutation in at least one of the positions of 3652928, 1423417, 4631898, 2454405.

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 CAZ and a mutation in at least one of the genes of gyrA, csiE, mnmC, recD is detected, or a mutation in at least one of the positions of 3652928, 4037047, 3757631, 4253544.

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 CFT and a mutation in at least one of the genes of csiE, mnmC, bioD, rlmG, SMWW4_v1c22750, recD is detected, or a mutation in at least one of the positions of 4037047, 3757631, 1423417, 4631898, 2454764, 2454405, 4253544.

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 IMP, MER and ETP and a mutation in at least one of the genes of SMWW4_v1c08980, SMWW4_v1c01000, SMWW4_v1c00940, SMWW4_v1c09000 is detected, or a mutation in at least one of the positions of 1008174, 106274, 101412, 1009779.

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 A/S and AM and a mutation in at least one of the genes of dhaR, rluC, SMWW4_v1c25060, SMWW4_v1c08620 is detected, or a mutation in at least one of the positions of 4554545, 2047091, 2719311, 2719308, 971081.

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
gyrA	3652928	T/S;CP;CAX;AZT; P/T;CPE;CAZ;LVX	2,71641E-31	YP_007407188.1
SMWW4_v1c28700	3102771	TE;LVX;CP	1,66842E-20	YP_007406684.1
nuoM	3684663	TE;LVX;CP	4,81035E-20	YP_007407217.1
SMWW4_v1c31130	3351244	TE;LVX;CP	6,93739E-15	YP_007406926.1
SMWW4_v1c11380	1267465	TE;LVX;CP	6,10558E-14	YP_007404961.1
SMWW4_v1c11380	1267467	TE;LVX;CP	6,10558E-14	YP_007404961.1
SMWW4_v1c21000	2274687	CP;LVX	2,13307E-13	YP_007405920.1
ybcJ	1266246	CP;LVX	1,01082E-11	YP_007404959.1

SMWW4_v1c01360	143262	TE;LVX	7,6238E-30	YP_007403963.1
SMWW4_v1c24150	2608399	TE;LVX	2,11161E-26	YP_007406233.1
csiE	4036990	TE;LVX	4,35341E-24	YP_007407543.1
tmcA	3902870	TE;LVX	7,86789E-23	YP_007407422.1
SMWW4_v1c31090	3347837	TE;LVX	7,23732E-21	YP_007406922.1
yjjX	742354	TE;LVX	4,59367E-18	YP_007404495.1
yafE	1072696	TE;LVX	4,08141E-15	YP_007404787.1
SMWW4_v1c13160	1459283	TE;LVX	2,20905E-14	YP_007405139.1

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 gyrA, SMWW4_v1c28700, nuoM, SMWW4_v1c31130, SMWW4_v1c11380, SMWW4_v1c21000, ybcJ is detected, or a mutation in at least one of the positions of 3652928, 3102771, 3684663, 3351244, 1267465, 1267467, 2274687, 1266246.

According to certain embodiments of the method of the twelfth and/or thirteenth aspect of the present invention, as well as also of the eighteenth aspect of the present invention, the antibiotic is LVX and a mutation in at least one of the genes of SMWW4_v1c01360, SMWW4_v1c24150, csiE, tmcA, SMWW4_v1c31090, yjjX, yafE, SMWW4_v1c13160 is detected, or a mutation in at least one of the positions of 143262, 2608399, 4036990, 3902870, 3347837, 742354, 1072696, 1459283.

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
SMWW4_v1c42330	4593940	TO	6,39209E-11	YP_007408039.1

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 TO and a mutation in SMWW4_v1c42330 is detected, or a mutation in position 4593940.

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

Table 9: List of polyketides, preferably tetracycline

gene name	POS	antibiotic	p-value (FDR)	genbank protein accession number
actP	342947	TE	7,35941E-48	YP_007404126.1
SMWW4_v1c03050	352212	TE	7,35941E-48	YP_007404131.1
amiD	1816830	TE	7,35941E-48	YP_007405479.1
SMWW4_v1c03050	352221	TE	1,37918E-47	YP_007404131.1
amiD	1817267	TE	1,80252E-47	YP_007405479.1
SMWW4_v1c38520	4149382	TE	1,80252E-47	YP_007407658.1
selB	86770	TE	3,66497E-47	YP_007403906.1
selB	86742	TE	3,70866E-47	YP_007403906.1
selB	86744	TE	3,70866E-47	YP_007403906.1
SMWW4_v1c13480	1489672	TE	3,70866E-47	YP_007405171.1
SMWW4_v1c13480	1489673	TE	3,70866E-47	YP_007405171.1

SMWW4_v1c13480	1489681	TE	3,70866E-47	YP_007405171.1
bglX	1490996	TE	3,70866E-47	YP_007405172.1
SMWW4_v1c14040	1545409	TE	3,70866E-47	YP_007405227.1
SMWW4_v1c13470	1487651	TE	1,23812E-46	YP_007405170.1
SMWW4_v1c13480	1489693	TE;AM	1,23812E-46	YP_007405171.1
SMWW4_v1c38510	4148368	TE	1,23812E-46	YP_007407657.1
SMWW4_v1c07960	897774	TE	1,70034E-46	YP_007404622.1
SMWW4_v1c19810	2154027	TE	1,70034E-46	YP_007405801.1
SMWW4_v1c19810	2154042	TE	1,70034E-46	YP_007405801.1
SMWW4_v1c19810	2154044	TE	1,70034E-46	YP_007405801.1
folX	3716584	TE	1,76369E-46	YP_007407245.1
SMWW4_v1c00800	87742	TE	1,91346E-46	YP_007403907.1
SMWW4_v1c13910	1532249	TE	1,91346E-46	YP_007405214.1
actP	342947	TE	7,35941E-48	YP_007404126.1
SMWW4_v1c03050	352212	TE	7,35941E-48	YP_007404131.1

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 TE and a mutation in at least one of the genes of actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910 is detected, or a mutation in at least one of the positions of 342947, 352212, 1816830, 352221, 1817267, 4149382, 86770, 86742, 86744, 1489672, 1489673, 1489681, 1490996, 1545409, 1487651, 1489693, 4148368, 897774, 2154027, 2154042, 2154044, 3716584, 87742, 1532249.

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 T/S and a mutation in at least one of the genes listed in Table 10 is detected, or a mutation in at least one

of the positions (denoted POS in the tables) listed in Table 10.

