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(54) Title: GENETIC TESTING FOR ALIGNMENT-FREE PREDICTING RESISTANCE OF MICROORGANISMS AGAINST ANTIMICROBIAL AGENTS

(57) Abstract: The present invention relates to a method of determining an infection of a patient with at least one microorganism, particularly a bacterial microorganism, potentially resistant to antimicrobial drug treatment, a method of selecting a treatment of a patient suffering from an infection with at least one microorganism, particularly bacterial microorganism, and a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for at least one microorganism, particularly bacterial microorganism, as well as computer program products used in these methods.



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Description

Genetic testing for alignment-free predicting resistance of microorganisms against antimicrobial agents

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

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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 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.

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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

30

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%.

Staphylococcus is a genus of Gram-positive, facultative anaerobe bacteria of the family Staphylococcaceae, which are spherical, immobile and form grape-like clusters. The genus includes at least 40 species.

Staphylococcus aureus is the most common species of staphylococcus to cause Staph infections. It is frequently found in the human respiratory tract and on the skin. Although Staphylococcus aureus is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning as well as life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Staphylococcus aureus can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain. The position of *S. aureus* as one of the most important opportunistic human pathogens is largely attributable to the combination of its virulence potential and its ubiquitous occurrence as a colonizer in humans, domestic animals, and livestock.

Staphylococcus aureus is the second most common overall cause of healthcare-associated infections reported to the National Healthcare Safety Network (NHSN). And current estimates suggest that 49-65% of healthcare-associated Staphylococcus aureus infections reported to NHSN are caused by methicil-

lin-resistant *Staphylococcus aureus* (MRSA). MRSA is troublesome in hospitals, prisons, and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of nosocomial infection than the
5 general public. MRSA began as a hospital-acquired infection, but has developed limited endemic status and is now sometimes community-acquired. Healthcare-associated MRSA (HA-MRSA) is related to prolonged length of hospital stay and is currently one of the most frequently identified pathogens in hospitals
10 in many parts of the world. Furthermore, community acquired MRSA (CAMRSA) has demonstrated increasing trends, hence guidelines for prevention and surveillance have been issued by several healthcare officials.

15 In 2011 the CDC estimates 80,461 invasive MRSA infections and 11,285 related deaths occurred in the US. An unknown but much higher number of less severe infections occurred in both the community and in healthcare settings. MRSA is difficult to treat because of its resistance to most antibiotics. Treatment with vancomycin, a glycopeptide antibiotic often consid-
20 ered a last line of defense against MRSA, has led to the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA), against which few agents are effective. In addition, the use of teicoplanin, an antibiotic derived from
25 vancomycin, has given rise to teicoplanin-resistant MRSA strains. There are other agents available to treat MRSA infection, though many have limited therapeutic benefit, primarily because of severe side effects.

30 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.

Resistance to methicillin and related drugs is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding β -lactams (penicillins, cephalosporins, and carbapenems). This allows for resistance to all β -lactam antibiotics, and obviates their clinical use during MRSA infections. As such, the glycopeptide vancomycin is often deployed against MRSA.

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. Automated systems exist from several companies, e.g. Biomeriux (Vitek), Beckman Coulter (Microscan). 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.

5 The use of molecular techniques for direct detection of MRSA has become more commonplace especially for screening purposes. Resistance to methicillin is mediated via the mec operon which is part of the staphylococcal cassette chromosome mec (SCCmec). Recently PCR tests were introduced that are based
10 on the detection of the right extremity sequence of the SCCmec in combination with *S. aureus* specific marker. Initial reports exist that describe culture based susceptibility reports despite detection of the presence of a resistance conferring gene.

15 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
20 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.

25 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
30 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
5 *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
10 Identification of single nucleotide 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*.

Gordon et al (Gordon et al (2014), Prediction of *Staphylococcus aureus* Antimicrobial Resistance by Whole-Genome Sequencing, J. Clin.Microbiol. 2014, 52(4):1182) and Dordel et al (Dordel, J., Kim, C. et al (2014). Novel Determinants of Antibiotic Resistance: Identification of Mutated Loci in Highly
15 Methicillin-Resistant Subpopulations of Methicillin-Resistant *Staphylococcus aureus*. mBio, 5(2), e01000-13; doi:10.1128/mBio.01000-13) focused in their identification of new markers on already known genes.
20

The fast and accurate detection of infections with microorganisms, particularly microbial species, e.g. *Staphylococcus aureus*, and the prediction of response to anti-microbial therapy, particularly also without reference to known genes, represent a high unmet clinical need.
25

30 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 microorganisms, particularly bacterial microorganisms, particularly *Staphylococcus aureus* clinical isolates, and comparing the genetic mutation profile to resistant phenotypes of isolates and/or 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 bacterial species, particularly *Staphylococcus* species, particularly *Staphylococcus aureus*, either susceptible or resistant to antimicrobial, e.g. antibiotic, drugs, particularly being susceptible or resistant to methicillin and related drugs. Based on this information, it is now possible to provide a detailed analysis on the resistance pattern of *Staphylococcus*, particularly *Staphylococcus aureus*, strains based on individual 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 microbial, e.g. *Staphylococcus*, particularly *Staphylococcus aureus*, infection in a

patient and thus will largely improve the quality of diagnosis and treatment.

The present approach is based on the use of reference free
5 SNP calling and association testing to cover the different sources of genetic resistance as well as the different ways of how bacteria can become resistant. This way the detection of resistances is not limited to reference genomes anymore

10 In contrast to other works (e.g. Gordon et al, Dordel et al, see above) the identification of new markers was not focused on already known genes, but a reference free SNP calling was performed, and the annotation is based on several reference genomes.

15

According to a first aspect, the present invention relates to a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for a microorganism, particularly a bacterial microorganism, comprising:

20 obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of the microorganism;

wherein at least a part of the gene sequences of the first data set are assembled;

analyzing the gene sequences of the first data set for genet-

25 ic variants to obtain a third data set of genetic variants;

providing a second data set of antimicrobial drug, e.g. antibiotic, resistance and/or susceptibility of the plurality of clinical isolates of the microorganism;

correlating the third data set with the second data set and

30 statistically analyzing the correlation; and

determining the genetic sites in the genome of the microorganism with antimicrobial drug, e.g. antibiotic, resistance.

In a second aspect the present invention discloses a diagnostic method of determining an infection of a patient with a microorganism, particularly a bacterial microorganism potentially resistant to antimicrobial drug treatment, comprising the steps of:

- a) obtaining or providing a sample containing or suspected of containing a microorganism, particularly a bacterial microorganism, from the patient;
- b) determining the presence of at least one genetic variant in at least one position of the microorganism, particularly the bacterial microorganism, as determined by the method of the first aspect, wherein the presence of said at least one genetic variant is indicative of an infection with an antimicrobial drug resistant microorganism in said patient.

A third aspect of the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant microorganism, particularly bacterial microorganism, comprising the steps of:

- a) obtaining or providing a sample containing or suspected of containing a microorganism, particularly a bacterial microorganism, from the patient;
- b) determining the presence of at least one genetic variant in at least one position of the microorganism, particularly bacterial microorganism, as determined by the method of the first aspect, wherein the presence of said at least one genetic variant 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 the infection with the microorganism, particularly the bacterial microorganism.

A fourth aspect of the present invention relates to a method
5 of acquiring, respectively determining, an antimicrobial
drug, e.g. antibiotic, resistance profile for a clinical isolate
of a microorganism, particularly a bacterial microorganism,
comprising:

obtaining or providing at least one gene sequence of the
10 clinical isolate of the microorganism, particularly the bacterial
microorganism; and

determining the presence of genetic variants in the at least
one gene sequence of the clinical isolate of the microorganism,
particularly bacterial microorganism, as determined by
15 the method of the first aspect of the present invention.

Furthermore, a computer program product comprising computer
executable instructions which, when executed, perform a method
according to any one of the first to third aspects of the
20 present invention is disclosed in a fifth aspect of the present
invention.

In addition, a sixth aspect of the present invention relates
to a diagnostic method of determining an infection of a patient
25 with a Staphylococcus species, particularly Staphylococcus
aureus, 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 Staphylococcus species, particularly
30 larly Staphylococcus aureus, from the patient;

b) determining the presence of at least one genetic variation
in at least two positions from the group of positions
annotated with Nos. 1 - 50 with regard to the reference ge-

nomes with the genome names given in Table 1 below, wherein the presence of said at least two genetic variations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain in said patient, wherein for some positions more than one position in different reference genomes is annotated.

Table 1: List of positions with Nos. 1 - 50

No.	position	reference genome	genome name
1	534953 F	NC_017340.1	04_02981
	543821 F	NC_010079.1	USA300_TCH1516
2	210528 R	NC_022222.1	6850
	267448 R	NC_017340.1	04_02981
	269814 R	NC_010079.1	USA300_TCH1516
3	1362060 F	NC_017340.1	04_02981
4	1252703 R	NC_021670.1	Bmb9393
	1520285 F	NC_017351.1	11819_97
	1523326 F	NC_017340.1	04_02981
5	1619285 R	NC_017340.1	04_02981
	1661238 R	NC_010079.1	USA300_TCH1516
6	1641150 R	NC_017340.1	04_02981
7	170059 F	NC_002953.3	MSSA476
	142263 F	NC_017337.1	ED133
8	517571 F	NC_017340.1	04_02981
	554542 F	NC_018608.1	08BA02176
9	978538 F	NC_017340.1	04_02981
10	1434811 R	NC_017340.1	04_02981
11	953696 R	NC_022222.1	6850
	1010027 R	NC_010079.1	USA300_TCH1516
12	208285 R	NC_017340.1	04_02981
	161011 R	NC_007795.1	NCTC_8325
13	2179136 R	NC_017351.1	11819_97
	2149064 R	NC_017340.1	04_02981
	2107689 R	NC_018608.1	08BA02176
14	2358535 F	NC_017340.1	04_02981
15	2023012 R	NC_017340.1	04_02981
16	2777211 F	NC_007795.1	NCTC_8325
	2779170 F	NC_017340.1	04_02981

17	1801995 R 1790672 R	NC_018608.1 NC_017340.1	08BA02176 04_02981
18	976788 F 878040 F	NC_017340.1 NC_007795.1	04_02981 NCTC_8325
19	2101899 R 1972149 R	NC_021670.1 NC_017340.1	Bmb9393 04_02981
20	1875550 F 2006001 F 1959494 F	NC_022222.1 NC_010079.1 NC_017340.1	6850 USA300_TCH1516 04_02981
21	705667 R	NC_017340.1	04_02981
22	2268723 F 2221448 F	NC_010079.1 NC_017340.1	USA300_TCH1516 04_02981
23	1814108 R	NC_017340.1	04_02981
24	531649 R 531398 R	NC_017340.1 NC_017351.1	04_02981 11819_97
25	1754561 F 1691742 F	NC_017340.1 NC_007795.1	04_02981 NCTC_8325
26	1958403 R 2004910 R	NC_017340.1 NC_010079.1	04_02981 USA300_TCH1516
27	1242653 R 1294527 R 1299554 R	NC_022222.1 NC_010079.1 NC_017340.1	6850 USA300_TCH1516 04_02981
28	2590222 R 2637689 R	NC_017340.1 NC_010079.1	04_02981 USA300_TCH1516
29	1881161 R 1871101 R 1759861 R 1855493 R 1858794 R 1964828 R	NC_010079.1 NC_021059.1 NC_022222.1 NC_018608.1 NC_021554.1 NC_021670.1	USA300_TCH1516 M1 6850 08BA02176 CC45 Bmb9393
30	1050123 R 1147277 R	NC_007795.1 NC_017340.1	NCTC_8325 04_02981
31	2005634 F 2039052 F 2187801 F	NC_016912.1 NC_022222.1 NC_010079.1	VC40 6850 USA300_TCH1516
32	350202 F 402479 F 352104 F	NC_022222.1 NC_017340.1 NC_007795.1	6850 04_02981 NCTC_8325
33	920768 F 956878 F 956978 F 858255 F	NC_021059.1 NC_018608.1 NC_017340.1 NC_007795.1	M1 08BA02176 04_02981 NCTC_8325

34	1121847 R	NC_017340.1	04_02981
	1024692 R	NC_007795.1	NCTC_8325
35	429303 F	NC_017340.1	04_02981
36	1812380 R	NC_010079.1	USA300_TCH1516
	1775835 R	NC_017340.1	04_02981
	1714993 R	NC_007795.1	NCTC_8325
37	1928346 F	NC_017340.1	04_02981
38	1388095 R	NC_022226.1	CN1
39	559072 R	NC_017340.1	04_02981
	504007 R	NC_007795.1	NCTC_8325
40	2719339 R	NC_010079.1	USA300_TCH1516
	2668764 R	NC_017340.1	04_02981
41	1124668 F	NC_017340.1	04_02981
	1121585 F	NC_010079.1	USA300_TCH1516
42	158073 F	NC_022226.1	CN1
	193628 F	NC_021059.1	M1
	138357 F	NC_022222.1	6850
	196480 F	NC_010079.1	USA300_TCH1516
	189192 F	NC_017340.1	04_02981
43	1187805 F	NC_017340.1	04_02981
	1078815 F	NC_022113.1	55_2053
	1182930 F	NC_010079.1	USA300_TCH1516
44	1376396 R	NC_010079.1	USA300_TCH1516
	1323236 R	NC_022222.1	6850
	1315892 R	NC_022226.1	CN1
	1379143 R	NC_017340.1	04_02981
	1399306 F	NC_021670.1	Bmb9393
45	2398505 R	NC_017340.1	04_02981
46	2753541 F	NC_017340.1	04_02981
	2803165 F	NC_010079.1	USA300_TCH1516
47	1415365 R	NC_017340.1	04_02981
	1428821 R	NC_018608.1	08BA02176
	1318646 R	NC_007795.1	NCTC_8325
	1412563 R	NC_010079.1	USA300_TCH1516
	1381147 R	NC_021059.1	M1
48	1678734 R	NC_017340.1	04_02981
	1720315 R	NC_010079.1	USA300_TCH1516
49	854815 R	NC_007795.1	NCTC_8325
	953539 R	NC_017340.1	04_02981
	948900 R	NC_010079.1	USA300_TCH1516
50	1675156 R	NC_017340.1	04_02981

A seventh aspect of the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain, comprising the steps of:

- 5 a) obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus*, particularly *Staphylococcus aureus*, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions
10 annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1, wherein the presence of said at least two genetic variations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position
15 in different reference genomes is annotated;
- 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
20 suitable for the treatment of a *Staphylococcus*, particularly *Staphylococcus aureus*, infection.

Further, an eighth aspect of the present invention relates to a diagnostic method of determining an infection of a patient
25 with a *Staphylococcus* species, particularly *Staphylococcus aureus*, 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 *Staphylococcus*, particularly
30 *Staphylococcus aureus*, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference ge-

nomes with the genome names given in Tables 3a and/or 3b below, wherein the presence of said at least two genetic variations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain in said patient, wherein for some positions more than one position in different reference genomes is annotated.