Table 10: List of others antibiotics (benzene derived/sulfonamide)

gene name	POS	antibiotic	p-value (FDR)	genbank protein accession number
gyrA	3652928	T/S;CP;CAX;AZT; P/T;CPE;CAZ;LVX	2,71641E-31	YP_007407188.1
SMWW4_v1c34690	3748106	T/S;TE	1,73209E-15	YP_007407278.1
SMWW4_v1c06040	679311	T/S;TE	5,14974E-14	YP_007404430.1

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

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, preferably SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960,

SMWW4_v1c19810, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, nrdH, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, ydaN, yceA, yhjK, and SMWW4_v1c25770, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Serratia 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 Serratia infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, preferably SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, nrdH, SMWW4_v1c24620,

SMWW4_v1c24800, SMWW4_v1c20760, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, ydaN, yceA, yhjK, and SMWW4_v1c25770, 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 Serratia 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 Serratia infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350,

yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, preferably SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, nrdH, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, ydaN, yceA, yhjK, and SMWW4_v1c25770, 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 Serratia 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 Serratia infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB,

nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, 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 Serratia 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 Serratia infection, comprising the steps of:

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

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 11, preferably from the group of genes listed in Table 12, 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 Serratia infection; and

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

Table 11: List of genes

actP	alsK	alx	amiD
bglX	cnu	cysK	dppB
folX	galT	glrK	impL
kdpA	lysR	nrdH	rfaC
rihB	selB	SMWW4_v1c00800	SMWW4_v1c03050
SMWW4_v1c07960	SMWW4_v1c09360	SMWW4_v1c12350	SMWW4_v1c13350
SMWW4_v1c13470	SMWW4_v1c13480	SMWW4_v1c13910	SMWW4_v1c14040
SMWW4_v1c16540	SMWW4_v1c19810	SMWW4_v1c20760	SMWW4_v1c21930
SMWW4_v1c24620	SMWW4_v1c24800	SMWW4_v1c24810	SMWW4_v1c25040
SMWW4_v1c25770	SMWW4_v1c30050	SMWW4_v1c38510	SMWW4_v1c38520

SMWW4_v1c40850	SMWW4_v1c44490	vasD	ybiO
yceA	ydaN	yeaN	yhiN
yhjK	znuB	SMWW4_v1c06040	csiE
mnmC	bioD	rlmG	SMWW4_v1c08980
SMWW4_v1c01000	SMWW4_v1c22750	SMWW4_v1c00940	recD
SMWW4_v1c09000	dhaR	rluC	SMWW4_v1c25060
SMWW4_v1c28700	nuoM	SMWW4_v1c31130	SMWW4_v1c11380
SMWW4_v1c21000	ybcJ	SMWW4_v1c01360	SMWW4_v1c24150
tmcA	SMWW4_v1c31090	yjjX	yafE
SMWW4_v1c42330	SMWW4_v1c34690		

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.

Table 12: List of genes

actP	alsK	alx	amiD
bglX	cnu	SMWW4_v1c06040	SMWW4_v1c34690
SMWW4_v1c42330	galT	glrK	impL
yafE	SMWW4_v1c31090	nrdH	tmcA
rihB	selB	SMWW4_v1c00800	SMWW4_v1c03050
SMWW4_v1c07960	SMWW4_v1c09360	SMWW4_v1c12350	SMWW4_v1c13350
SMWW4_v1c13470	SMWW4_v1c13480	SMWW4_v1c13910	SMWW4_v1c14040
SMWW4_v1c16540	SMWW4_v1c19810	SMWW4_v1c20760	SMWW4_v1c21930
SMWW4_v1c24620	SMWW4_v1c24800	SMWW4_v1c24810	SMWW4_v1c25040
SMWW4_v1c25770	SMWW4_v1c30050	SMWW4_v1c38510	SMWW4_v1c38520
SMWW4_v1c40850	SMWW4_v1c44490	vasD	ybiO
yceA	ydaN	yeaN	yhiN
yhjK	SMWW4_v1c24150	SMWW4_v1c01360	csiE
mnmC	bioD	rlmG	SMWW4_v1c08980
SMWW4_v1c01000	SMWW4_v1c22750	SMWW4_v1c00940	ybcJ
SMWW4_v1c09000	dhaR	rluC	SMWW4_v1c25060
SMWW4_v1c28700	nuoM	SMWW4_v1c31130	SMWW4_v1c11380
SMWW4_v1c21000			

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

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 11, preferably from the group of genes listed in Table 12, wherein the presence of said at least one mutation is indicative of an antimicrobial drug, e.g. antibiotic, resistant Serratia 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 Serratia infection, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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 11, preferably from the group of genes listed in Table 12, 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 Serratia 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 438 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 Serratia isolates was measured.

The detailed procedure is given in the following:

Bacterial Strains

The inventors selected 438 Serratia strains from the microbiology strain collection at Siemens Healthcare Diagnostics (West Sacramento, CA) for susceptibility testing and whole genome sequencing.

Antimicrobial Susceptibility Testing (AST) Panels

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

Inoculum Preparation

Isolates were cultured on trypticase soy agar with 5% sheep blood (BBL, Cockeysville, Md.) and incubated in ambient air at 35±1°C for 18-24 h. Isolated colonies (4-5 large colonies or 5-10 small colonies) were transferred to a 3 ml Sterile Inoculum Water (Siemens) and emulsified to a final turbidity of a 0.5 McFarland standard. 2 ml of this suspension was added 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 438 Serratia samples were mapped against the Serratia reference (NC_020211) 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 Serratia 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 Serratia samples were considered. Serratia 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 Serratia 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_020211.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 Serratia 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:
Serratia 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 Ge-

nome Project Data Processing Subgroup (2009) The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics, 25, 2078-9. [PMID: 19505943]).

As reference genome, NC_020211 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 438 different clinical isolates of Serratia species, particularly Serratia marcescens, 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 438 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 Serratia 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_020211 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 438 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 the correlation between different genetic sites were calculated
3) For detecting gene dosage effects, e.g. loss or gain of genes (in the genome or on plasmids) the coverage (i.e. how many read map to the current position) at each site for resistant and not resistant isolates was calculated.

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 for the respective antibiotics.

In addition, the data with the best p-values for each antibiotic class with the most antibiotic drugs as well as each antibiotic, respectively, were evaluated, being disclosed in Tables 5 – 10.