Table 3a: List of positions with Nos. 1 - 50

No.	position	reference genome	genome name
1	1958403 R	NC_017340.1	04_02981
	2004910 R	NC_010079.1	USA300_TCH1516
2	1641150 R	NC_017340.1	04_02981
3	978538 F	NC_017340.1	04_02981
4	705667 R	NC_017340.1	04_02981
5	1434811 R	NC_017340.1	04_02981
6	953696 R	NC_022222.1	6850
	1010027 R	NC_010079.1	USA300_TCH1516
7	2101899 R	NC_021670.1	Bmb9393
	1972149 R	NC_017340.1	04_02981
8	208285 R	NC_017340.1	04_02981
	161011 R	NC_007795.1	NCTC_8325
9	2179136 R	NC_017351.1	11819_97
	2149064 R	NC_017340.1	04_02981
	2107689 R	NC_018608.1	08BA02176
10	2358535 F	NC_017340.1	04_02981
11	2023012 R	NC_017340.1	04_02981
12	2777211 F	NC_007795.1	NCTC_8325
	2779170 F	NC_017340.1	04_02981
13	1801995 R	NC_018608.1	08BA02176
	1790672 R	NC_017340.1	04_02981
14	1754561 F	NC_017340.1	04_02981
	1691742 F	NC_007795.1	NCTC_8325
15	1362060 F	NC_017340.1	04_02981
16	1242653 R	NC_022222.1	6850
	1294527 R	NC_010079.1	USA300_TCH1516
	1299554 R	NC_017340.1	04_02981
17	1252703 R	NC_021670.1	Bmb9393
	1520285 F	NC_017351.1	11819_97
	1523326 F	NC_017340.1	04_02981

18	1619285 R 1661238 R	NC_017340.1 NC_010079.1	04_02981 USA300_TCH1516
19	1875550 F 2006001 F 1959494 F	NC_022222.1 NC_010079.1 NC_017340.1	6850 USA300_TCH1516 04_02981
20	976788 F 878040 F	NC_017340.1 NC_007795.1	04_02981 NCTC_8325
21	2590222 R 2637689 R	NC_017340.1 NC_010079.1	04_02981 USA300_TCH1516
22	210528 R 267448 R 269814 R	NC_022222.1 NC_017340.1 NC_010079.1	6850 04_02981 USA300_TCH1516
23	1814108 R	NC_017340.1	04_02981
24	170059 F 142263 F	NC_002953.3 NC_017337.1	MSSA476 ED133
25	534953 F 543821 F	NC_017340.1 NC_010079.1	04_02981 USA300_TCH1516
26	517571 F 554542 F	NC_017340.1 NC_018608.1	04_02981 08BA02176
27	531649 R 531398 R	NC_017340.1 NC_017351.1	04_02981 11819_97
28	1050123 R 1147277 R	NC_007795.1 NC_017340.1	NCTC_8325 04_02981
29	1881161 R 1871101 R 1759861 R 1855493 R 1858794 R 1964828 R	NC_010079.1 NC_021059.1 NC_022222.1 NC_018608.1 NC_021554.1 NC_021670.1	USA300_TCH1516 M1 6850 08BA02176 CC45 Bmb9393
30	2268723 F 2221448 F	NC_010079.1 NC_017340.1	USA300_TCH1516 04_02981
31	920768 F 956878 F 956978 F 858255 F	NC_021059.1 NC_018608.1 NC_017340.1 NC_007795.1	M1 08BA02176 04_02981 NCTC_8325
32	2005634 F 2039052 F 2187801 F	NC_016912.1 NC_022222.1 NC_010079.1	VC40 6850 USA300_TCH1516
33	429303 F	NC_017340.1	04_02981
34	350202 F 402479 F 352104 F	NC_022222.1 NC_017340.1 NC_007795.1	6850 04_02981 NCTC_8325

35	158073 F	NC_022226.1	CN1
	193628 F	NC_021059.1	M1
	138357 F	NC_022222.1	6850
	196480 F	NC_010079.1	USA300_TCH1516
	189192 F	NC_017340.1	04_02981
36	1121847 R	NC_017340.1	04_02981
	1024692 R	NC_007795.1	NCTC_8325
37	2719339 R	NC_010079.1	USA300_TCH1516
	2668764 R	NC_017340.1	04_02981
38	1388095 R	NC_022226.1	CN1
39	1415365 R	NC_017340.1	04_02981
	1428821 R	NC_018608.1	08BA02176
	1318646 R	NC_007795.1	NCTC_8325
	1412563 R	NC_010079.1	USA300_TCH1516
	1381147 R	NC_021059.1	M1
40	1678734 R	NC_017340.1	04_02981
	1720315 R	NC_010079.1	USA300_TCH1516
41	1928346 F	NC_017340.1	04_02981
42	1376396 R	NC_010079.1	USA300_TCH1516
	1323236 R	NC_022222.1	6850
	1315892 R	NC_022226.1	CN1
	1379143 R	NC_017340.1	04_02981
	1399306 F	NC_021670.1	Bmb9393
43	1338943 R	NC_017340.1	04_02981
44	1124668 F	NC_017340.1	04_02981
	1121585 F	NC_010079.1	USA300_TCH1516
45	559072 R	NC_017340.1	04_02981
	504007 R	NC_007795.1	NCTC_8325
46	1675156 R	NC_017340.1	04_02981
47	1187805 F	NC_017340.1	04_02981
	1078815 F	NC_022113.1	55_2053
	1182930 F	NC_010079.1	USA300_TCH1516
48	1356138 F	NC_017340.1	04_02981
49	854815 R	NC_007795.1	NCTC_8325
	953539 R	NC_017340.1	04_02981
	948900 R	NC_010079.1	USA300_TCH1516
50	2459738 F	NC_017340.1	04_02981
	2364478 F	NC_022222.1	6850

Table 3b: List of positions with Nos. 1 - 50

No.	position	reference genome	genome name
1	1958403 R	NC_017340.1	04_02981

	2004910 R	NC_010079.1	USA300_TCH1516
2	1641150 R	NC_017340.1	04_02981
3	978538 F	NC_017340.1	04_02981
4	705667 R	NC_017340.1	04_02981
5	1434811 R	NC_017340.1	04_02981
6	953696 R	NC_022222.1	6850
	1010027 R	NC_010079.1	USA300_TCH1516
7	2101899 R	NC_021670.1	Bmb9393
	1972149 R	NC_017340.1	04_02981
8	208285 R	NC_017340.1	04_02981
	161011 R	NC_007795.1	NCTC_8325
9	2179136 R	NC_017351.1	11819_97
	2149064 R	NC_017340.1	04_02981
	2107689 R	NC_018608.1	08BA02176
10	2358535 F	NC_017340.1	04_02981
11	2023012 R	NC_017340.1	04_02981
12	2777211 F	NC_007795.1	NCTC_8325
	2779170 F	NC_017340.1	04_02981
13	1801995 R	NC_018608.1	08BA02176
	1790672 R	NC_017340.1	04_02981
14	1754561 F	NC_017340.1	04_02981
	1691742 F	NC_007795.1	NCTC_8325
15	1362060 F	NC_017340.1	04_02981
16	1242653 R	NC_022222.1	6850
	1294527 R	NC_010079.1	USA300_TCH1516
	1299554 R	NC_017340.1	04_02981
17	1252703 R	NC_021670.1	Bmb9393
	1520285 F	NC_017351.1	11819_97
	1523326 F	NC_017340.1	04_02981
18	1619285 R	NC_017340.1	04_02981
	1661238 R	NC_010079.1	USA300_TCH1516
19	1875550 F	NC_022222.1	6850
	2006001 F	NC_010079.1	USA300_TCH1516
	1959494 F	NC_017340.1	04_02981
20	976788 F	NC_017340.1	04_02981
	878040 F	NC_007795.1	NCTC_8325
21	2590222 R	NC_017340.1	04_02981
	2637689 R	NC_010079.1	USA300_TCH1516
22	210528 R	NC_022222.1	6850
	267448 R	NC_017340.1	04_02981
	269814 R	NC_010079.1	USA300_TCH1516
23	1814108 R	NC_017340.1	04_02981

24	170059 F	NC_002953.3	MSSA476
	142263 F	NC_017337.1	ED133
25	534953 F	NC_017340.1	04_02981
	543821 F	NC_010079.1	USA300_TCH1516
26	517571 F	NC_017340.1	04_02981
	554542 F	NC_018608.1	08BA02176
27	531649 R	NC_017340.1	04_02981
	531398 R	NC_017351.1	11819_97
28	1050123 R	NC_007795.1	NCTC_8325
	1147277 R	NC_017340.1	04_02981
29	1881161 R	NC_010079.1	USA300_TCH1516
	1871101 R	NC_021059.1	M1
	1759861 R	NC_022222.1	6850
	1855493 R	NC_018608.1	08BA02176
	1858794 R	NC_021554.1	CC45
	1964828 R	NC_021670.1	Bmb9393
30	2268723 F	NC_010079.1	USA300_TCH1516
	2221448 F	NC_017340.1	04_02981
31	920768 F	NC_021059.1	M1
	956878 F	NC_018608.1	08BA02176
	956978 F	NC_017340.1	04_02981
	858255 F	NC_007795.1	NCTC_8325
32	2005634 F	NC_016912.1	VC40
	2039052 F	NC_022222.1	6850
	2187801 F	NC_010079.1	USA300_TCH1516
33	429303 F	NC_017340.1	04_02981
34	350202 F	NC_022222.1	6850
	402479 F	NC_017340.1	04_02981
	352104 F	NC_007795.1	NCTC_8325
35	158073 F	NC_022226.1	CN1
	193628 F	NC_021059.1	M1
	138357 F	NC_022222.1	6850
	196480 F	NC_010079.1	USA300_TCH1516
	189192 F	NC_017340.1	04_02981
36	1121847 R	NC_017340.1	04_02981
	1024692 R	NC_007795.1	NCTC_8325
37	2719339 R	NC_010079.1	USA300_TCH1516
	2668764 R	NC_017340.1	04_02981
38	1388095 R	NC_022226.1	CN1
39	1415365 R	NC_017340.1	04_02981
	1428821 R	NC_018608.1	08BA02176
	1318646 R	NC_007795.1	NCTC_8325
	1412563 R	NC_010079.1	USA300_TCH1516

	1381147 R	NC_021059.1	M1
40	1678734 R	NC_017340.1	04_02981
	1720315 R	NC_010079.1	USA300_TCH1516
41	1928346 F	NC_017340.1	04_02981
42	1376396 R	NC_010079.1	USA300_TCH1516
	1323236 R	NC_022222.1	6850
	1315892 R	NC_022226.1	CN1
	1379143 R	NC_017340.1	04_02981
	1399306 F	NC_021670.1	Bmb9393
43	1124668 F	NC_017340.1	04_02981
	1121585 F	NC_010079.1	USA300_TCH1516
44	559072 R	NC_017340.1	04_02981
	504007 R	NC_007795.1	NCTC_8325
45	1675156 R	NC_017340.1	04_02981
46	1187805 F	NC_017340.1	04_02981
	1078815 F	NC_022113.1	55_2053
	1182930 F	NC_010079.1	USA300_TCH1516
47	1356138 F	NC_017340.1	04_02981
48	854815 R	NC_007795.1	NCTC_8325
	953539 R	NC_017340.1	04_02981
	948900 R	NC_010079.1	USA300_TCH1516
49	2459738 F	NC_017340.1	04_02981
	2364478 F	NC_022222.1	6850
50	1812380 R	NC_010079.1	USA300_TCH1516
	1775835 R	NC_017340.1	04_02981
	1714993 R	NC_007795.1	NCTC_8325

A ninth aspect of the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain, comprising the steps of:

- obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus*, particularly *Staphylococcus aureus*, strain from the patient;
- determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b, wherein the presence of said at least two genetic variations

is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;

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 Staphylococcus, particularly Staphylococcus aureus, infection.

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
5 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 embodi-
10 ments, 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
15 molecules with incorporated nucleotide analogs or cDNA.

The term "nucleic acid sequence information" relates to an information which can be derived from the sequence of a nucleic acid molecule, such as the sequence itself or a variation in the sequence as compared to a reference sequence.
20

In general, the term "genetic variation" relates to a position in the genome where at least two random organisms of a species are different. The term "genetic variation" particularly relates to a variation in the sequence as compared to one or more reference sequences, e.g. single nucleotide polymorphisms (SNPs), mutations, copy number variations, etc. Such reference sequences can be sequences determined in a predominant wild type organism or a reference organism, e.g. a defined and known bacterial strain or substrain, e.g. of a bacterial species like *Staphylococcus aureus*, which can have large variations in gene content among closely related
25
30

strains. A genetic variation is for example a deletion of one or multiple nucleotides, an insertion of one or multiple nucleotides, or substitution of one or multiple nucleotides, duplication of one or a sequence of multiple nucleotides, translocation of one or a sequence of multiple nucleotides, and, in particular, a single nucleotide polymorphism (SNP).

For clearly identifying the variations, these can then be annotated to one or more reference sequences, e.g. a defined and known bacterial strain or substrain, but also a pan-genome of a microorganism like *Staphylococcus aureus*. The pan-genome generally includes the genes present in all strains of the microorganism, e.g. the bacterial species, as well as genes present in two or more strains, and genes specific to single strains.

According to certain embodiments, genetic variations were obtained with alignment-free methods, e.g. for detecting single base exchanges, for example based on contigs that were constructed by assemblies. For example, reads obtained from sequencing can be assembled to contigs and the contigs can be compared to each other.

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 Staphylococcus aureus strains.

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.

5 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, 10 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 15 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, 25 Forth, Henschler, Rummel "Allgemeine und spezielle Pharmakologie und Toxikologie", 9th edition, 2005 might be used as a guideline. Regarding the formulation of a ready-to-use medicament, reference is made to "Remington, The Science and Practice of Pharmacy", 22nd edition, 2013. 30

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

According to a first aspect, the present invention relates to a method of determining an antimicrobial drug, e.g. antibiotic, resistance profile for a microorganism, particularly a bacterial microorganism, comprising:

obtaining or providing a first data set of gene sequences of a plurality of clinical isolates of the microorganism; wherein at least a part of the gene sequences of the first data set are assembled;

analyzing the gene sequences of the first data set for genetic variants to obtain a third data set of genetic variants; providing a second data set of antimicrobial drug, e.g. antibiotic, resistance and/or susceptibility of the plurality of clinical isolates of the microorganism;

correlating the third data set with the second data set and statistically analyzing the correlation; and determining the genetic sites in the genome of the microorganism with antimicrobial drug, e.g. antibiotic, resistance.

In this method, as well as the other methods of the invention, the first data set of gene sequences of a plurality of clinical isolates can be provided or obtained in any way, preferably non-invasive, and can be e.g. provided from *in vitro* samples.

According to certain embodiments, the obtaining or providing of gene sequences of a plurality of clinical isolates 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 a bacterial microorganism, e.g. of the species *Staphylococcus aureus*. 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 of the microorganism, e.g. of *Staphylococcus aureus* 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 microorganism, particularly *Staphylococcus aureus* - discarding additional data from other sources, e.g. the vertebrate. According to certain embodiments, at least a part of the gene sequences of the first data set are assembled, wherein assembly can be carried out by any known method and is not particularly limited. According to certain embodiments, the data of the gene sequences are essentially all or all assembled. However, also data from genomes of known species, e.g. from bacterial species like *Staphylococcus aureus*, that are already known, e.g. from databases like at the NCBI, can be used in the first data set.