In Tables 3 - 10 the columns are designated as follows:
Gene name: affected gene;

POS: genomic position of the SNP / variant in the Serratia 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
342947	polyketide (tetracycline)	1	7,35941E-48	actP	YP_007404126.1
352212	polyketide (tetracycline)	1	7,35941E-48	SMWW4_v1c03050	YP_007404131.1
1816830	polyketide (tetracycline)	1	7,35941E-48	amiD	YP_007405479.1
352221	polyketide (tetracycline)	1	1,37918E-47	SMWW4_v1c03050	YP_007404131.1
1817267	polyketide (tetracycline)	1	1,80252E-47	amiD	YP_007405479.1
4149382	polyketide (tetracycline)	1	1,80252E-47	SMWW4_v1c38520	YP_007407658.1
86770	polyketide (tetracycline)	1	3,66497E-47	selB	YP_007403906.1
86742	polyketide (tetracycline)	1	3,70866E-47	selB	YP_007403906.1
86744	polyketide (tetracycline)	1	3,70866E-47	selB	YP_007403906.1
1489672	polyketide (tetracycline)	1	3,70866E-47	SMWW4_v1c13480	YP_007405171.1
1489673	polyketide (tetracycline)	1	3,70866E-47	SMWW4_v1c13480	YP_007405171.1
1489681	polyketide (tetracycline)	1	3,70866E-47	SMWW4_v1c13480	YP_007405171.1
1490996	polyketide (tetracycline)	1	3,70866E-47	bglX	YP_007405172.1
1545409	polyketide (tetracycline)	1	3,70866E-47	SMWW4_v1c14040	YP_007405227.1
1487651	polyketide (tetracycline)	1	1,23812E-46	SMWW4_v1c13470	YP_007405170.1
1489693	polyketide (tetracycline); Lactams	2	1,23812E-46	SMWW4_v1c13480	YP_007405171.1
4148368	polyketide (tetracycline)	1	1,23812E-46	SMWW4_v1c38510	YP_007407657.1
897774	polyketide (tetracycline)	1	1,70034E-46	SMWW4_v1c07960	YP_007404622.1
2154027	polyketide (tetracycline)	1	1,70034E-46	SMWW4_v1c19810	YP_007405801.1

2154042	polyketide (tetracycline)	1	1,70034E-46	SMWW4_v1c19810	YP_007405801.1
2154044	polyketide (tetracycline)	1	1,70034E-46	SMWW4_v1c19810	YP_007405801.1
3716584	polyketide (tetracycline)	1	1,76369E-46	folX	YP_007407245.1
87742	polyketide (tetracycline)	1	1,91346E-46	SMWW4_v1c00800	YP_007403907.1
1532249	polyketide (tetracycline)	1	1,91346E-46	SMWW4_v1c13910	YP_007405214.1
4148381	polyketide (tetracycline)	1	1,91346E-46	SMWW4_v1c38510	YP_007407657.1
1049796	polyketide (tetracycline)	1	2,81969E-46	SMWW4_v1c09360	YP_007404759.1
1601495	polyketide (tetracycline)	1	2,81969E-46	ybiO	YP_007405284.1
4148825	polyketide (tetracycline)	1	2,91358E-46	SMWW4_v1c38510	YP_007407657.1
2715811	polyketide (tetracycline)	1	3,69091E-46	SMWW4_v1c25040	YP_007406320.1
3025014	polyketide (tetracycline)	1	4,2298E-46	znuB	YP_007406603.1
4143093	polyketide (tetracycline)	1	4,2298E-46	nrdH	YP_007407651.1
4284592	polyketide (tetracycline)	1	4,2298E-46	lysR	YP_007407763.1
2154037	polyketide (tetracycline)	1	4,33782E-46	SMWW4_v1c19810	YP_007405801.1
1489972	polyketide (tetracycline)	1	5,73298E-46	bglX	YP_007405172.1
2662382	polyketide (tetracycline)	1	5,84504E-46	SMWW4_v1c24620	YP_007406278.1
2687128	polyketide (tetracycline)	1	5,84504E-46	SMWW4_v1c24800	YP_007406296.1
2250726	polyketide (tetracycline)	1	6,98841E-46	SMWW4_v1c20760	YP_007405896.1
4148361	polyketide (tetracycline)	1	6,98841E-46	SMWW4_v1c38510	YP_007407657.1
5161374	polyketide (tetracycline)	1	7,35922E-46	rfaC	YP_007408564.1
5161396	polyketide (tetracycline)	1	7,35922E-46	rfaC	YP_007408564.1
2371667	polyketide (tetracycline)	1	8,11844E-46	SMWW4_v1c21930	YP_007406013.1
1371641	polyketide (tetracycline)	1	9,02953E-46	SMWW4_v1c12350	YP_007405058.1

1398352	polyketide (tetracycline)	1	9,02953E-46	galT	YP_007405081.1
4339539	polyketide (tetracycline)	1	1,07097E-45	alsK	YP_007407817.1
2687789	polyketide (tetracycline)	1	1,10597E-45	SMWW4_v1c24810	YP_007406297.1
4057459	polyketide (tetracycline)	1	1,40374E-45	glrK	YP_007407560.1
2716368	polyketide (tetracycline)	1	1,45717E-45	SMWW4_v1c25040	YP_007406320.1
4712441	polyketide (tetracycline)	1	1,55547E-45	rihB	YP_007408169.1
5025276	polyketide (tetracycline)	1	1,55547E-45	yhiN	YP_007408451.1
4636300	polyketide (tetracycline)	1	2,05052E-45	alx	YP_007408086.1
4812879	polyketide (tetracycline)	1	2,51465E-45	SMWW4_v1c44490	YP_007408255.1
3231402	polyketide (tetracycline)	1	2,83885E-45	cnu	YP_007406808.1
3243004	polyketide (tetracycline)	1	2,83885E-45	SMWW4_v1c30050	YP_007406818.1
3244657	polyketide (tetracycline)	1	2,83885E-45	vasD	YP_007406821.1
3249370	polyketide (tetracycline)	1	2,83885E-45	impL	YP_007406824.1
3249507	polyketide (tetracycline)	1	2,83885E-45	impL	YP_007406824.1
2716411	polyketide (tetracycline)	1	3,33452E-45	SMWW4_v1c25040	YP_007406320.1
1814748	polyketide (tetracycline)	1	3,63807E-45	SMWW4_v1c16540	YP_007405477.1
1476885	polyketide (tetracycline)	1	3,76125E-45	SMWW4_v1c13350	YP_007405158.1
1049699	polyketide (tetracycline)	1	4,1218E-45	SMWW4_v1c09360	YP_007404759.1
4296135	polyketide (tetracycline)	1	4,1218E-45	yeaN	YP_007407775.1
4419488	polyketide (tetracycline)	1	4,1218E-45	SMWW4_v1c40850	YP_007407891.1
1347521	polyketide (tetracycline)	1	4,55375E-45	kdpA	YP_007405036.1
1347533	polyketide (tetracycline)	1	4,55375E-45	kdpA	YP_007405036.1
156541	polyketide (tetracycline)	1	4,59793E-45	dppB	YP_007403975.1