For some organisms, it might be 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, and particularly also genetic variations that are not on genes, particularly known genes, can be missed when aligning sequence data to a reference genome. One possibility to overcome this is to fall back on a virtual pan-genome which contains all sequences of a certain genus or to perform reference free variation calling. 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) can be applied and the data can be compared to several reference genomes. In such a case, $n \times k$ complete alignments are carried out. Having a big number of references, stable results can be obtained, as is the case for e.g. *Staphylococcus aureus*. Further, due to the high division rate under stress / an exogenous signal a jump in the mutation rate can be observed.

According to the invention, the gene sequence of the first data set are 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.

5 According to certain embodiments, the data of nucleic acids of different origin than the microorganism of interest, e.g. a bacterial microorganism like *Staphylococcus aureus*, can be removed after the nucleic acids of interest are identified, e.g. by filtering the data out. Such data can e.g. include
10 nucleic acids of a 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-
15 tools are available. This way the original data amount from the sample can be drastically reduced.

After such removal of "excess" data, obtaining the third data set can be carried out for the microorganism, e.g. *Staphylococcus aureus*, as described above.
20

Using these techniques, genetic variations in the gene sequences of the microorganism of interest, e.g. a bacterial microorganism like *Staphylococcus aureus*, can be obtained for
25 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
30 be cross-referenced/correlated with the genetic variations in

the genome of the respective microorganism, e.g. *Staphylococcus aureus*. Using several, e.g. 50 or more than 50, 100 or more than 100, 200 or more than 200, 400 or more than 400, 800 or more than 800, or 900 or more than 900 different isolates of the same or different species of a microorganism, e.g. of *Staphylococcus aureus*, statistical analysis can be carried out on the obtained cross-referenced data between genetic variations and antimicrobial drug, e.g. antibiotic, susceptibility for these microorganisms, using known methods.

Regarding culturing methods, samples of microorganisms 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.

Also, resistance testing can be carried out by determining e.g. known resistance genes in the different isolates, e.g. in case of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin susceptible *Staphylococcus aureus* (MSSA), but also regarding resistances of *Staphylococcus* to one or more (different) drugs, e.g. antibiotics. For determining resistances, respectively susceptibility, the data from culturing methods and/or from determining known resistance genes, as well as data obtained in different ways, e.g. based on mass spectrometry (possibly also in connection with culturing) can be used.

Correlation of the genetic variations 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 genetic variances in the whole
5 genome of the respective microorganism or only parts thereof, for example only coding parts of the genome. In some cases even only genetic variations in genes, e.g. certain genes, or certain mutations, e.g. SNPs, in genes can be determined. After correlation, statistical analysis can be carried out.

10

According to certain embodiments, the genetic variants in the gene sequences of the first data set are single nucleotide polymorphisms (SNPs).

15 According to certain embodiments, the data of the first data set, particularly SNPs, can be filtered prior to a possible annotation to a pan-genome and/or reference genome(s) and the correlation with the resistance/susceptibility data.

20 For example, to reduce the number of similar annotations they can be filtered and aggregated by one or more of the following:

- Only annotations for which the considered SNP lies on a protein can be kept and the further data discarded
- 25 • Only annotations which do not contain "hypothetical proteins" can be kept
- Annotations can be sorted by SNP identification number (ID) and gene product
- For a unique pair of SNP IDs and gene products only the
30 first annotation can be kept

Also, according to certain embodiments, the following SNPs can be excluded:

- SNPs without any annotation or SNPs whose all annotations contain flag "synonymous", so that only SNPs with at least one non-synonymous annotation, e.g. a non-synonymous coding, are considered
- 5 • Constant SNPs, i.e. with the same value for all samples
- Almost constant SNPs: SNPs whose most frequent value has a frequency $\geq 95\%$, i.e. min. 95% of all samples have the same SNP value
- SNPs with a missing value ("–") for more than 10% of
10 samples can also be removed

According to certain embodiments, the SNPs are detected alignment-free. This way also SNPs can be found that are not found in one or more certain reference genomes. For example,
15 the assembled gene sequences can be compared to each other, as described above. Nevertheless, as noted above, it is also possible to include known gene sequences of microorganisms of e.g. the same species, e.g. *Staphylococcus* species, particularly *Staphylococcus aureus*, that are e.g. deposited for the
20 public, e.g. at the NCBI, and use also these data for finding genetic variations.

According to certain embodiments, the SNPs are annotated to a pan-genome of the microorganism and/or annotated to one or
25 more reference genomes of the microorganism, e.g. a *Staphylococcus* species, particularly *Staphylococcus aureus*. For example, according to certain embodiments the microorganism used in the above method is a *Staphylococcus* species, particularly *Staphylococcus aureus*, and the antimicrobial drug is methi-
30 cillin, and/or one or more of the antibiotics described below. For such embodiments, the 50 genetic variations with the highest statistical probability (particularly using 49 finished *S. aureus* genomes from NCBI including the chromosome

and available plasmids and 995 *S. aureus* de novo assemblies which have an assembly) determined according to the present method obtained are the ones given in Table 1. In Table 1, the position of the genetic variation (named "position"; with
 5 R being reverse direction and F being forward direction) are given for each variation (given with consecutive numbers 1 - 50) with reference to one or more known reference genomes from the NCBI (with the NCBI number given in the column "reference genome" and the genome name given in the column "genome name"). The reference genomes are attached to this ap-
 10 plication as sequence listing.

The reference genomes used in Table 1 for annotation thereby were obtained from the following *Staphylococcus aureus*
 15 strains and are as follows: NC_017340, NC_010079, NC_022222, NC_021670, NC_017351, NC_002953, NC_017337, NC_018608, NC_007795, NC_021059, NC_021554, NC_016912, NC_022226, and NC_022113, given in the following in the same order in more detail:

20
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_017340
 LOCUS NC_017340 2821452 bp DNA circular CON 15-JUL-2015
 DEFINITION *Staphylococcus aureus* 04-02981, complete genome.
 ACCESSION NC_017340
 25 VERSION NC_017340.1 GI:387149188
 DBLINK BioProject: PRJNA224116
 BioSample: SAMN02603764
 Assembly: GCF_000025145.1
 KEYWORDS RefSeq.
 30 SOURCE *Staphylococcus aureus* 04-02981
 ORGANISM *Staphylococcus aureus* 04-02981
 Bacteria; Firmicutes; Bacilli; Bacillales; *Staphylococcus*.

REFERENCE 1 (bases 1 to 2821452)

AUTHORS Nubel,U., Dordel,J., Kurt,K., Strommenger,B.,
Westh,H., Shukla,S.K., Zemlickova,H., Leblois,R., Wirth,T.,
Jombart,T., Balloux,F. and Witte,W.

5 TITLE A timescale for evolution, population expansion,
and spatial spread of an emerging clone of methicillin-
resistant *Staphylococcus aureus*

JOURNAL PLoS Pathog. 6 (4), E1000855 (2010)

PUBMED 20386717

10 REMARK Publication Status: Online-Only

REFERENCE 2 (bases 1 to 2821452)

AUTHORS Nuebel,U., Dordel,J., Kurt,K., Strommenger,B.,
Westh,H., Shukla,S.K., Zemlickova,H., Leblois,R., Wirth,T.,
Jombart,T., Balloux,F. and Witte,W.

15 TITLE Direct Submission

JOURNAL Submitted (05-NOV-2010) Nosocomial Infections,
Robert Koch Institute, Burgstr. 37, Wernigerode 38855, Germa-
ny

REMARK Sequence update by submitter

20 REFERENCE 3 (bases 1 to 2821452)

AUTHORS Nuebel,U., Dordel,J., Kurt,K., Strommenger,B.,
Westh,H., Shukla,S.K., Zemlickova,H., Leblois,R., Wirth,T.,
Jombart,T., Balloux,F. and Witte,W.

TITLE Direct Submission

25 JOURNAL Submitted (22-DEC-2009) Nosocomial Infections,
Robert Koch Institute, Burgstr. 37, Wernigerode 38855, Germa-
ny

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_010079

30 LOCUS NC_010079 2872915 bp DNA circular CON 15-JUL-2015

DEFINITION *Staphylococcus aureus* subsp. *aureus*

USA300_TCH1516, complete genome.

ACCESSION NC_010079

VERSION NC_010079.1 GI:161508266
 DBLINK BioProject: PRJNA224116
 BioSample: SAMN00253845
 Assembly: GCF_000017085.1

5 KEYWORDS RefSeq.
 SOURCE Staphylococcus aureus subsp. aureus
 USA300_TCH1516
 ORGANISM Staphylococcus aureus subsp. aureus
 USA300_TCH1516

10 Bacteria; Firmicutes; Bacilli; Bacillales; Sta-
 phylococcus.

REFERENCE 1 (bases 1 to 2872915)
 AUTHORS Highlander,S.K., Hulten,K.G., Qin,X., Jiang,H.,
 Yerrapragada,S., Mason,E.O. Jr., Shang,Y., Williams,T.M.,
 15 Fortunov,R.M., Liu,Y., Igboeli,O., Petrosino,J., Tiruma-
 lai,M., Uzman,A., Fox,G.E., Cardenas,A.M., Muzny,D.M., Hem-
 phill,L., Ding,Y., Dugan,S., Blyth,P.R., Buhay,C.J.,
 Dinh,H.H., Hawes,A.C., Holder,M., Kovar,C.L., Lee,S.L.,
 Liu,W., Nazareth,L.V., Wang,Q., Zhou,J., Kaplan,S.L. and
 20 Weinstock,G.M.
 TITLE Subtle genetic changes enhance virulence of
 methicillin resistant and sensitive Staphylococcus aureus
 JOURNAL BMC Microbiol. 7, 99 (2007)
 PUBMED 17986343

25 REMARK Publication Status: Online-Only
 REFERENCE 2 (bases 1 to 2872915)
 AUTHORS Muzny,D., Qin,X., Buhay,C., Dugan-Rocha,S.,
 Ding,Y., Chen,G., Hawes,A., Holder,M., Jhangiani,S., John-
 son,A., Khan,Z., Li,Z., Liu,W., Liu,X., Perez,L., Shen,H.,
 30 Wang,Q., Watt,J., Xi,L., Xin,Y., Zhou,J., Deng,J., Jiang,H.,
 Liu,Y., Qu,J., Song,X.-Z., Zhang,L., Villasana,D., John-
 son,A., Liu,J., Liyanage,D., Lorensuhewa,L., Robinson,T.,
 Song,A., Song,B.-B., Dinh,H., Thornton,R., Coyle,M., Francis-

co,L., Jackson,L., Javaid,M., Korchina,V., Kovar,C., Mata,R.,
 Mathew,T., Ngo,R., Nguyen,L., Nguyen,N., Okwuonu,G.,
 Ongerii,F., Pham,C., Simmons,D., Wilczek-Boney,K., Hale,W.,
 Jakkamsetti,A., Pham,P., Ruth,R., San Lucas,F., Warren,J.,
 5 Zhang,J., Zhao,Z., Zhou,C., Zhu,D., Lee,S., Bess,C.,
 Blankenburg,K., Forbes,L., Fu,Q., Gubbala,S., Hirani,K.,
 Jayaseelan,J.C., Lara,F., Munidasa,M., Palculict,T.,
 Patil,S., Pu,L.-L., Saada,N., Tang,L., Weissenberger,G.,
 Zhu,Y., Hemphill,L., Shang,Y., Youmans,B., Ayvaz,T., Ross,M.,
 10 Santibanez,J., Aqrawi,P., Gross,S., Joshi,V., Fowler,G., Naz-
 areth,L., Reid,J., Worley,K., Petrosino,J., Highlander,S. and
 Gibbs,R.

TITLE Direct Submission

JOURNAL Submitted (20-JUN-2007) Molecular Virology and
 15 Microbiology and the Human Genome Sequencing Center, Baylor
 College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_022222

LOCUS NC_022222 2736560 bp DNA circular CON 17-FEB-2015

20 DEFINITION Staphylococcus aureus subsp. aureus 6850, com-
 plete genome.

ACCESSION NC_022222

VERSION NC_022222.1 GI:537441500

DBLINK BioProject: PRJNA224116

25 BioSample: SAMN02604264

Assembly: GCF_000462955.1

KEYWORDS RefSeq.

SOURCE Staphylococcus aureus subsp. aureus 6850

ORGANISM Staphylococcus aureus subsp. aureus 6850
 30 Bacteria; Firmicutes; Bacilli; Bacillales; Staph-
 ylococcus.

REFERENCE 1 (bases 1 to 2736560)

AUTHORS Fraunholz,M., Bernhardt,J., Schuldes,J., Daniel,R., Hecker,M. and Sinha,B.

TITLE Complete Genome Sequence of Staphylococcus aureus 6850, a Highly Cytotoxic and Clinically Virulent Methicillin-Sensitive Strain with Distant Relatedness to Prototype Strains

JOURNAL Genome Announc 1 (5) (2013)

PUBMED 24072870

REMARK Publication Status: Online-Only

10 REFERENCE 2 (bases 1 to 2736560)

AUTHORS Fraunholz,M.J., Bernhardt,J., Hecker,M., Schuldes,J., Daniel,R. and Sinha,B.

TITLE Direct Submission

15 JOURNAL Submitted (26-AUG-2013) Department of Microbiology, University of Wuerzburg, Am Hubland, Wuerzburg 97074, Germany

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_021670

LOCUS NC_021670 2980548 bp DNA circular CON 07-FEB-2015

20 DEFINITION Staphylococcus aureus Bmb9393, complete genome.

ACCESSION NC_021670

VERSION NC_021670.1 GI:521210823

DBLINK BioProject: PRJNA224116

BioSample: SAMN02603524

25 Assembly: GCF_000418345.1

KEYWORDS RefSeq.

SOURCE Staphylococcus aureus Bmb9393

ORGANISM Staphylococcus aureus Bmb9393

30 Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1 (bases 1 to 2980548)

AUTHORS Costa,M.O., Beltrame,C.O., Ferreira,F.A., Botelho,A.M., Lima,N.C., Souza,R.C., de Almeida,L.G., Vasconcelos,A.T., Nicolas,M.F. and Figueiredo,A.M.

TITLE Complete Genome Sequence of a Variant of the
5 Methicillin-Resistant Staphylococcus aureus ST239 Lineage,
Strain BMB9393, Displaying Superior Ability To Accumulate
ica-Independent Biofilm

JOURNAL Genome Announc 1 (4) (2013)

PUBMED 23929475

10 REMARK Publication Status: Online-Only

REFERENCE 2 (bases 1 to 2980548)

AUTHORS Costa,M.O.C., Beltrame,C.O., Lima,N.C.B., Almeida,L.G.P., Vasconcelos,A.T.R., Ferreira,F.A., Nicolas,M.F.
and Figueiredo,A.M.S.

15 TITLE Direct Submission

JOURNAL Submitted (15-APR-2013) Labinfo, LNCC -
Laboratorio Nacional de Computacao Cientifica, Rua Getulio
Vargas 333, Petropolis, RJ 25651070, Brazil

20 http://www.genome.jp/dbget-bin/www_bget?refseq+NC_017351
LOCUS NC_017351 2846546 bp DNA circular CON 07-FEB-2015
DEFINITION Staphylococcus aureus subsp. aureus 11819-97,
complete genome.