2816076	polyketide (tetracycline)	1	4,59793E-45	ydaN	YP_007406409.1
3844397	polyketide (tetracycline)	1	4,59793E-45	cysK	YP_007407367.1
2018803	polyketide (tetracycline)	1	5,6407E-45	yceA	YP_007405655.1
176654	polyketide (tetracycline)	1	5,88767E-45	yhjK	YP_007403988.1
176722	polyketide (tetracycline)	1	5,88767E-45	yhjK	YP_007403988.1
176784	polyketide (tetracycline)	1	5,88767E-45	yhjK	YP_007403988.1
2796043	polyketide (tetracycline)	1	5,88767E-45	SMWW4_v1c25770	YP_007406393.1
2796045	polyketide (tetracycline)	1	5,88767E-45	SMWW4_v1c25770	YP_007406393.1

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

POS	drug	#drugs	drug class	#drug classes
342947	TE	1	polyketide (tetracycline)	1
352212	TE	1	polyketide (tetracycline)	1
1816830	TE	1	polyketide (tetracycline)	1
352221	TE	1	polyketide (tetracycline)	1
1817267	TE	1	polyketide (tetracycline)	1
4149382	TE	1	polyketide (tetracycline)	1
86770	TE	1	polyketide (tetracycline)	1
86742	TE	1	polyketide (tetracycline)	1
86744	TE	1	polyketide (tetracycline)	1
1489672	TE	1	polyketide (tetracycline)	1
1489673	TE	1	polyketide (tetracycline)	1

1489681	TE	1	polyketide (tetracycline)	1
1490996	TE	1	polyketide (tetracycline)	1
1545409	TE	1	polyketide (tetracycline)	1
1487651	TE	1	polyketide (tetracycline)	1
1489693	TE;AM	2	polyketide (tetracycline);Lactams	2
4148368	TE	1	polyketide (tetracycline)	1
897774	TE	1	polyketide (tetracycline)	1
2154027	TE	1	polyketide (tetracycline)	1
2154042	TE	1	polyketide (tetracycline)	1
2154044	TE	1	polyketide (tetracycline)	1
3716584	TE	1	polyketide (tetracycline)	1
87742	TE	1	polyketide (tetracycline)	1
1532249	TE	1	polyketide (tetracycline)	1
4148381	TE	1	polyketide (tetracycline)	1
1049796	TE	1	polyketide (tetracycline)	1
1601495	TE	1	polyketide (tetracycline)	1
4148825	TE	1	polyketide (tetracycline)	1
2715811	TE	1	polyketide (tetracycline)	1
3025014	TE	1	polyketide (tetracycline)	1
4143093	TE	1	polyketide (tetracycline)	1
4284592	TE	1	polyketide (tetracycline)	1
2154037	TE	1	polyketide (tetracycline)	1
1489972	TE	1	polyketide (tetracycline)	1

2662382	TE	1	polyketide (tetracycline)	1
2687128	TE	1	polyketide (tetracycline)	1
2250726	TE	1	polyketide (tetracycline)	1
4148361	TE	1	polyketide (tetracycline)	1
5161374	TE	1	polyketide (tetracycline)	1
5161396	TE	1	polyketide (tetracycline)	1
2371667	TE	1	polyketide (tetracycline)	1
1371641	TE	1	polyketide (tetracycline)	1
1398352	TE	1	polyketide (tetracycline)	1
4339539	TE	1	polyketide (tetracycline)	1
2687789	TE	1	polyketide (tetracycline)	1
4057459	TE	1	polyketide (tetracycline)	1
2716368	TE	1	polyketide (tetracycline)	1
4712441	TE	1	polyketide (tetracycline)	1
5025276	TE	1	polyketide (tetracycline)	1
4636300	TE	1	polyketide (tetracycline)	1
4812879	TE	1	polyketide (tetracycline)	1
3231402	TE	1	polyketide (tetracycline)	1
3243004	TE	1	polyketide (tetracycline)	1
3244657	TE	1	polyketide (tetracycline)	1
3249370	TE	1	polyketide (tetracycline)	1
3249507	TE	1	polyketide (tetracycline)	1
2716411	TE	1	polyketide (tetracycline)	1

1814748	TE	1	polyketide (tetracycline)	1
1476885	TE	1	polyketide (tetracycline)	1
1049699	TE	1	polyketide (tetracycline)	1
4296135	TE	1	polyketide (tetracycline)	1
4419488	TE	1	polyketide (tetracycline)	1
1347521	TE	1	polyketide (tetracycline)	1
1347533	TE	1	polyketide (tetracycline)	1
156541	TE	1	polyketide (tetracycline)	1
2816076	TE	1	polyketide (tetracycline)	1
3844397	TE	1	polyketide (tetracycline)	1
2018803	TE	1	polyketide (tetracycline)	1
176654	TE	1	polyketide (tetracycline)	1
176722	TE	1	polyketide (tetracycline)	1
176784	TE	1	polyketide (tetracycline)	1
2796043	TE	1	polyketide (tetracycline)	1
2796045	TE	1	polyketide (tetracycline)	1

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)/ sul-fonamide**

342947	TE	0	0	0	1	0	
352212	TE	0	0	0	1	0	
1816830	TE	0	0	0	1	0	
352221	TE	0	0	0	1	0	
1817267	TE	0	0	0	1	0	
4149382	TE	0	0	0	1	0	
86770	TE	0	0	0	1	0	
86742	TE	0	0	0	1	0	
86744	TE	0	0	0	1	0	
1489672	TE	0	0	0	1	0	
1489673	TE	0	0	0	1	0	
1489681	TE	0	0	0	1	0	
1490996	TE	0	0	0	1	0	
1545409	TE	0	0	0	1	0	
1487651	TE	0	0	0	1	0	
1489693	TE	1	0	0	1	0	
4148368	TE	0	0	0	1	0	
897774	TE	0	0	0	1	0	
2154027	TE	0	0	0	1	0	
2154042	TE	0	0	0	1	0	
2154044	TE	0	0	0	1	0	
3716584	TE	0	0	0	1	0	
87742	TE	0	0	0	1	0	