ACCESSION NC_017351

25 VERSION NC_017351.1 GI:385780298

DBLINK BioProject: PRJNA224116

BioSample: SAMN02603886

Assembly: GCF_000239235.1

KEYWORDS RefSeq.

30 SOURCE Staphylococcus aureus subsp. aureus 11819-97

ORGANISM Staphylococcus aureus subsp. aureus 11819-97

Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1 (bases 1 to 2846546)

AUTHORS Stegger,M., Price,L.B., Larsen,A.R.,
Gillece,J.D., Waters,A.E., Skov,R. and Andersen,P.S.

TITLE Genome sequence of Staphylococcus aureus strain
5 11819-97, an ST80-IV European community-acquired methicillin-
resistant isolate

JOURNAL J. Bacteriol. 194 (6), 1625-1626 (2012)

PUBMED 22374956

REFERENCE 2 (bases 1 to 2846546)

10 AUTHORS Stegger,M., Price,L.B., Larsen,A.R.,
Gillece,J.D., Waters,A.E., Skov,R. and Andersen,P.S.

TITLE Direct Submission

JOURNAL Submitted (13-DEC-2011) Department of Microbio-
logical Surveillance and Research, Statens Serum Institut,
15 Oerestads Boulevard 5, 2300 Copenhagen S, 5 Artillerivej,
Denmark

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_002953

LOCUS NC_002953 2799802 bp DNA circular CON 07-FEB-2015

20 DEFINITION Staphylococcus aureus strain MSSA476, complete
genome.

ACCESSION NC_002953

VERSION NC_002953.3 GI:49484912

DBLINK BioProject: PRJNA224116

25 Assembly: GCF_000011525.1

KEYWORDS RefSeq; complete genome.

SOURCE Staphylococcus aureus subsp. aureus MSSA476

ORGANISM Staphylococcus aureus subsp. aureus MSSA476
Bacteria; Firmicutes; Bacilli; Bacillales; Sta-
30 phylococcus.

REFERENCE 1 (bases 1 to 2799802)

AUTHORS Holden,M.T., Feil,E.J., Lindsay,J.A., Pea-
cock,S.J., Day,N.P., Enright,M.C., Foster,T.J., Moore,C.E.,

Hurst,L., Atkin,R., Barron,A., Bason,N., Bentley,S.D., Chillingworth,C., Chillingworth,T., Churcher,C., Clark,L., Corton,C., Cronin,A., Doggett,J., Dowd,L., Feltwell,T., Hance,Z., Harris,B., Hauser,H., Holroyd,S., Jagels,K.,
 5 James,K.D., Lennard,N., Line,A., Mayes,R., Moule,S., Mungall,K., Ormond,D., Quail,M.A., Rabinowitsch,E., Rutherford,K., Sanders,M., Sharp,S., Simmonds,M., Stevens,K., Whitehead,S., Barrell,B.G., Spratt,B.G. and Parkhill,J.

TITLE Complete genomes of two clinical *Staphylococcus*
 10 aureus strains: evidence for the rapid evolution of virulence and drug resistance

JOURNAL Proc. Natl. Acad. Sci. U.S.A. 101 (26), 9786-9791
 (2004)

PUBMED 15213324

15 REFERENCE 2 (bases 1 to 2799802)

AUTHORS Holden,M.T.G.

TITLE Direct Submission

JOURNAL Submitted (23-JUN-2004) Submitted on behalf of
 the Pathogen Sequencing Unit, Sanger Institute, Wellcome
 20 Trust Genome Campus, Hinxton, Cambridge CB10 1SA, E-mail:
 mh3@sanger.ac.uk

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_017337

LOCUS NC_017337 2832478 bp DNA circular CON 07-FEB-2015

25 DEFINITION *Staphylococcus aureus* subsp. *aureus* ED133, complete genome.

ACCESSION NC_017337

VERSION NC_017337.1 GI:384546269

DBLINK BioProject: PRJNA224116

30 BioSample: SAMN02604166

Assembly: GCF_000210315.1

KEYWORDS RefSeq.

SOURCE *Staphylococcus aureus* subsp. *aureus* ED133

ORGANISM Staphylococcus aureus subsp. aureus ED133
 Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1 (bases 1 to 2832478)

5 AUTHORS Guinane,C.M., Ben Zakour,N.L., Tormo-Mas,M.A.,
 Weinert,L.A., Lowder,B.V., Cartwright,R.A., Smyth,D.S.,
 Smyth,C.J., Lindsay,J.A., Gould,K.A., Witney,A., Hinds,J.,
 Bollback,J.P., Rambaut,A., Penades,J.R. and Fitzgerald,J.R.

TITLE Evolutionary genomics of Staphylococcus aureus
 10 reveals insights into the origin and molecular basis of ruminant host adaptation

JOURNAL Genome Biol Evol 2, 454-466 (2010)

PUBMED 20624747

REMARK Publication Status: Online-Only

15 REFERENCE 2 (bases 1 to 2832478)

AUTHORS Guinane,C.M., Ben Zakour,N.L., Tormo-Mas,M.A.,
 Weinert,L.A., Lowder,B.V., Cartwright,R.A., Smyth,D.S.,
 Smyth,C.J., Lindsay,J., Gould,K.A., Witney,A., Hinds,J.,
 Bollback,J.P., Rambaut,A., Penades,J. and Fitzgerald,J.R.

20 TITLE Direct Submission

JOURNAL Submitted (29-MAR-2010) The Roslin Institute and
 Centre for Infectious Diseases, Royal (Dick) School of Veterinary
 Studies, University of Edinburgh, The Chancellor's
 Building, New Royal Infirmary, 49 Little France Crescent, Edinburgh
 25 EH16 4SB, United Kingdom

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_018608

LOCUS NC_018608 2782313 bp DNA circular CON 07-FEB-2015

DEFINITION Staphylococcus aureus 08BA02176, complete genome.

30 ACCESSION NC_018608

VERSION NC_018608.1 GI:404477334

DBLINK BioProject: PRJNA224116

BioSample: SAMN02603722

Assembly: GCF_000296595.1

KEYWORDS RefSeq.

SOURCE Staphylococcus aureus 08BA02176

ORGANISM Staphylococcus aureus 08BA02176

5 Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1 (bases 1 to 2782313)

AUTHORS Golding,G.R., Bryden,L., Levett,P.N., McDonald,R.R., Wong,A., Graham,M.R., Tyler,S., Van Domselaar,G.,
10 Mabon,P., Kent,H., Butaye,P., Smith,T.C., Kadlec,K., Schwarz,S., Weese,S.J. and Mulvey,M.R.

TITLE whole-genome sequence of livestock-associated st398 methicillin-resistant staphylococcus aureus Isolated from Humans in Canada

15 JOURNAL J. Bacteriol. 194 (23), 6627-6628 (2012)

PUBMED 23144384

REFERENCE 2 (bases 1 to 2782313)

AUTHORS Golding,G.R., Bryden,L., Levett,P.N., McDonald,R.R., Wong,A., Graham,M.R., Tyler,S., Van Domselaar,G.,
20 Mabon,P., Kent,H., Butaye,P., Smith,T.C., Kadlec,K., Schwarz,S., Weese,S.J. and Mulvey,M.R.

TITLE Direct Submission

JOURNAL Submitted (31-AUG-2012) Antimicrobial Resistance and Nosocomial Infections, Public Health Agency of Canada,
25 National Microbiology Laboratory, 1015 Arlington Street, Winnipeg, Manitoba R3E 3R2, Canada

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_007795

LOCUS NC_007795 2821361 bp DNA circular CON 16-DEC-2014

30 DEFINITION Staphylococcus aureus subsp. aureus NCTC 8325 chromosome, complete genome.

ACCESSION NC_007795

VERSION NC_007795.1 GI:88193823

DBLINK BioProject: PRJNA57795

KEYWORDS RefSeq.

SOURCE Staphylococcus aureus subsp. aureus NCTC 8325

ORGANISM Staphylococcus aureus subsp. aureus NCTC 8325

5 Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1 (bases 1 to 2821361)

AUTHORS Gillaspay,A.F., Worrell,V., Orvis,J., Roe,B.A., Dyer,D.W. and Iandolo,J.J.

10 TITLE The Staphylococcus aureus NCTC8325 Genome

JOURNAL (in) Fischetti,V., Novick,R., Ferretti,J., Portnoy,D. and Rood,J. (Eds.); GRAM POSITIVE PATHOGENS; ASM Press (2006)

REFERENCE 2 (bases 1 to 2821361)

15 CONSRM NCBI Genome Project

TITLE Direct Submission

JOURNAL Submitted (18-FEB-2006) National Center for Biotechnology Information, NIH, Bethesda, MD 20894, USA

REFERENCE 3 (bases 1 to 2821361)

20 AUTHORS Gillaspay,A.F., Worrell,V., Orvis,J., Roe,B.A., Dyer,D.W. and Iandolo,J.J.

TITLE Direct Submission

JOURNAL Submitted (27-JAN-2006) Microbiology and Immunology, The University of Oklahoma Health Sciences Center, 940 Stanton L. Young Boulevard, Oklahoma City, OK 73104, USA

25 http://www.genome.jp/dbget-bin/www_bget?refseq+NC_021059

LOCUS NC_021059 2864125 bp DNA circular CON 01-MAR-2015

DEFINITION Staphylococcus aureus M1, complete genome.

30 ACCESSION NC_021059

VERSION NC_021059.1 GI:479328021

DBLINK BioProject: PRJNA224116

Assembly: GCF_000367745.1

KEYWORDS RefSeq; complete genome.

SOURCE Staphylococcus aureus M1

ORGANISM Staphylococcus aureus M1

Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1

AUTHORS Larner-Svensson, H., Worning, P., Bartels, M.D., Hestbjerg Hansen, L., Boye, K. and Westh, H.

TITLE Complete Genome Sequence of Staphylococcus aureus Strain M1, a Unique t024-ST8-IVa Danish Methicillin-Resistant S. aureus Clone

JOURNAL Genome Announc 1 (3) (2013)

PUBMED 23792746

REMARK Publication Status: Online-Only

REFERENCE 2 (bases 1 to 2864125)

AUTHORS Worning, P.

TITLE Direct Submission

JOURNAL Submitted (18-MAR-2013) Dept. of Clinical Microbiology, Hvidovre Hospital, Kettegaard Alle 30, DK-2650, DENMARK

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_021554

LOCUS NC_021554 2850503 bp DNA circular CON 07-FEB-2015

DEFINITION Staphylococcus aureus CA-347, complete genome.

ACCESSION NC_021554

VERSION NC_021554.1 GI:514064966

DBLINK BioProject: PRJNA224116

BioSample: SAMN02603909

Assembly: GCF_000412775.1

KEYWORDS RefSeq.

SOURCE Staphylococcus aureus CA-347

ORGANISM Staphylococcus aureus CA-347

Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1 (bases 1 to 2850503)

AUTHORS Stegger,M., Driebe,E.M., Roe,C., Lemmer,D., Bowers,J.R., Engelthaler,D.M., Keim,P. and Andersen,P.S.

TITLE Genome Sequence of Staphylococcus aureus Strain CA-347, a USA600 Methicillin-Resistant Isolate

JOURNAL Genome Announc 1 (4) (2013)

PUBMED 23887918

10 REMARK Publication Status: Online-Only

REFERENCE 2 (bases 1 to 2850503)

AUTHORS Stegger,M., Driebe,E.M., Roe,C., Lemmer,D., Engelthaler,D.M., Keim,P. and Andersen,P.S.

TITLE Direct Submission

15 JOURNAL Submitted (10-JUN-2013) CPHCP, TGen North, 3051 W. Shamrell Blvd., Ste. 106, Flagstaff, AZ 86001, USA

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_016912

LOCUS NC_016912 2692570 bp DNA circular CON 07-FEB-2015

20 DEFINITION Staphylococcus aureus subsp. aureus VC40, complete genome.

ACCESSION NC_016912

VERSION NC_016912.1 GI:379013365

DBLINK BioProject: PRJNA224116

25 BioSample: SAMN02603393

Assembly: GCF_000245495.1

KEYWORDS RefSeq.

SOURCE Staphylococcus aureus subsp. aureus VC40

ORGANISM Staphylococcus aureus subsp. aureus VC40

30 Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcus.

REFERENCE 1 (bases 1 to 2692570)

AUTHORS Sass,P., Berscheid,A., Jansen,A., Oedenkoven,M.,
Szekat,C., Strittmatter,A., Gottschalk,G. and Bierbaum,G.

TITLE Genome sequence of Staphylococcus aureus VC40, a
vancomycin- and daptomycin-resistant strain, to study the ge-
netics of development of resistance to currently applied
last-resort antibiotics

JOURNAL J. Bacteriol. 194 (8), 2107-2108 (2012)

PUBMED 22461548

REFERENCE 2 (bases 1 to 2692570)

10 AUTHORS Sass,P., Berscheid,A., Jansen,A., Oedenkoven,M.,
Szekat,C., Strittmatter,A., Gottschalk,G. and Bierbaum,G.

TITLE Direct Submission

JOURNAL Submitted (25-AUG-2011) Institute of Medical Mi-
crobiology, Immunology and Parasitology, University of Bonn,
15 Sigmund-Freud-Str. 25, Bonn 53105, Germany

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_022226

LOCUS NC_022226 2751266 bp DNA circular CON 01-MAR-2015

DEFINITION Staphylococcus aureus subsp. aureus CN1, complete
20 genome.

ACCESSION NC_022226

VERSION NC_022226.1 GI:537459744

DBLINK BioProject: PRJNA224116

BioSample: SAMN02603420

25 Assembly: GCF_000463055.1

KEYWORDS RefSeq.

SOURCE Staphylococcus aureus subsp. aureus CN1

ORGANISM Staphylococcus aureus subsp. aureus CN1

Bacteria; Firmicutes; Bacilli; Bacillales; Staph-
30 ylococcus.

REFERENCE 1 (bases 1 to 2751266)

AUTHORS Chen,Y., Chatterjee,S.S., Porcella,S.F., Yu,Y.S.
and Otto,M.

TITLE Complete genome sequence of a Panton-Valentine leukocidin-negative community-associated methicillin-resistant *Staphylococcus aureus* strain of sequence type 72 from Korea

- 5 JOURNAL PLoS ONE 8 (8), E72803 (2013)
 PUBMED 23977354
 REMARK Publication Status: Online-Only
 REFERENCE 2 (bases 1 to 2751266)
 AUTHORS Otto,M. and Porcella,S.F.
- 10 TITLE Direct Submission
 JOURNAL Submitted (04-DEC-2012) Laboratory of Human Bacterial Pathogenesis, NIAID/NIH, 9000 Rockville Pike, Bethesda, MD 20892, USA
- 15 http://www.genome.jp/dbget-bin/www_bget?refseq+NC_022113
 LOCUS NC_022113 2756919 bp DNA circular CON 07-FEB-2015
 DEFINITION *Staphylococcus aureus* subsp. *aureus* 55/2053, complete genome.
 ACCESSION NC_022113 NZ_ACJR01000000-NZ_ACJR01000094
 20 NZ_GG700533-NZ_GG700558
 VERSION NC_022113.1 GI:532358222
 DBLINK BioProject: PRJNA224116
 BioSample: SAMN00103091
 Assembly: GCF_000160335.2
- 25 KEYWORDS RefSeq.
 SOURCE *Staphylococcus aureus* subsp. *aureus* 55/2053
 ORGANISM *Staphylococcus aureus* subsp. *aureus* 55/2053
 Bacteria; Firmicutes; Bacilli; Bacillales; *Staphylococcus*.
- 30 REFERENCE 1 (bases 1 to 2756919)
 AUTHORS Feldgarden,M., Robinson,A., Wong,A., Smyth,D., Young,S.K., Zeng,Q., Gargeya,S., Fitzgerald,M., Haas,B., Abouelleil,A., Alvarado,L., Arachchi,H.M., Berlin,A.,

Brown,A., Chapman,S.B., Chen,Z., Dunbar,C., Gearin,G., Gold-
 berg,J., Griggs,A., Gujja,S., Heiman,D., Howarth,C., Lar-
 son,L., Lui,A., MacDonald,P.J.P., Montmayeur,A., Murphy,C.,
 Neiman,D., Pearson,M., Priest,M., Roberts,A., Saif,S.,
 5 Shea,T., Sisk,P., Stolte,C., Sykes,S., Wortman,J., Nusbaum,C.
 and Birren,B.

CONSRTM The Broad Institute Genome Sequencing Platform

TITLE The Genome Sequence of Staphylococcus aureus
 strain 55-2053

10 JOURNAL Unpublished

REFERENCE 2 (bases 1 to 2756919)

AUTHORS Feldgarden,M., Robinson,A., Wong,A., Smyth,D.,
 Young,S.K., Zeng,Q., Gargeya,S., Fitzgerald,M., Haas,B.,
 Abouelleil,A., Alvarado,L., Arachchi,H.M., Berlin,A.,

15 Brown,A., Chapman,S.B., Chen,Z., Dunbar,C., Gearin,G., Gold-
 berg,J., Griggs,A., Gujja,S., Heiman,D., Howarth,C., Lar-
 son,L., Lui,A., MacDonald,P.J.P., Montmayeur,A., Murphy,C.,
 Neiman,D., Pearson,M., Priest,M., Roberts,A., Saif,S.,
 Shea,T., Sisk,P., Stolte,C., Sykes,S., Wortman,J., Nusbaum,C.
 20 and Birren,B.

CONSRTM The Broad Institute Genome Sequencing Platform

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In addition, the genetic variations can also be annotated to a pan-genome constructed from the genomes used, and can be numbered using consecutive numbers. In the present method, the construction of a pan-genome is not particularly limited and can be done using known methods.

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However, other suitable reference genomes (e.g. used in the Examples, but also for other microorganisms) can be found at publicly available data bases like at the NCBI.

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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), Student's t-test or Fisher's exact test, for example with a sample size n of 50, 100, 200, 300, 400, 800 or 900, 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 genetic variation 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.

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For statistically sound results a multitude of individuals should be sampled, with $n = 50, 100, 200, 300, 400, 800$ or 900, 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

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to certain embodiments, particularly significant results can be obtained for $n = 200, 300, 400, 800$ or 900 .

For statistically sound results a multitude of individuals should be sampled, with $n = 50$ or more, 100 or more, 200 or more, 300 or more, 400 or more, 800 or more or 900 or more, and a level of significance (α -error-level) of e.g. 0.05 or smaller, e.g. 0.05 , preferably 0.01 or smaller. According to certain embodiments, particularly significant results can be obtained for $n = 200$ or more, 300 or more, 400 or more, 800 or more or 900 or more.

After the above procedure has been carried out for more than 900 , e.g. 987 , individual strains of *Staphylococcus* species, particularly *Staphylococcus aureus*, the data disclosed in Tables 1 and 2 were obtained for the statistically best correlations between genetic variations and antimicrobial drug, e.g. antibiotic, resistances, particularly methicillin resistance. Thus, genetic variations in the positions given in Tables 1 and 2, with regard to the several reference genomes as above, were proven as valid markers for antimicrobial drug, e.g. antibiotic, resistance.

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 as-

pect 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,
5 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 $p < 10^{-9}$.

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The method of the first aspect of the present invention, as well as related methods, e.g. according to the 2nd, 3rd and 4th aspect, can, according to certain embodiments, comprise correlating different genetic sites to each other. This way even
15 higher statistical significance can be achieved.

According to certain embodiments of the method of the first aspect and related methods - as above, the second data set can be provided by culturing the clinical isolates of the microorganism on agar plates provided with antimicrobial drugs, e.g. antibiotics, at different concentrations, and the second
20 data can be obtained by taking the minimal concentration of the plates that inhibits growth of the respective microorganism, e.g. Staphylococcus aureus.

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According to certain embodiments of the method of the first aspect and related methods, the antimicrobial drug, e.g. antibiotic drug, is selected from the group consisting of β -lactams, β -lactam inhibitors, quinolones and derivatives thereof, e.g. fluoroquinolones, aminoglycosides, glycopeptides, lincosamides, macrolides, nitrofuranes, oxazolidinones
30 polyketides, respectively tetracyclines, and folate synthesis inhibitors, e.g. benzene derived/sulfonamide antibiotics,

preferably from the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Methicillin, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin.

According to a second aspect, the present invention discloses a diagnostic method of determining an infection of a patient with a microorganism, particularly a bacterial microorganism potentially resistant to antimicrobial drug treatment, comprising the steps of:

- a) obtaining or providing a sample containing or suspected of containing a microorganism, particularly a bacterial microorganism, from the patient;
- b) determining the presence of at least one genetic variant in at least one position of the microorganism, particularly the bacterial microorganism, as determined by the method of the first aspect of the invention, wherein the presence of said at least one genetic variant is indicative of an infection with an antimicrobial drug resistant microorganism in said patient.

Again, the microorganism can be a Staphylococcus species, particularly Staphylococcus aureus, according to certain embodiments, and the drug methicillin and/or a drug as described below, e.g. with regard to the eight and ninth aspect.

With this method, any mutations in the genome of a microorganism, e.g. a Staphylococcus species, particularly Staphylococcus aureus, e.g. a clinical isolate with an unknown strain
5 of the microorganism, particularly bacterial microorganism, correlated with antimicrobial drug, e.g. antibiotic, resistance can be determined and a thorough antimicrobial drug, e.g. antibiotic, resistance profile can be established.

10 Again, the different steps can herein be carried out as described with regard to the first aspect of the present invention.

According to this aspect, an infection with a microorganism,
15 particularly a bacterial microorganism, e.g. a Staphylococcus, particularly Staphylococcus aureus, infection, in a patient can be determined using sequencing methods, as well as a resistance to antimicrobial drugs, e.g. antibiotics, of the microorganism, e.g. a Staphylococcus species, particularly
20 Staphylococcus aureus, can be determined in a short amount of time compared to conventional methods.

In a third aspect, the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant microorganism, particularly bacterial microorganism, comprising the steps of:

- a) obtaining or providing a sample containing or suspected of containing a microorganism, particularly a bacterial microorganism, from the patient;
- 30 b) determining the presence of at least one genetic variant in at least one position of the microorganism, particularly bacterial microorganism, as determined by the method of the first aspect of the invention, wherein the presence of said

at least one genetic variant is indicative of a resistance to one or more antimicrobial drugs;

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

5 d) selecting one or more antimicrobial drugs different from the ones identified in step c) and being suitable for the treatment of the infection with the microorganism, particularly the bacterial microorganism.

10 This method can be carried out similarly to the second aspect of the invention and enables a fast way to select a suitable treatment with antibiotics for any infection with an unknown microorganism, particularly bacterial microorganism, e.g. *Staphylococcus aureus*.

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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 genetic variations and antimicrobial
20 drug, e.g. antibiotic, resistances can be determined. The first data set can be assembled, for example, using known techniques.

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

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A fourth aspect of the present invention relates to a method of acquiring, respectively determining, an antimicrobial drug, e.g. antibiotic, resistance profile for a clinical iso-

late of a microorganism, particularly a bacterial microorganism, comprising:

obtaining or providing at least one gene sequence of the clinical isolate of the microorganism, particularly the bacterial microorganism; and

determining the presence of genetic variants in the at least one gene sequence of the clinical isolate of the microorganism, particularly bacterial microorganism, as determined by the method of the first aspect of the invention.

With this method, antimicrobial drug, e.g. antibiotic, resistances in an unknown isolate of a microorganism, e.g. *Staphylococcus aureus*, can be determined.

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 containing several reference genomes and/or a pan-genome as obtained by the

method of the first aspect, i.e. selected sequences or the whole sequence are/is compared to one or more reference sequences and/or a pan-genome, and genetic variations (SNPs, sequence- gene additions/deletions, etc.) are correlated with susceptibility/resistance profile of reference strains in the reference library. The reference library 4 herein contains many genomes and/or a pan-genome and is different from a reference genome. Then the result 5 is reported which can comprise ID (pathogen identification), i.e. a list of all (path-

ogenic) species identified in the sample, and AST (antimicrobial susceptibility testing), i.e. a list including a susceptibility /resistance profile for all species listed.

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

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Again, in the third and fourth aspect, the different steps can herein be carried out as described with regard to the first aspect of the present invention, and the microorganism can be a Staphylococcus species, particularly Staphylococcus aureus, according to certain embodiments, and the antibiotic can be methicillin and/or another antibiotic as described below according to certain embodiments. In this regard, it should be noted that resistance to methicillin can indicate, particular in Staphylococcus species, particularly Staphylococcus aureus, resistance to β -lactam antibiotics.

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In a fifth aspect the present invention relates to one or more computer program products comprising computer executable instructions which, when executed, perform a method according to any one of the first to the fourth aspect of the present invention.

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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. As noted above, the computer program products of the present

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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. assembly of the genome of the microorganisms, at least in part and optionally annotating the genomes to one or more reference genomes and/or a pan-genome, or, in the second, third and/or fourth aspect, alignment of the sequence data of the clinical isolate to be determined with one or more reference genomes and/or a pan-genome, and correlation of genetic variations found in every sample, e.g. from each patient, respectively an unknown clinical isolate, with all references and drugs, e.g. antibiotics, or only one or some of them, and search for mutations which occur in one or several drug and one or several strains.

Using the above steps a list of genetic variations as well as of positions with regard to one or more reference genomes and/or a pan-genome 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 genetic variations in at least one or more positions. Statistical models that can be trained can be combined from genetic variations and positions. 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 genetic variation or at least one position, but also combinations of genetic variations, 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 *Staphylococcus aureus*, against all or only some or one 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 tenth aspect of the present invention relates to the use of the computer program product according to the fifth aspect, e.g. for acquiring an antimicrobial drug, e.g. antibiotic, resistance profile for microorganisms in the fourth aspect of the invention and/or for use in the diagnostic method of the second method of the invention and/or for selecting a treatment in the third aspect of the present invention and/or in the method of the first aspect of the present invention.

A sixth aspect of the present invention discloses a diagnostic method of determining an infection of a patient with a *Staphylococcus* species, particularly *Staphylococcus aureus*,

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 Staphylococcus, particularly Staphylococcus aureus, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1, wherein the presence of said at least two genetic variations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant Staphylococcus, particularly Staphylococcus aureus, strain in said patient wherein for some positions more than one position in different reference genomes is annotated.

As noted above, in Table 1, the position of the genetic variation (named "position"; with R being reverse direction and F being forward direction) are given for each variation (given with consecutive numbers 1 - 50) with reference to one or more known reference genomes from the NCBI (with the NCBI number given in the column "reference genome" and the genome name given in the column "genome name").

An infection of a patient with Staphylococcus, particularly Staphylococcus aureus, potentially resistant to antimicrobial drug treatment herein means an infection of a patient with Staphylococcus aureus wherein it is unclear if the Staphylococcus, particularly Staphylococcus aureus, strain is susceptible to treatment with a specific antimicrobial drug or if it is resistant to the antimicrobial drug.

In step b) above, as well as corresponding steps, at least one genetic variation in at least two positions is determined, so that in total at least two genetic variations are determined, wherein the two genetic variations are in different positions. Again, it should be noted that in Table 1 a certain position can be annotated to more than one reference gene, so that also here only different positions are used, and not the same position that is annotated to different reference genomes.

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, genetic variations in at least two, three, four, five, six, seven, eight, nine or ten positions are determined in any of the methods of the present invention, e.g. in at least two positions or in at least three positions. Instead of testing only single positions, 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 genetic variation in 2, 3, 4, 5, 6, 7, 8 or 9 (or more) positions selected from Table 1.

For the above positions, i.e. the positions denoted in Table 1, the highest probability of a resistance to at least one antimicrobial drug, e.g. antibiotic, could be observed, with p-values smaller than 10^{-140} , particularly smaller than 10^{-160} , indicating the high significance of the values ($n = 987$; $\alpha = 10^{-9}$). Details regarding Table 1 can be taken from Table 2,

respectively Tables 2a and 2b, disclosed in the Examples.

Having at least two positions with genetic variations determined, a high probability of an antimicrobial drug, e.g. antibiotic, resistance could be determined. The genes in Table

5 1 thereby represent the 50 best genes for which a genetic variation was observed in the genomes of *Staphylococcus*, particularly *Staphylococcus aureus*, with regard to methicillin resistance/susceptibility as described above and below.

10 According to certain embodiments, the obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus* species from the patient in this method - as well as the other methods of the invention - can comprise the following:

15 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 *Staphylococcus*, particularly *Staphylococcus aureus*. 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.

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The data obtained by the sequencing can be in any format, and can then be analyzed as described with regard to the first to fourth aspect of the present invention.

- 5 In a seventh aspect, the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain, comprising the steps of:
- 10 a) obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus*, particularly *Staphylococcus aureus*, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions
- 15 annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1, wherein the presence of said at least two genetic variations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;
- 20 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
- 25 suitable for the treatment of a *Staphylococcus*, particularly *Staphylococcus aureus*, infection.

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

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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 genetic variations. 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 sixth and seventh aspect also apply to the 11th, 12th, 13th and 14th aspect, referring to the same positions, unless clear from the context that they don't apply.

According to certain embodiments of the sixth and or seventh aspect, the antimicrobial drug, e.g. antibiotic, in the method of the sixth or seventh aspect is at least one from the group consisting of β -lactams, β -lactam inhibitors, quinolones and derivatives thereof, e.g. fluoroquinolones, aminoglycosides, glycopeptides, lincosamides, macrolides, nitrofurans, oxazolidinones polyketides, respectively tetracyclines, and folate synthesis inhibitors, e.g. benzene derived/sulfonamide antibiotics, particularly from the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Methicillin, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin, particularly Methicillin.

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

According to certain embodiments of the sixth and/or seventh aspect of the invention, determining the nucleic acid sequence information or the presence of a genetic variation comprises determining the presence of a single nucleotide at a single position. 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 of the sixth and/or seventh aspect of the invention, the resistance of a Staphylococcus aureus strain against 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 17, 18, 19, 20 or more antibiotic drugs is determined.