1532249	TE	0	0	0	1	0	
4148381	TE	0	0	0	1	0	
1049796	TE	0	0	0	1	0	
1601495	TE	0	0	0	1	0	
4148825	TE	0	0	0	1	0	
2715811	TE	0	0	0	1	0	
3025014	TE	0	0	0	1	0	
4143093	TE	0	0	0	1	0	
4284592	TE	0	0	0	1	0	
2154037	TE	0	0	0	1	0	
1489972	TE	0	0	0	1	0	
2662382	TE	0	0	0	1	0	
2687128	TE	0	0	0	1	0	
2250726	TE	0	0	0	1	0	
4148361	TE	0	0	0	1	0	
5161374	TE	0	0	0	1	0	
5161396	TE	0	0	0	1	0	
2371667	TE	0	0	0	1	0	
1371641	TE	0	0	0	1	0	
1398352	TE	0	0	0	1	0	
4339539	TE	0	0	0	1	0	
2687789	TE	0	0	0	1	0	
4057459	TE	0	0	0	1	0	

2716368	TE	0	0	0	1	0	
4712441	TE	0	0	0	1	0	
5025276	TE	0	0	0	1	0	
4636300	TE	0	0	0	1	0	
4812879	TE	0	0	0	1	0	
3231402	TE	0	0	0	1	0	
3243004	TE	0	0	0	1	0	
3244657	TE	0	0	0	1	0	
3249370	TE	0	0	0	1	0	
3249507	TE	0	0	0	1	0	
2716411	TE	0	0	0	1	0	
1814748	TE	0	0	0	1	0	
1476885	TE	0	0	0	1	0	
1049699	TE	0	0	0	1	0	
4296135	TE	0	0	0	1	0	
4419488	TE	0	0	0	1	0	
1347521	TE	0	0	0	1	0	
1347533	TE	0	0	0	1	0	
156541	TE	0	0	0	1	0	
2816076	TE	0	0	0	1	0	
3844397	TE	0	0	0	1	0	
2018803	TE	0	0	0	1	0	
176654	TE	0	0	0	1	0	

176722	TE	0	0	0	1	0
176784	TE	0	0	0	1	0
2796043	TE	0	0	0	1	0
2796045	TE	0	0	0	1	0

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

POS	p-value	gene name	genbank protein accession number
342947	7,35941E-48	actP	YP_007404126.1
352212	7,35941E-48	SMWW4_v1c03050	YP_007404131.1
1816830	7,35941E-48	amiD	YP_007405479.1
352221	1,37918E-47	SMWW4_v1c03050	YP_007404131.1
1817267	1,80252E-47	amiD	YP_007405479.1
4149382	1,80252E-47	SMWW4_v1c38520	YP_007407658.1
86770	3,66497E-47	selB	YP_007403906.1
86742	3,70866E-47	selB	YP_007403906.1
86744	3,70866E-47	selB	YP_007403906.1
1489672	3,70866E-47	SMWW4_v1c13480	YP_007405171.1
1489673	3,70866E-47	SMWW4_v1c13480	YP_007405171.1
1489681	3,70866E-47	SMWW4_v1c13480	YP_007405171.1

1490996	3,70866E-47	bglX	YP_007405172.1
1545409	3,70866E-47	SMWW4_v1c14040	YP_007405227.1
1487651	1,23812E-46	SMWW4_v1c13470	YP_007405170.1
1489693	1,23812E-46	SMWW4_v1c13480	YP_007405171.1
4148368	1,23812E-46	SMWW4_v1c38510	YP_007407657.1
897774	1,70034E-46	SMWW4_v1c07960	YP_007404622.1
2154027	1,70034E-46	SMWW4_v1c19810	YP_007405801.1
2154042	1,70034E-46	SMWW4_v1c19810	YP_007405801.1
2154044	1,70034E-46	SMWW4_v1c19810	YP_007405801.1
3716584	1,76369E-46	folX	YP_007407245.1
87742	1,91346E-46	SMWW4_v1c00800	YP_007403907.1
1532249	1,91346E-46	SMWW4_v1c13910	YP_007405214.1
4148381	1,91346E-46	SMWW4_v1c38510	YP_007407657.1
1049796	2,81969E-46	SMWW4_v1c09360	YP_007404759.1
1601495	2,81969E-46	ybiO	YP_007405284.1
4148825	2,91358E-46	SMWW4_v1c38510	YP_007407657.1
2715811	3,69091E-46	SMWW4_v1c25040	YP_007406320.1
3025014	4,2298E-46	znuB	YP_007406603.1
4143093	4,2298E-46	nrdH	YP_007407651.1
4284592	4,2298E-46	lysR	YP_007407763.1
2154037	4,33782E-46	SMWW4_v1c19810	YP_007405801.1
1489972	5,73298E-46	bglX	YP_007405172.1
2662382	5,84504E-46	SMWW4_v1c24620	YP_007406278.1

2687128	5,84504E-46	SMWW4_v1c24800	YP_007406296.1
2250726	6,98841E-46	SMWW4_v1c20760	YP_007405896.1
4148361	6,98841E-46	SMWW4_v1c38510	YP_007407657.1
5161374	7,35922E-46	rfaC	YP_007408564.1
5161396	7,35922E-46	rfaC	YP_007408564.1
2371667	8,11844E-46	SMWW4_v1c21930	YP_007406013.1
1371641	9,02953E-46	SMWW4_v1c12350	YP_007405058.1
1398352	9,02953E-46	galT	YP_007405081.1
4339539	1,07097E-45	alsK	YP_007407817.1
2687789	1,10597E-45	SMWW4_v1c24810	YP_007406297.1
4057459	1,40374E-45	glrK	YP_007407560.1
2716368	1,45717E-45	SMWW4_v1c25040	YP_007406320.1
4712441	1,55547E-45	rihB	YP_007408169.1
5025276	1,55547E-45	yhiN	YP_007408451.1
4636300	2,05052E-45	alx	YP_007408086.1
4812879	2,51465E-45	SMWW4_v1c44490	YP_007408255.1
3231402	2,83885E-45	cnu	YP_007406808.1
3243004	2,83885E-45	SMWW4_v1c30050	YP_007406818.1
3244657	2,83885E-45	vasD	YP_007406821.1
3249370	2,83885E-45	impL	YP_007406824.1
3249507	2,83885E-45	impL	YP_007406824.1
2716411	3,33452E-45	SMWW4_v1c25040	YP_007406320.1
1814748	3,63807E-45	SMWW4_v1c16540	YP_007405477.1