According to certain embodiments of the sixth and/or seventh aspect of the invention, a detected genetic variation is a genetic variation leading to an altered amino acid sequence, e.g. in a polypeptide derived from a respective gene, in which the detected genetic variation is located. According to this aspect, the detected genetic variation can thus lead 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 sixth and/or seventh aspect of the invention, determining the nucleic acid se-

quence information with the positions having a genetic variation or the presence of a genetic variation comprises determining a partial sequence or an entire sequence comprising the position with the genetic variation.

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According to certain embodiments of the sixth and/or seventh aspect of the invention, determining the nucleic acid sequence information with the positions having a genetic variation or the presence of a genetic variation comprises using a next generation sequencing or high throughput sequencing method. According to preferred embodiments of the sixth and/or seventh aspect of the invention, a partial or entire genome sequence of a Staphylococcus, particularly Staphylococcus aureus, strain is determined by using a next generation sequencing or high throughput sequencing method.

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According to certain embodiments of the sixth and/or seventh aspect, determining the nucleic acid sequence information or the presence of a genetic variation comprises determining a partial or entire sequence of the genome of the Staphylococcus species, particularly Staphylococcus aureus, wherein said partial or entire sequence of the genome comprises at least one of the positions with the genetic variation.

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

- a) obtaining or providing a sample containing or suspected of containing at least one Staphylococcus, particularly Staphylococcus aureus, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions

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annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1, wherein the presence of said at least two genetic variations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;

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 Staphylococcus, particularly Staphylococcus aureus, infection; and

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

Herein, steps a) to d) can be carried out as described with respect to the seventh aspect. Step e) can be sufficiently carried out without being restricted and can be done e.g. non-invasively.

A twelfth aspect of the present invention discloses a diagnostic method of determining an infection of a patient with a Staphylococcus species, particularly Staphylococcus aureus, 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 Staphylococcus, particularly Staphylococcus aureus, strain from the patient;

b) determining the presence of at least one genetic variation in at least one position from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1, wherein the presence of said at least one genetic variation is indicative of an

infection with an antimicrobial drug, e.g. antibiotic, resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain in said patient, wherein for some positions more than one position in different reference genomes is annotated.

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In a thirteenth aspect, the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain, comprising the steps of:

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a) obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus*, particularly *Staphylococcus aureus*, strain from the patient;

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b) determining the presence of at least one genetic variation in at least one position from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1, wherein the presence of said at least one genetic variation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;

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

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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 *Staphylococcus*, particularly *Staphylococcus aureus*, infection.

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Again, in the twelfth and the thirteenth aspect the steps correspond to those in the sixth or seventh aspect, although only a mutation in at least one gene is determined.

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

- 5 a) obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus aureus* strain from the patient;
- b) determining the presence of at least one genetic variation in at least one position from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes
10 with the genome names given in Table 1, wherein the presence of said at least one genetic variation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in
15 different reference genomes is annotated;
- 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
20 suitable for the treatment of a *Staphylococcus*, particularly *Staphylococcus aureus*, infection; and
- e) treating the patient with said one or more antimicrobial, e.g. antibiotic, drugs.

25 Also in the fourteenth aspect of the invention, steps a) to d) are analogous to the steps in the method of the eleventh aspect of the present invention. Step e) can again be sufficiently carried out without being restricted and can be done e.g. non-invasively.

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An eighth aspect of the present invention discloses a diagnostic method of determining an infection of a patient with a *Staphylococcus* species, particularly *Staphylococcus aureus*,

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 Staphylococcus, particularly

5 Staphylococcus aureus, strain from the patient;

b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b,

10 particular with regard to the reference genomes with the genome names given in Table 3b, wherein the presence of said at least two genetic variations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant Staphylococcus, particularly Staphylococcus aureus, strain in said
15 patient wherein for some positions more than one position in different reference genomes is annotated.

As noted above, in Tables 3a and 3b, the position of the genetic variation (named "position"; with R being reverse direction and F being forward direction) are given for each
20 variation (given with consecutive numbers 1 - 50) with reference to one or more known reference genomes from the NCBI (with the NCBI number given in the column "reference genome" and the genome name given in the column "genome name").

25

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

In step b) above, as well as corresponding steps, at least one genetic variation in at least two positions is determined, so that in total at least two genetic variations are
5 determined, wherein the two genetic variations are in different positions. Again, it should be noted that in Tables 3a and 3b a certain position can be annotated to more than one reference gene, so that also here only different positions are used, and not the same position that is annotated to different
10 reference genomes.

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*
15 *vitro* sample or prepared as *in vitro* sample.

According to certain aspects, genetic variations in at least two, three, four, five, six, seven, eight, nine or ten positions are determined in any of the methods of the present invention, e.g. in at least two positions or in at least three
20 positions. Instead of testing only single positions, 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 genetic variation in 2, 3, 4, 5, 6, 7, 8 or 9 (or more) positions selected
25 from Tables 3a and/or 3b.

For the above positions, i.e. the positions denoted in Tables
30 3a and/or 3b, the highest probability of a resistance to at least one antimicrobial drug, e.g. antibiotic, could be observed, with p-values smaller than 10^{-160} , particularly smaller than 10^{-190} , indicating the high significance of the values

($n = 985$; $\alpha = 10^{-9}$). Details regarding Tables 3a and 3b can be taken from Table 4, particularly Tables 4a-d with regard to Table 3a and Tables 4e-h with regard to Table 3b, disclosed in the Examples. Having at least two positions with genetic variations determined, a high probability of an antimicrobial drug, e.g. antibiotic, resistance could be determined. The genes in Table 3a thereby represent the 50 best genes for which a mutation was observed in the genomes of *Staphylococcus* species, particularly *S. aureus*, particularly with regard to resistance to the antibiotics described below, i.e. the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin, whereas the genes in Table 3b represent the 50 best genes for which a cross-correlation could be observed for the antimicrobial drug, e.g. antibiotic, susceptibility testing, particularly with regard to resistance to the antibiotics as above with regard to Table 3a, for *Staphylococcus* species, particularly *S. aureus*, as described below.

According to certain embodiments, the obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus* 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 the *Staphylococcus* species, particularly *Staphylococcus aureus*. 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 analyzed as described with regard to the first to fourth aspect of the present invention.

In a ninth aspect, the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain, comprising the steps of:

- a) obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus*, particularly *Staphylococcus aureus*, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b,

particular with regard to the reference genomes with the genome names given in Table 3b, wherein the presence of said at least two genetic variations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein
5 for some positions more than one position in different reference genomes is annotated;

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
10 suitable for the treatment of a Staphylococcus, particularly Staphylococcus aureus, infection.

In this method, the steps a) of obtaining or providing a sample and b) of determining the presence of at least one genetic variation are as in the method of the eighth aspect.
15

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,
20 e.g. antibiotic, drug(s) that correlate(s) with the genetic variations. 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
25 suitable for treatment.

In the description, references to the eighth and ninth aspect also apply to the 15th, 16th, 17th and 18th aspect, referring to the same positions, unless clear from the context that
30 they don't apply.

According to certain embodiments of the eighth and/or ninth aspect, the antimicrobial drug, e.g. antibiotic, in the meth-

od of the eighth or ninth aspect, as well as in the other methods of the invention, is at least one from the group consisting of β -lactams, β -lactam inhibitors, quinolines and derivatives thereof, e.g. fluoroquinolones, aminoglycosides, glycopeptides, lincosamides, macrolides, nitrofuranes, oxazolidinones polyketides, respectively tetracyclines, and folate synthesis inhibitors, e.g. benzene derived/sulfonamide antibiotics, particularly from the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Methicillin, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin. In the eighth and/or ninth aspect, as well as the fifteenth to eighteenth aspect, , the antimicrobial drug, e.g. antibiotic is preferably at least one from the group consisting of β -lactams, β -lactam inhibitors, quinolines and derivatives thereof, e.g. fluoroquinolones, aminoglycosides, glycopeptides, lincosamides, macrolides, nitrofuranes, oxazolidinones polyketides, respectively tetracyclines, and folate synthesis inhibitors, e.g. benzene derived/sulfonamide antibiotics, particularly from the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Moxifloxacin, Mupirocin, Nitrofurantoin,

Norfloxacin, Ofloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin.

5

In the methods of the invention the resistance of a Staphylococcus species, particularly Staphylococcus aureus, to one or more antimicrobial, e.g. antibiotic, drugs can be determined according to certain embodiments.

10

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

15

According to certain embodiments of the eighth and/or ninth aspect of the invention, the resistance of a Staphylococcus, particularly Staphylococcus aureus, strain against 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 17, 18, 19, 20 or more antibiotic drugs is determined.

20

According to certain embodiments of the eighth and/or ninth aspect of the invention, a detected genetic variation is a genetic variation leading to an altered amino acid sequence, e.g. in a polypeptide derived from a respective gene, in which the detected genetic variation is located. According to this aspect, the detected genetic variation can thus lead to a truncated version of the polypeptide (wherein a new stop codon is created by the mutation) or a mutated version of the

25

30

polypeptide having an amino acid exchange at the respective position.

According to certain embodiments of the eighth and/or ninth aspect of the invention, determining the nucleic acid sequence information with the positions having a genetic variation or the presence of a genetic variation comprises determining a partial sequence or an entire sequence comprising the position with the genetic variation.

According to certain embodiments of the eighth and/or ninth aspect of the invention, determining the nucleic acid sequence information with the positions having a genetic variation or the presence of a genetic variation comprises using a next generation sequencing or high throughput sequencing method. According to preferred embodiments of the eighth and/or ninth aspect of the invention, a partial or entire genome sequence of a Staphylococcus, particularly Staphylococcus aureus, strain is determined by using a next generation sequencing or high throughput sequencing method.

According to certain embodiments of the eighth and/or ninth aspect, determining the nucleic acid sequence information or the presence of a genetic variation comprises determining a partial or entire sequence of the genome of the Staphylococcus species, particularly Staphylococcus aureus, wherein said partial or entire sequence of the genome comprises at least one of the positions with the genetic variation.

According to certain embodiments of the eighth and/or ninth aspect of the invention, as well as 15th, 16th, 17th and/or 18th aspect, the position is from Table 3a, and the antibiotic class is at least one of the ones (column:

sign_phenos_class) given for the respective position in Table 4a and/or the antibiotic is at least one of the ones (column: sign_phenos) given for the respective position in Table 4a.

5 According to certain embodiments of the eighth and/or ninth aspect of the invention, as well as 15th, 16th, 17th and/or 18th aspect, the position is from Table 3a, and at least one antibiotic is from the antibiotic class (column: best_pheno_class) given for the respective position in Table
10 4d and/or at least one antibiotic is the antibiotic (column: best_pheno) given for the respective position in Table 4d.

According to certain embodiments of the eighth and/or ninth aspect of the invention, as well as 15th, 16th, 17th and/or
15 18th aspect, the position is from Table 3b, and the antibiotic class is at least one of the ones (column: sign_phenos_class) given for the respective position in Table 4e and/or the antibiotic is at least one of the ones (column: sign_phenos) given for the respective position in Table 4e.

20 According to certain embodiments of the eighth and/or ninth aspect of the invention, as well as 15th, 16th, 17th and/or 18th aspect, the position is from Table 3b, and at least one antibiotic is from the antibiotic class (column: best_pheno_class) given for the respective position in Table
25 4h and/or at least one antibiotic is the antibiotic (column: best_pheno) given for the respective position in Table 4h.

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

- a) obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus*, particularly *Staphylococcus aureus*, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b, particular with regard to the reference genomes with the genome names given in Table 3b, wherein the presence of said at least two genetic variations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;
- 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 the *Staphylococcus*, particularly *Staphylococcus aureus*, infection; and
- e) treating the patient with said one or more antimicrobial, e.g. antibiotic, drugs.

Herein, steps a) to d) can be carried out as described with respect to the ninth aspect. Step e) can be sufficiently carried out without being restricted and can be done e.g. non-invasively.

A sixteenth aspect of the present invention discloses a diagnostic method of determining an infection of a patient with a *Staphylococcus* species, particularly *Staphylococcus aureus*, 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 Staphylococcus, particularly Staphylococcus aureus, strain from the patient;

b) determining the presence of at least one genetic variation in at least one position from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b, particularly with regard to the reference genomes with the genome names given in Table 3b, wherein the presence of said at least one genetic variation is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant Staphylococcus, particularly Staphylococcus aureus, strain in said patient, wherein for some positions more than one position in different reference genomes is annotated.

In a seventeenth aspect, the present invention relates to a method of selecting a treatment of a patient suffering from an infection with a potentially resistant Staphylococcus, particularly Staphylococcus aureus, strain, comprising the steps of:

a) obtaining or providing a sample containing or suspected of containing at least one Staphylococcus, particularly Staphylococcus aureus, strain from the patient;

b) determining the presence of at least one genetic variation in at least one position from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b, particularly with regard to the reference genomes with the genome names given in Table 3b, wherein the presence of said at least one genetic variation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;

- 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
5 suitable for the treatment of a Staphylococcus, particularly Staphylococcus aureus, infection.

Again, in the sixteenth and the seventeenth aspect the steps correspond to those in the eighth or ninth aspect, although
10 only a mutation in at least one gene is determined.

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 Staphylococcus, particularly Staphylococcus aureus, infection, comprising the steps
15 of:

- a) obtaining or providing a sample containing or suspected of containing at least one Staphylococcus, particularly Staphylococcus aureus, strain from the patient;
- 20 b) determining the presence of at least one genetic variation in at least one position from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b, particularly with regard to the reference genomes with the genome
25 names given in Table 3b, wherein the presence of said at least one genetic variation is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;
- 30 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 Staphylococcus, particularly Staphylococcus aureus, infection; and

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

5

Also in the eighteenth aspect of the invention, steps a) to d) are analogous to the steps in the method of the fifteenth aspect of the present invention. Step e) can again be sufficiently carried out without being restricted and can be done e.g. non-invasively.

10

Examples

15 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.

20 Example 1: Determination of genetic resistance profile for MRSA/MSSA phenotype

Whole genome sequencing was carried out in addition to classical antimicrobial susceptibility testing of the same isolates for a cohort of 1001 specimens of S. aureus, of which 25 995 had an assembly and 987 had an assembly and an MRSA/MSSA phenotype. These 987 samples were used for further analysis. The whole genome sequencing allowed performing genome wide correlation studies to find genetic variants (e.g. point mutations, small insertions and deletion, larger structural 30 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 al-

so allowed 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.

The detailed procedure is given in the following:

Bacterial Strains

The inventors selected 1001 specimens of *S. aureus* from the microbiology strain collection at Siemens Healthcare Diagnostics (West Sacramento, CA) for susceptibility testing and whole genome sequencing, of which 987 were further analyzed, as stated above. To include data on the different ways how resistance mechanisms are acquired *Staphylococcus aureus* isolates collected over more than three decades were analyzed such that also horizontal gene transfer could potentially be discovered.

Determination of Methicillin resistance / susceptibility
MRSA and MSSA strains were determined by culturing according to standard procedures, determining the phenotype of the strains, and confirmed by further tests using e.g. the genetic information.