1476885	3,76125E-45	SMWW4_v1c13350	YP_007405158.1
1049699	4,1218E-45	SMWW4_v1c09360	YP_007404759.1
4296135	4,1218E-45	yeaN	YP_007407775.1
4419488	4,1218E-45	SMWW4_v1c40850	YP_007407891.1
1347521	4,55375E-45	kdpA	YP_007405036.1
1347533	4,55375E-45	kdpA	YP_007405036.1
156541	4,59793E-45	dppB	YP_007403975.1
2816076	4,59793E-45	ydaN	YP_007406409.1
3844397	4,59793E-45	cysK	YP_007407367.1
2018803	5,6407E-45	yceA	YP_007405655.1
176654	5,88767E-45	yhjK	YP_007403988.1
176722	5,88767E-45	yhjK	YP_007403988.1
176784	5,88767E-45	yhjK	YP_007403988.1
2796043	5,88767E-45	SMWW4_v1c25770	YP_007406393.1
2796045	5,88767E-45	SMWW4_v1c25770	YP_007406393.1

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 30.051 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 significance that was reached was 10^{-48}
- Besides these, insertions or deletions of up to four bases were discovered
- Further, potential genetic tests for five different drug classes relating to resistances were discovered
    - β-lactams (includes Penicillins, Cephalosporins, Carbapenems, Monobactams)
    - Quinolones, particularly Fluoroquinolones
    - Aminoglycosides
    - Polyketides, particularly Tetracyclines
    - 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 3.718 different genes

While in the tables only the best mutations in each gene are represented, a manifold of different SNPs has been found for each gene. Examples for multiple SNPs for two of the genes given in Table 3 are shown in the following Tables 13 and 14.

Table 13: Statistically significant SNPs in gene selB (genbank protein accession number YP_007403906.1) (headers as in Tables 3 and 4, respectively)

POS	drug	#drugs	drug class	best drug	p-value
86770	TE	1	Polyketide*	TE	3.6650E-047
86410	TE	1	Polyketide*	TE	5.9659E-031
86266	TE	1	Polyketide*	TE	6.3107E-022
86743	TE	1	Polyketide*	TE	6.5562E-043
86377	TE	1	Polyketide*	TE	1.2601E-018
87028	TE	1	Polyketide*	TE	8.7798E-025
86406	TE	1	Polyketide*	TE	2.8903E-014
86448	TE	1	Polyketide*	TE	1.8623E-013
86043	TE	1	Polyketide*	TE	7.2832E-016
86154	TE	1	Polyketide*	TE	4.7708E-015
86744	TE	1	Polyketide*	TE	3.7087E-047
86342	TE	1	Polyketide*	TE	1.4386E-016
86787	TE	1	Polyketide*	TE	1.6621E-015
86611	TE	1	Polyketide*	TE	3.9534E-010
86379	TE	1	Polyketide*	TE	4.1536E-011
87315	TE	1	Polyketide*	TE	7.2700E-017
86341	TE	1	Polyketide*	TE	8.2622E-042
86155	TE;AM	2	polyketide*;Lactams	TE	7.0844E-027
86482	TE	1	Polyketide*	TE	1.8840E-023
87219	TE	1	Polyketide*	TE	5.6863E-014
86158	TE;AM	2	Polyketide*;Lactams	TE	1.5954E-026
86016	TE	1	Polyketide*	TE	1.9196E-017
86860	TE	1	Polyketide*	TE	6.6181E-012

87027	TE	1	Polyketide*	TE	1.0395E-015
86803	TE	1	Polyketide*	TE	8.2435E-044
86030	TE	1	Polyketide*	TE	5.5168E-042
86742	TE	1	Polyketide*	TE	3.7087E-047
86446	TE	1	Polyketide*	TE	2.7424E-010
86684	TE	1	Polyketide*	TE	1.4554E-016
87500	TE	1	Polyketide*	TE	2.2039E-014

*: (tetracycline)

Table 14: Statistically significant SNPs in gene SMWW4_v1c00800 (genbank protein accession number YP_007403907.1) (headers as in Tables 3 and 4, respectively)

POS	drug	#drugs	drug class	best drug	p-value
87790	TE	1	Polyketide*	TE	9.4526E-011
88055	TE	1	Polyketide*	TE	1.4844E-018
87559	TE	1	Polyketide*	TE	5.0631E-014
87777	TE	1	Polyketide*	TE	6.8034E-019
87780	TE	1	Polyketide*	TE	6.8873E-014
87742	TE	1	Polyketide*	TE	1.9135E-046
87606	TE	1	Polyketide*	TE	3.6313E-016
88111	TE	1	Polyketide*	TE	3.0716E-011
87551	TE	1	Polyketide*	TE	4.0293E-043
88337	TE	1	Polyketide*	TE	1.0046E-011

*: (tetracycline)

Similar results were obtained for other genes but are omitted for the sake of brevity.

Further, a synergistic effect of individual SNPs was demonstrated by exhaustively comparing significance levels for association of single SNPs with antibiotic susceptibility/resistance and significance levels for association of combinations of SNPs with antibiotic susceptibility/resistance. For a representative example of 2 SNPs the

significance level for synergistic association of two SNPs was improved with the values given in Table 15 compared to the association of either SNP alone, given for exemplary different antibiotics.

Table 15: Synergistic increase for association of two SNPs

drug	POS 1	Ref	Alt	POS 2	Ref	Alt	Improv [%]
CAX	1398352	C	T	86770	G	A	7553.8
CFT	1398352	C	T	86770	G	A	4906.1
CP	1398352	C	T	86770	G	A	7217.7
LVX	1476885	C	G	1487651	G	T	1016.6
CAX	1476885	C	G	86770	G	A	35186.3
CFT	1476885	C	G	86770	G	A	5234.5
CP	1476885	C	G	86770	G	A	16666.2
LVX	1476885	C	G	86770	G	A	791.3
AM	1487651	G	T	1490996	T	G	27110310435.0
A/S	1487651	G	T	1490996	T	G	7387639463717348.0
AUG	1487651	G	T	1490996	T	G	22882381295241392.0
LVX	1487651	G	T	2371667	C	T	203.3
AM	1487651	G	T	3249370	A	G	4745162369.1
A/S	1487651	G	T	3249370	A	G	151190127735.9
AUG	1487651	G	T	3249370	A	G	15974939963.6
AM	1487651	G	T	3244657	A	C	16268568579657476096.0
A/S	1487651	G	T	3244657	A	C	4856552041684392738816.0
AUG	1487651	G	T	3244657	A	C	1559036069143894357442560
CAX	1487651	G	T	3244657	A	C	106.2
LVX	1487651	G	T	3244657	A	C	397.9
LVX	1487651	G	T	3243004	C	G	108.5
LVX	1487651	G	T	3231402	C	A	108.5
AM	1487651	G	T	3025014	T	G	3083339089536906.5
A/S	1487651	G	T	3025014	T	G	909065603608585338880
AUG	1487651	G	T	3025014	T	G	14107165708602796032.0
CVX	1487651	G	T	3025014	T	G	2699.8
LVX	1487651	G	T	2816076	A	C	17548.3
T/S	1487651	G	T	2816076	A	C	389.5
LVX	1487651	G	T	2687128	G	C	106.3
AM	1487651	G	T	1347521	A	G	11992584461895092224.0
A/S	1487651	G	T	1347521	A	G	4643772663859210878976.0