30

DNA extraction

DNA extraction and purification was carried out using the MagAttract HMW DNA Kit (Qiagen) procedure with the following

changes. After up to 2×10^9 bacteria (1ml culture) were centrifuged in a 2 ml tube (10 min, 5000 x g) and the supernatant was discharged, it was again centrifuged 1 min and the sample was taken. The resulting pellet was dispersed in 160
5 μ l P1, 20 μ l lysozyme (100 mg/ml) and 4 μ l lysostaphin were added and mixed, and the suspension was incubated at 37°C at 900 rpm for 30 mins in a thermal mixer. Afterwards 300 μ l lysis buffer and proteinase K (30 μ l) (both from the blood kit for Maxwell of Promega) were added and the whole again
10 incubated for 30 mins at 56°C and 900 rpm. The samples as a whole (~ 510 μ l) were then transferred to the Maxwell cartridges for further processing, using the Tissue LEV Total RNA Kit AS1220 or the XAS1220 Custom Kit (Promega).

15 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
20 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

25 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 sequencing chemistry (Illumina). Basic sequencing quality parameters were determined using the FastQC quality control
30 tool for high throughput sequence data (Babraham Bioinformatics Institute).

Data analysis

Trimmomatic (version 0.32, Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114-2120.

5 doi:10.1093/bioinformatics/btu170) was used for adapter and quality trimming of raw reads with following parameters
ILLUMINACLIP:NexteraPE-PE.fa:1:50:30 LEADING:3 TRAILING:3
SLIDINGWINDOW:4:15 MINLEN:36. *De novo* assemblies were constructed using SPAdes (version 3.0.0, Bankevich A, Nurk S,
10 Antipov D, et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology. 2012;19(5):455-477.
doi:10.1089/cmb.2012.0021) with parameters -t 20 -m 256 -k 21,33,55,77 --careful -1 fp.fastq.gz -2 rp.fastq.gz. To de-
15 termine the quality of the assemblies we ran QUAST (version 2.3) with minimal length threshold of 500 bp. Resulting metric values not matching the RefSeq assembly quality criteria (N50 > 5000, L50 < 20, # contigs < 1000) were highlighted.

20 SNP calling:

Reference-free SNP calling was performed using tool kSNP3 which applies k-mer analysis, i.e. the tool considers all possible k-mers found in given data. The central base of a k-mer is the SNP (example k=21 "AAAGTTTCGCAGTTGGTAATA", SNP=A),
25 the bases on its left and right site are SNP's context.

- Tool URL: <http://sourceforge.net/projects/ksnp/files/>

The following input was used:

- 30 • 49 finished *S. aureus* genomes from NCBI including the chromosome and available plasmids
- 995 *S. aureus* *de novo* assemblies (see above)
 - In total: 995+49=1044 samples

The finished genomes were used to choose the parameter k , the chosen value was 21, as determined by the tool.

5 SNP calling results:

The output contained 487,415 SNPs annotated using given finished genomes resulting in 9,419,797 annotations in total. SNPs can have following values: bases A/T/C/G or "-" (missing), the latter means, that the considered genomic part is
10 missing (e.g. gene absence). With the results, the annotations used in Table 2 were obtained along with other annotations (see Table 2 and annotations for details)

Extracting SNP annotations:

15 To reduce the number of similar annotations they were filtered and aggregated as follows:

- Only annotations for which the considered SNP lies on a protein were kept
- Only annotations whose "product" entry and "note" entry
20 do not contain "hypothetical protein" were kept
- Annotations were sorted by SNP ID ("LocusNum") and gene product ("product")
- For each unique pair of SNP ID and gene product only the first line was kept

25

Association testing:

In addition, the following SNPs were not considered:

- SNPs without any annotation or SNPs whose all annotations contained a flag "synonymous" were not considered → on-
30 ly SNPs with at least one non-synonymous annotation were considered
- Constant SNPs, i.e. same value for all samples were also not considered

- Almost constant SNPs: SNPs whose most frequent value had a frequency $\geq 95\%$, i.e. min. 95% of all samples have the same SNP value, were not considered as well
- SNPs with missing value ("–") for more than 10% of samples were also removed

In total 14,856 SNPs were kept for association tests.

Fisher's exact two-sided test was applied with subsequent p-value adjustment using FDR and p-value threshold of 10^{-9} . In

total 7,925 SNPs have a significant adjusted p-value, 7101 of them have at least one annotation.

Annotation:

Annotation of the found SNPs was carried out using 49 refer-

ence genomes available at NCBI, with the names of the genomes and the reference sequence ID at NCBI (for chromosomes; respectively plasmids) given in the following Table 5.

Genome name	RefSeq ID Chr; plasmids
04_02981	NC_017340.fna;
08BA02176	NC_018608.fna;
11819_97	NC_017351.fna;NC_017350.fna
55_2053	NC_022113.fna;NC_022126.fna
6850	NC_022222.fna;
71193	NC_017673.fna;
Bmb9393	NC_021670.fna;NC_021657.fna
CC45	NC_021554.fna;NC_021552.fna
CN1	NC_022226.fna;NC_022227.fna, NC_022228.fna
COL	NC_002951.fna;NC_006629.fna
ECT_R_2	NC_017343.fna;NC_017346.fna, NC_017344.fna
ED133	NC_017337.fna;
ED98	NC_013450.fna;NC_013451.fna, NC_013452.fna, NC_013453.fna
HO_5096_0412	NC_017763.fna;
JH1	NC_009632.fna;NC_009619.fna
JH9	NC_009487.fna;NC_009477.fna
JKD6008	NC_017341.fna;
JKD6159	NC_017338.fna;NC_017339.fna
LGA251	NC_017349.fna;NC_017348.fna
M013	NC_016928.fna;
M1	NC_021059.fna;NC_021060.fna
MRSA252	NC_002952.fna;
MSHR1132	NC_016941.fna;NC_016942.fna
MSSA476	NC_002953.fna;NC_005951.fna
Mu3	NC_009782.fna;
Mu50	NC_002758.fna;NC_002774.fna
MW2	NC_003923.fna;

N315	NC_002745.fna;NC_003140.fna
NCTC_8325	NC_007795.fna;
Newman	NC_009641.fna;
RF122	NC_007622.fna;
SA40	NC_022443.fna;
SA957	NC_022442.fna;
ST228_10388	NC_020529.fna;NC_020530.fna
ST228_10497	NC_020564.fna;NC_020531.fna
ST228_15532	NC_020532.fna;NC_020565.fna
ST228_16035	NC_020533.fna;NC_020534.fna
ST228_18412	NC_020537.fna;NC_020538.fna
ST398	NC_017333.fna;NC_017335.fna, NC_017334.fna, NC_017336.fna
T0131	NC_017347.fna;
TCH60	NC_017342.fna;NC_017345.fna
TW20	NC_017331.fna;NC_017332.fna, NC_017352.fna
uid193758	NC_020566.fna;NC_020535.fna
uid193759	NC_020536.fna;NC_020567.fna
uid193761	NC_020568.fna;NC_020539.fna
USA300_FPR3757	NC_007793.fna;NC_007792.fna, NC_007791.fna, NC_007790.fna
USA300_TCH1516	NC_010079.fna;NC_010063.fna, NC_012417.fna
VC40	NC_016912.fna;
Z172	NC_022604.fna;NC_022610.fna, NC_022605.fna

From the data, the 50 genes with the best p-value were chosen for the list of genetic variants with regard to methicillin resistance.

5

A full list of all positions, p-values, affected genes etc. is provided in Table 2, respectively Tables 2a and 2b, which corresponds to Table 1, and represents the genes having the lowest p-values after correlating the genetic variations with antibiotic resistance.

10

In Table 2, respectively Tables 2a and 2b, the positions are numbered according to the best p-value results, ranging from 1 to 50. Further, the positions are also annotated with regard to one or more reference genomes of the 49 finished *S. aureus* genomes from NCBI, wherein the found reference genomes are the following as annotated at the NCBI:

15

NC_017340, NC_010079, NC_022222, NC_021670, NC_017351,
 NC_002953, NC_017337, NC_018608, NC_007795, NC_021059,
 NC_021554, NC_016912, NC_022226, NC_022113

20

http://www.genome.jp/dbget-bin/www_bget?refseq+NC_017340
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_010079
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_022222
5 http://www.genome.jp/dbget-bin/www_bget?refseq+NC_021670
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_017351
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_002953
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_017337
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_018608
10 http://www.genome.jp/dbget-bin/www_bget?refseq+NC_007795
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_021059
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_021554
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_016912
http://www.genome.jp/dbget-bin/www_bget?refseq+NC_022226
15 http://www.genome.jp/dbget-bin/www_bget?refseq+NC_022113

In Table 2, respectively Tables 2a and 2b, more than one annotation per SNP is possible for various positions with regard to the reference genomes for the following reason: the SNPs were annotated using all given finished genomes, thus a SNP may have multiple annotations even after the annotation aggregation, which was mentioned above. The reasons why a SNP may have more than one annotation after the aggregation can be as follows:

- 25 ◦ The gene products have very similar but not equal information, e.g. "potassium-transporting ATPase A chain" and "potassium-transporting ATPase subunit A". In this case it may be not possible to apply a straightforward approach to remove such duplicates.
- 30 ◦ The annotations may differ in the genes/gene products, then it may be not possible not say which of the annotations is the correct one.

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Table 2a: List of positions (corresponding to Table 1)

No.	p-value (FDR)	GenomeName	fasta_header	SNPPositioninGenome	gene
1	2,8455E-163	04_02981 USA300 TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	534953 F 543821 F	
2	2,8455E-163	6850 04_02981 USA300 TCH1516	gi 537441500 ref NC_022222.1 gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	210528 R 267448 R 269814 R	
3	3,348E-163	04_02981	gi 387149188 ref NC_017340.1	1362060 F	
4	3,348E-163	Bmb9393 11819_97 04_02981	gi 521210823 ref NC_021670.1 gi 385780298 ref NC_017351.1 gi 387149188 ref NC_017340.1	1252703 R 1520285 F 1523326 F	
5	3,5068E-163	04_02981 USA300 TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	1619285 R 1661238 R	
6	4,5499E-163	04_02981	gi 387149188 ref NC_017340.1	1641150 R	
7	4,5499E-163	MSSA476 ED133	gi 49484912 ref NC_002953.3 gi 384546269 ref NC_017337.1	170059 F 142263 F	
8	4,5499E-163	04_02981 08BA02176	gi 387149188 ref NC_017340.1 gi 404477334 ref NC_018608.1	517571 F 554542 F	
9	6,182E-163	04_02981	gi 387149188 ref NC_017340.1	978538 F	
10	6,182E-163	04_02981	gi 387149188 ref NC_017340.1	1434811 R	
11	6,182E-163	6850 USA300 TCH1516	gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1	953696 R 1010027 R	rluA1
12	6,182E-163	04_02981 NCTC 8325	gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	208285 R 161011 R	argC argC
13	6,182E-163	11819_97 04_02981 08BA02176	gi 385780298 ref NC_017351.1 gi 387149188 ref NC_017340.1 gi 404477334 ref NC_018608.1	2179136 R 2149064 R 2107689 R	

14	6, 182E-163	04 02981	gi 387149188 ref NC_017340.1	2358535 F	
15	6, 182E-163	04 02981	gi 387149188 ref NC_017340.1	2023012 R	
16	6, 182E-163	NCTC_8325	gi 88193823 ref NC_007795.1	2777211 F	
		04 02981	gi 387149188 ref NC_017340.1	2779170 F	
17	6, 182E-163	08BA02176	gi 404477334 ref NC_018608.1	1801995 R	
		04 02981	gi 387149188 ref NC_017340.1	1790672 R	
18	7, 2093E-163	04 02981	gi 387149188 ref NC_017340.1	976788 F	
		NCTC_8325	gi 88193823 ref NC_007795.1	878040 F	
19	7, 2093E-163	Bmb9393	gi 521210823 ref NC_021670.1	2101899 R	
		04 02981	gi 387149188 ref NC_017340.1	1972149 R	
20	1, 1926E-162	6850	gi 537441500 ref NC_022222.1	1875550 F	bcp
		USA300_TCH1516	gi 161508266 ref NC_010079.1	2006001 F	
		04 02981	gi 387149188 ref NC_017340.1	1959494 F	
21	1, 2012E-162	04 02981	gi 387149188 ref NC_017340.1	705667 R	
22	1, 2012E-162	USA300_TCH1516	gi 161508266 ref NC_010079.1	2268723 F	
		04 02981	gi 387149188 ref NC_017340.1	2221448 F	
23	1, 2797E-162	04 02981	gi 387149188 ref NC_017340.1	1814108 R	
24	2, 0719E-162	04 02981	gi 387149188 ref NC_017340.1	531649 R	
		11819_97	gi 385780298 ref NC_017351.1	531398 R	
25	2, 0719E-162	04 02981	gi 387149188 ref NC_017340.1	1754561 F	
		NCTC_8325	gi 88193823 ref NC_007795.1	1691742 F	
26	2, 0719E-162	04 02981	gi 387149188 ref NC_017340.1	1958403 R	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	2004910 R	
27	2, 2505E-162	6850	gi 537441500 ref NC_022222.1	1242653 R	ribC
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1294527 R	
		04 02981	gi 387149188 ref NC_017340.1	1299554 R	

28	2, 5515E-162	04_02981 USA300 TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	2590222 R 2637689 R	gntP
29	8, 302E-162	USA300 TCH1516 M1 6850 08BA02176 CC45 Bmb9393	gi 161508266 ref NC_010079.1 gi 479328021 ref NC_021059.1 gi 537441500 ref NC_022222.1 gi 404477334 ref NC_018608.1 gi 514064966 ref NC_021554.1 gi 521210823 ref NC_021670.1	1881161 R 1871101 R 1759861 R 1855493 R 1858794 R 1964828 R	fmtB1
30	1, 7865E-161	NCTC_8325 04_02981	gi 88193823 ref NC_007795.1 gi 387149188 ref NC_017340.1	1050123 R 1147277 R	
31	9, 7283E-147	VC40 6850 USA300 TCH1516	gi 379013365 ref NC_016912.1 gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1	2005634 F 2039052 F 2187801 F	rsbU
32	2, 0972E-146	6850 04_02981 NCTC_8325	gi 537441500 ref NC_022222.1 gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	350202 F 402479 F 352104 F	
33	6, 9539E-146	M1 08BA02176 04_02981 NCTC_8325	gi 479328021 ref NC_021059.1 gi 404477334 ref NC_018608.1 gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	920768 F 956878 F 956978 F 858255 F	rocD rocD rocD rocD
34	8, 7237E-146	04_02981 NCTC_8325	gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	1121847 R 1024692 R	
35	1, 2342E-145	04_02981	gi 387149188 ref NC_017340.1	429303 F	
36	2, 7651E-145	USA300 TCH1516 04_02981 NCTC_8325	gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	1812380 R 1775835 R 1714993 R	dnaE2
37	2, 9677E-145	04_02981	gi 387149188 ref NC_017340.1	1928346 F	