AUG	1487651	G	T	1347521	A	G	1848280988722277562449 92.0
CAX	1487651	G	T	4057459	T	G	125.8
AM	1487651	G	T	4143093	A	G	12911802015.4
A/S	1487651	G	T	4143093	A	G	12019751974.7
AUG	1487651	G	T	4143093	A	G	20840834.3
LVX	1487651	G	T	176654	T	G	133.4
AM	1487651	G	T	1490996	T	G	115930572249.5
A/S	1487651	G	T	1490996	T	G	53491140266121.4
AUG	1487651	G	T	1490996	T	G	2515400175210.6
LVX	1490996	T	G	3025014	T	G	2373.7
AM	1490996	T	G	3844397	A	C	283621833823.0
A/S	1490996	T	G	3844397	A	C	104788504079843856.0
AUG	1490996	T	G	3844397	A	C	466668336295372718080.0
A/S	1490996	T	G	4143093	A	G	141.3
AUG	1490996	T	G	4143093	A	G	618537.6
CAX	2371667	C	T	86770	G	A	1253.5
CFT	2371667	C	T	86770	G	A	1474.7
CP	2371667	C	T	86770	G	A	7023.8
AM	3249370	A	G	3844397	A	C	72173352.7
A/S	3249370	A	G	3844397	A	C	1900805046.5
AUG	3249370	A	G	3844397	A	C	218882023.8
CAX	3249370	A	G	86770	G	A	587.2
CFT	3249370	A	G	86770	G	A	219.3
CP	3249370	A	G	86770	G	A	884.9
AM	3244657	A	C	3844397	A	C	1909530141221417123 84.0
A/S	3244657	A	C	3844397	A	C	253290689783629002833 92.0
AUG	3244657	A	C	3844397	A	C	7167041178077088 6095732736.0
AUG	3244657	A	C	4143093	A	G	146873214.6
CAX	3244657	A	C	86770	G	A	1677.7
CFT	3244657	A	C	86770	G	A	1835.9
CP	3244657	A	C	86770	G	A	5013.9
CAX	3243004	C	G	86770	G	A	1677.7
CFT	3243004	C	G	86770	G	A	1835.9
CP	3243004	C	G	86770	G	A	5013.9
CAX	3231402	C	A	86770	G	A	1677.7
CFT	3231402	C	A	86770	G	A	1835.9
CP	3231402	C	A	86770	G	A	5013.9
AM	3025014	T	G	3844397	A	C	1908359560282912063488.0

A/S	3025014	T	G	3844397	A	C	351722837318441712529395968.0
AUG	3025014	T	G	3844397	A	C	589175898363033562145240383488.0
CAX	3025014	T	G	3844397	A	C	609.0
A/S	3025014	T	G	4143093	A	G	55269.5
AUG	3025014	T	G	4143093	A	G	2734964708.4
CAX	3025014	T	G	86770	G	A	5471.0
CFT	3025014	T	G	86770	G	A	898.6
CP	3025014	T	G	86770	G	A	75921.3
LVX	3025014	T	G	86770	G	A	1916.7
LVX	2816076	A	C	4149382	G	C	268.1
CAX	2816076	A	C	86770	G	A	184.4
CFT	2816076	A	C	86770	G	A	168.2
CP	2816076	A	C	86770	G	A	28668.1
LVX	2816076	A	C	86770	G	A	2334.7
LVX	2816076	A	C	4339539	G	C	458.4
LVX	2816076	A	C	5025276	A	C,G	2713.8
CAX	2687128	G	C	86770	G	A	1916.6
CFT	2687128	G	C	86770	G	A	1847.8
CP	2687128	G	C	86770	G	A	7145.1
AM	1347521	A	G	3844397	A	C	653844900357359488.0
A/S	1347521	A	G	3844397	A	C	903648514199348346880
AUG	1347521	A	G	3844397	A	C	752376082264290 6169868288.0
AUG	1347521	A	G	4143093	A	G	3963922539.7
CAX	1347521	A	G	86770	G	A	308.6
CFT	1347521	A	G	86770	G	A	205.9
CP	1347521	A	G	86770	G	A	1005.6
CAX	3716584	G	A	86770	G	A	176781.7
CFT	3716584	G	A	86770	G	A	141473.9
CP	3716584	G	A	86770	G	A	117836.8
AM	3844397	A	C	4057459	T	G	1732.3
A/S	3844397	A	C	4057459	T	G	64489837.0
AUG	3844397	A	C	4057459	T	G	3267527.0
AM	3844397	A	C	4143093	A	G	6257499746192352.0
A/S	3844397	A	C	4143093	A	G	143764130498273056.0
AUG	3844397	A	C	4143093	A	G	40284225184437.6
CP	3844397	A	C	86770	G	A	1725.6

LVX	3844397	A	C	86770	G	A	190.4
AM	3844397	A	C	1490996	T	G	2185306317.5
A/S	3844397	A	C	1490996	T	G	601032319774.7
AUG	3844397	A	C	1490996	T	G	5658475370741.3
CFT	4636300	T	C	4149382	G	C	100.3
CAX	4636300	T	C	86770	G	A	491347.8
CFT	4636300	T	C	86770	G	A	364274.8
CP	4636300	T	C	86770	G	A	181329.0
CAX	4057459	T	G	86770	G	A	30600.0
CFT	4057459	T	G	86770	G	A	979.7
CP	4057459	T	G	86770	G	A	7592.1
LVX	4057459	T	G	86770	G	A	138.6
CAX	4143093	A	G	86770	G	A	811.9
CFT	4143093	A	G	86770	G	A	192.7
CP	4143093	A	G	86770	G	A	24441.6
LVX	4143093	A	G	86770	G	A	11804.8
CAX	86770	G	A	176654	T	G	12106.4
CFT	86770	G	A	176654	T	G	11346.3
CP	86770	G	A	176654	T	G	4412.3
CAX	86770	G	A	4296135	A	C,G	2156.
CFT	86770	G	A	4296135	A	C,G	1515.8
CP	86770	G	A	4296135	A	C,G	8097.4

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

For example, the improvement of 8097.4 % in the last example with positions 86770 and 4296135 for CP results from a p-value change from 1.90043e-11 to 2.34696e-13.

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

A genetic test for the combined pathogen identification and antimicrobial susceptibility testing direct from the patient sample can reduce the time-to actionable result significantly

from several days to hours, thereby enabling targeted treatment. Furthermore, this approach will not be restricted to central labs, but point of care devices can be developed that allow for respective tests. Such technology along with the present methods and computer program products could revolutionize the care, e.g. in intense care units or for admissions to hospitals in general. Furthermore, even applications like real time outbreak monitoring can be achieved using the present methods.

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

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

The present method allows computing a best breakpoint for the separation of isolates into resistant and susceptible groups. The inventors designed a flexible software tool that allows to consider - besides the best breakpoints - also values 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 Serratia 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 Serratia 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 actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, wherein the presence of said at least two mutations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant Serratia strain in said patient.

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

a) obtaining or providing a sample containing or suspected of containing at least one Serratia 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

actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX, SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and SMWW4_v1c25770, 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 Serratia infection.

3. The method of one or more of the preceding claims, where the method involves determining the resistance of Serratia to one or more antimicrobial, e.g. antibiotic, drugs.

4. The method of any one of claims 1 to 3, wherein the antimicrobial, e.g. antibiotic, drug is selected from lactam antibiotics and the presence of a mutation in the following genes is determined: SMWW4_v1c13480; and/or wherein the antimicrobial, e.g. antibiotic, drug is selected from polyketide antibiotics, preferably tetracycline antibiotics, and the presence of a mutation in the following genes is determined: actP, SMWW4_v1c03050, amiD, SMWW4_v1c38520, selB, SMWW4_v1c13480, bglX,

SMWW4_v1c14040, SMWW4_v1c13470, SMWW4_v1c38510, SMWW4_v1c07960, SMWW4_v1c19810, folX, SMWW4_v1c00800, SMWW4_v1c13910, SMWW4_v1c09360, ybiO, SMWW4_v1c25040, znuB, nrdH, lysR, SMWW4_v1c24620, SMWW4_v1c24800, SMWW4_v1c20760, rfaC, SMWW4_v1c21930, SMWW4_v1c12350, galT, alsK, SMWW4_v1c24810, glrK, rihB, yhiN, alx, SMWW4_v1c44490, cnu, SMWW4_v1c30050, vasD, impL, SMWW4_v1c16540, SMWW4_v1c13350, yeaN, SMWW4_v1c40850, kdpA, dppB, ydaN, cysK, yceA, yhjK, and/or SMWW4_v1c25770.

5. 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).

6. The method of any one of claims 1 to 5, wherein the antibiotic drug is AM and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_020211: 1489693; and/or
wherein the antibiotic drug is TE and a mutation in at least one of the following nucleotide positions is detected with regard to reference genome NC_020211: 342947, 352212, 1816830, 352221, 1817267, 4149382, 86770, 86742, 86744, 1489672, 1489673, 1489681, 1490996, 1545409, 1487651, 1489693, 4148368, 897774, 2154027, 2154042, 2154044, 3716584, 87742, 1532249, 4148381, 1049796,

1601495, 4148825, 2715811, 3025014, 4143093, 4284592, 2154037, 1489972, 2662382, 2687128, 2250726, 4148361, 5161374, 5161396, 2371667, 1371641, 1398352, 4339539, 2687789, 4057459, 2716368, 4712441, 5025276, 4636300, 4812879, 3231402, 3243004, 3244657, 3249370, 3249507, 2716411, 1814748, 1476885, 1049699, 4296135, 4419488, 1347521, 1347533, 156541, 2816076, 3844397, 2018803, 176654, 176722, 176784, 2796043, 2796045.

7. The method of any one of claims 1 to 6, wherein the resistance of a bacterial microorganism belonging to the species Serratia 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.

8. 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.

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 or entire sequence of the genome of the Serratia species, wherein said partial or entire sequence of the genome comprises at least a partial sequence of said 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 using a next generation sequencing or high throughput sequencing method, preferably wherein a partial or entire genome sequence of

the bacterial organism of Serratia species is determined by using a next generation sequencing or high throughput sequencing method.

11. A method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for bacterial microorganisms of Serratia species, comprising:
obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of Serratia species;
providing a second data set of antimicrobial drug, e.g. antibiotic, resistance of the plurality of clinical isolates of Serratia species;
aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Serratia, 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 Serratia associated with antimicrobial drug, e.g. antibiotic, resistance.

12. A diagnostic method of determining an infection of a patient with Serratia 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 Serratia 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 Serratia as determined by the

method of claim 11, wherein the presence of said at least one mutation is indicative of an infection with an anti-microbial drug resistant Serratia strain in said patient.

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

a) obtaining or providing a sample containing or suspected of containing a bacterial microorganism belonging to the species Serratia 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 Serratia as determined by the method of claim 11, 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 Serratia infection.

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

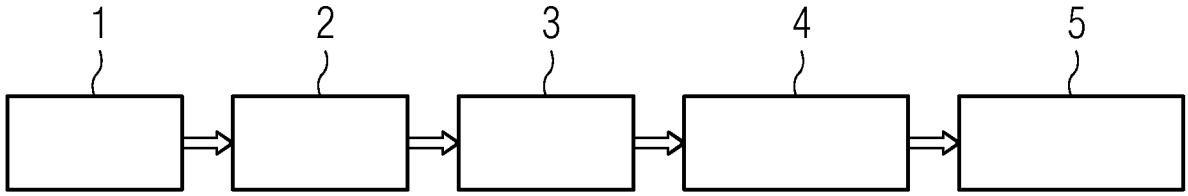
obtaining or providing a first data set of gene sequences of a clinical isolate of Serratia species;

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

aligning the gene sequences of the first data set to at least one, preferably one, reference genome of Serratia, 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 Serratia of the first data set associated with antimicrobial drug, e.g. antibiotic, resistance.

15. Computer program product comprising computer executable instructions which, when executed, perform a method according to any one of claims 11 to 14.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