38	1, 6935E-144	CN1	gi 537459744 ref NC_022226.1	1388095 R	
39	1, 7849E-144	04_02981 NCTC_8325	gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	559072 R 504007 R	
40	1, 8015E-144	USA300_TCH1516 04_02981	gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	2719339 R 2668764 R	
41	4, 3308E-144	04_02981 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	1124668 F 1121585 F	
42	5, 541E-144	CN1 M1 6850 USA300_TCH1516 04_02981	gi 537459744 ref NC_022226.1 gi 479328021 ref NC_021059.1 gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	158073 F 193628 F 138357 F 196480 F 189192 F	
43	6, 1938E-144	04_02981 55_2053 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 532358222 ref NC_022113.1 gi 161508266 ref NC_010079.1	1187805 F 1078815 F 1182930 F	
44	1, 7019E-143	USA300_TCH1516 6850 CN1 04_02981 Bmb9393	gi 161508266 ref NC_010079.1 gi 537441500 ref NC_022222.1 gi 537459744 ref NC_022226.1 gi 387149188 ref NC_017340.1 gi 521210823 ref NC_021670.1	1376396 R 1323236 R 1315892 R 1379143 R 1399306 F	sbcC
45	2, 694E-143	04_02981	gi 387149188 ref NC_017340.1	2398505 R	
46	3, 3496E-143	04_02981 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	2753541 F 2803165 F	
47	3, 5029E-143	04_02981 08EA02176 NCTC_8325 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 404477334 ref NC_018608.1 gi 88193823 ref NC_007795.1 gi 161508266 ref NC_010079.1	1415365 R 1428821 R 1318646 R 1412563 R	femB

		M1	gi 479328021 ref NC_021059.1	1381147 R	
48	3,5029E-143	04_02981 USA300 TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	1678734 R 1720315 R	
49	8,2809E-143	NCTC_8325 04_02981 USA300 TCH1516	gi 88193823 ref NC_007795.1 gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	854815 R 953539 R 948900 R	prsA1
50	8,2809E-143	04_02981	gi 387149188 ref NC_017340.1	1675156 R	

Table 2b: list of positions (corresponding to Table 1, continued)

No.	Amino Acids	Codons	GenomeGI	Protein_GI
1	F_L F_L	TTA_TTT TTA_TTT	387149188 161508266	446874184 161508745
2	A_V A_V A_V	GCA_GTA GCA_GTA GCA_GTA	537441500 387149188 161508266	537465126 447077358 161508491
3	I_N	AAT_ATT	387149188	447178207
4	L_S L_S L_S	TCA_TTA TCA_TTA TCA_TTA	521210823 385780298 387149188	521258120 446060496 446060495
5	N_S N_S	AAT_AGT AAT_AGT	387149188 161508266	446940596 161509778
6	E_K	AAA_GAA	387149188	446032753
7	G_R G_R	AGA_GGA AGA_GGA	49484912 384546269	487756815 446093782
8	K_R K_R	AAA_AGA AAA_AGA	387149188 404477334	446973880 446973883

9	I_M	ATC_ATG	387149188	446312722	
10	A_S	GCA_TCA	387149188	446180863	
11	L_P	CCT_CTT	537441500	537465549	
	L_P	CCT_CTT	161508266	161509205	
12	K_T	AAA_ACA	387149188	446556386	
	K_T	AAA_ACA	88193823	88193961	
13	M_V	ATG_GTG	385780298	446324804	
	M_V	ATG_GTG	387149188	446324797	
	M_V	ATG_GTG	404477334	446324791	
14	K_N	AAA_AAC	387149188	445930822	
15	E_V	GAA_GTA	387149188	446943955	
16	F_L	TTG_TTT	88193823	88196623	
	F_L	TTG_TTT	387149188	446800117	
17	Q_R	CAG_CGG	404477334	446795417	
	Q_R	CAG_CGG	387149188	446795407	
18	F_L	TTA_TTT	387149188	447047252	
	F_L	TTA_TTT	88193823	88194665	
19	M_V	ATG_GTG	521210823	752533903	
	M_V	ATG_GTG	387149188	446753128	
20	D_Y	GAT_TAT	537441500	537465893	
	D_Y	GAT_TAT	161508266	161510081	
	D_Y	GAT_TAT	387149188	446862272	
21	E_K	AAG_GAG	387149188	446725640	
22	L_S	TCA_TTA	161508266	161510359	
	L_S	TCA_TTA	387149188	446293068	
23	A_V	GCG_GTG	387149188	446784840	

24	N_T N_T	AAT_ACT AAT_ACT	387149188 385780298	446076361 446076373
25	L_V L_V	GTA_TTA GTA_TTA	387149188 88193823	446028277 88195494
26	A_T A_T	ACA_GCA ACA_GCA	387149188 161508266	446792191 161510080
27	I_T I_T I_T	ACC_ATC ACC_ATC ACC_ATC	537441500 161508266 387149188	537465687 161509438 446786934
28	K_T K_T	AAA_ACA AAA_ACA	387149188 161508266	446403560 161510698
29	K_T K_T K_T K_T K_T K_T K_T	AAG_ACG AAG_ACG AAG_ACG AAG_ACG AAG_ACG AAG_ACG AAG_ACG	161508266 479328021 537441500 404477334 514064966 521210823	161509974 505394769 537465850 446973259 514074897 521258173
30	F_L F_L	TTA_TTT TTA_TTT	88193823 387149188	88194836 446593607
31	I_V I_V I_V	ATA_GTA ATA_GTA ATA_GTA	379013365 537441500 161508266	487720346 537465949 161510279
32	K_T K_T K_T	AAA_ACA AAA_ACA AAA_ACA	537441500 387149188 88193823	537465192 446129782 88194138
33	E_K E_K	AAA_GAA AAA_GAA	479328021 404477334	505394709 446089469

	E_K	AAA_GAA	387149188	446089454
	E_K	AAA_GAA	88193823	88194651
34	I_T	ACA_ATA	387149188	446104798
	I_T	ACA_ATA	88193823	88194808
35	G_V	GGA_GTA	387149188	446343556
36	D_E	GAA_GAT	161508266	161509916
	D_E	GAA_GAT	387149188	446149063
	D_E	GAA_GAT	88193823	88195511
37	A_G	GCA_GGA	387149188	446506832
38	C_Y	TAT_TGT	537459744	686312170
39	A_V	GCC_GTC	387149188	446804811
	A_V	GCC_GTC	88193823	88194284
40	P_T	ACA_CCA	161508266	161510779
	P_T	ACA_CCA	387149188	446083969
41	I_L	ATA_TTA	387149188	446710589
	I_L	ATA_TTA	161508266	161509291
42	I_V	ATT_GTT	537459744	537467717
	I_V	ATT_GTT	479328021	505394663
	I_V	ATT_GTT	537441500	537465062
	I_V	ATT_GTT	161508266	161508437
	I_V	ATT_GTT	387149188	446513509
43	I_T	ACA_ATA	387149188	446462960
	I_T	ACA_ATA	532358222	532479591
	I_T	ACA_ATA	161508266	161509351
44	I_T	ACT_ATT	161508266	161509514
	I_T	ACT_ATT	537441500	537465718
	I_T	ACT_ATT	537459744	537467986

	I_T	ACT_ATT	387149188	446725826
	I_T	ACT_ATT	521210823	521258127
45	G_S	AGT_GGT	387149188	446921498
46	S_Y	TAT_TCT	387149188	446080575
	S_Y	TAT_TCT	161508266	161510854
47	L_S	TCA_TTA	387149188	446595763
	L_S	TCA_TTA	404477334	446595752
	L_S	TCA_TTA	88193823	88195101
	L_S	TCA_TTA	161508266	161509542
	L_S	TCA_TTA	479328021	505394733
48	I_L	ATT_CTT	387149188	446059917
	I_L	ATT_CTT	161508266	161509840
49	A_V	GCA_GTA	88193823	88194648
	A_V	GCA_GTA	387149188	445957208
	A_V	GCA_GTA	161508266	161509155
50	D_N	AAT_GAT	387149188	446305320

In Table 2, respectively Tables 2a and 2b, the annotations obtained by the analysis contain the following information:

- No.: consecutive number
- p-value (FDR): significance value calculated for MRSA-MSSA using Fishers exact test and adjusted by FDR (Benjamini Hochberg method (Benjamini Hochberg, 1995))
- GenomeName: Name of the reference genome used for the annotation
- fasta_header: Header of the reference genome fasta file (including GI and NCBI RefSeq ID)
- SNPPositioninGenome: SNP position in the reference genome (F = forward; R = reverse)
- AminoAcids: Amino acids coded by the codon in which the SNP occurs (only one value for synonymous SNPs, otherwise at least 2), separated by "_"
- Codons: All found codons for the SNP, separated by "_"
- GenomeGI: GI number of the genome sequence
- Protein_GI: GI number of the protein sequence
- gene: gene symbol (if applicable)
- product: Gene product
- protein_id: GenBank accession of the protein

Further, in Table 2, all SNP are non-synonymous (1=yes, 0=no), and the SNPs lie within a coding region (are "OnProtein")

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.

- 5 The following results were obtained
- A total of 7.101 non-synonymous SNPs associated with the MRSA/MSSA phenotypes (FDR adjusted p-value $< 10^{-9}$) was detected.
 - The biggest part of these were point mutations (i.e. single
10 base exchanges)
 - The highest significance reached was $< 3 \cdot 10^{-163}$.

Example 2: Determination of genetic resistance profile

The same bacteria used in Example 1, i.e. the cohort of 1001
15 specimens of *S. aureus*, were used in Example 2. Of those 985 had an assembly, a unique Kiel NGS ID (NGS data and assembly ID, a unique resistance profile (no different resistance profiles with different outcomes, and at least one drug with non-missing resistance value, so that these were further ana-
20 lyzed. The experiments were carried out as in Example 1, except that instead of a determination of Methicillin resistance / susceptibility, resistance / susceptibility was determined for the following antibiotics as described below:

Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam,
25 Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Moxifloxacin, Mupirocin,
30 Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin.

For testing, standard procedures were used, i.e. VITEK 2 system and AST cards (Biomerieux), Microscan system and AST panels (Beckmann Coulter).

5

Data analysis was carried out as in Example 1.

For the resistance profiles only drugs with non-missing data for at least 10% of the samples were kept, so that only 16 drugs remained: Ampicillin, Ampicillin/Sulbactam, Cefepime, Cefotaxime, Cefuroxime, Ciprofloxacin, Clindamycin, Erythromycin, Imipenem, Levofloxacin, Moxifloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Tetracycline, and Tobramycin.

15

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 3a and 3b.

20 For correlation, the data was filtered by the following drug class ratio and the annotation product:

$$\text{drugclassratio} = \frac{\text{number of significant drug of that class}}{\text{number of tested drug of that class}}$$

The genes in Table 3a thereby represent the 50 best genes for which a mutation was observed in the genomes of *S. aureus*, whereas the genes in Table 3b represent the 50 best genes for which a cross-correlation could be observed for the antimicrobial drug, e.g. antibiotic, susceptibility testing. Details for Table 3a are given in Tables 4a-d, and details for Tables 3b in Tables 4e-h. The found reference genomes were as in Example 1.

30

Table 4a: List of positions (corresponding to Table 3a)

No.	position	reference genome	genome name	sign_phenos	sign_phenos_class
1	1958403 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
2	2004910 R	NC_010079.1	USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
3	1641150 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
4	978538 F	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
5	705667 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
6	1434811 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
6	953696 R	NC_022222.1	6850	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	1010027 R	NC_010079.1	USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide

7	2101899 R	NC_021670.1	Bmb9393	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
8	1972149 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
9	208285 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	161011 R	NC_007795.1	NCTC_8325	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	2179136 R	NC_017351.1	11819_97	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	2149064 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
10	2107689 R	NC_018608.1	08BA02176	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	2358535 F	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
11	2023012 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
12	2777211 F	NC_007795.1	NCTC_8325	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	2779170 F	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
13	1801995 R	NC_018608.1	08BA02176	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	1790672 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide

14	1754561 F	NC_017340.1			Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	mid; macrolide
	1691742 F	NC_007795.1		04_02981 NCTC_8325	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
15	1362060 F	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
16	1242653 R 1294527 R	NC_022222.1 NC_010079.1		6850 USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
17	1252703 R 1520285 F	NC_021670.1 NC_017351.1		Bmb9393 11819_97	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
18	1523326 F 1619285 R	NC_017340.1 NC_017340.1		04_02981 04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1661238 R	NC_010079.1		USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
19	1875550 F 2006001 F	NC_022222.1 NC_010079.1		6850 USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1959494 F	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
20	976788 F	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide

	878040 F	NC_007795.1		NCTC_8325	Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	fluoroquinolone;lactam; lincosamide;macrolide
21	2590222 R	NC_017340.1		04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
22	2637689 R	NC_010079.1		USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	210528 R	NC_022222.1		6850	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	267448 R	NC_017340.1		04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	269814 R	NC_010079.1		USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
23	1814108 R	NC_017340.1		04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
24	170059 F	NC_002953.3		MSSA476	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	142263 F	NC_017337.1		ED133	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
25	534953 F	NC_017340.1		04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	543821 F	NC_010079.1		USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
26	517571 F	NC_017340.1		04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	554542 F	NC_018608.1		08BA02176	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide

27	531649 R	NC_017340.1	04_02981	G; Tobramycin	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
28	531398 R	NC_017351.1	11819_97			
	1050123 R	NC_007795.1	NCTC_8325		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1147277 R	NC_017340.1	04_02981			
29	1881161 R	NC_010079.1	USA300_TCH1516			
	1871101 R	NC_021059.1	M1		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1759861 R	NC_022222.1	6850			
	1855493 R	NC_018608.1	08BA02176			
	1858794 R	NC_021554.1	CC45			
	1964828 R	NC_021670.1	Bmb9393			
30	2268723 F	NC_010079.1	USA300_TCH1516		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2221448 F	NC_017340.1	04_02981			
31	920768 F	NC_021059.1	M1		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	956878 F	NC_018608.1	08BA02176			
	956978 F	NC_017340.1	04_02981			
	858255 F	NC_007795.1	NCTC_8325			
32	2005634 F	NC_016912.1	VC40		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2039052 F	NC_022222.1	6850			
	2187801 F	NC_010079.1	USA300_TCH1516			

33	429303 F	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
34	350202 F 402479 F	NC_022222.1 NC_017340.1	6850 04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
35	352104 F 158073 F 193628 F 138357 F 196480 F 189192 F	NC_007795.1 NC_022226.1 NC_021059.1 NC_022222.1 NC_010079.1 NC_017340.1	NCTC_8325 CN1 M1 6850 USA300_TCH1516 04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
36	1121847 R 1024692 R	NC_017340.1 NC_007795.1	04_02981 NCTC_8325	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
37	2719339 R 2668764 R	NC_010079.1 NC_017340.1	USA300_TCH1516 04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
38	1388095 R	NC_022226.1	CN1	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
39	1415365 R 1428821 R	NC_017340.1 NC_018608.1	04_02981 08BA02176	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin;	aminoglycoside; fluoroquinolone

	1318646 R	NC_007795.1	NCTC_8325	Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	ne; lactam; lincosamide; macrolide
40	1412563 R	NC_010079.1	USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1381147 R	NC_021059.1	M1		
	1678734 R	NC_017340.1	04_02981		
	1720315 R	NC_010079.1	USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
41	1928346 F	NC_017340.1	04_02981		
42	1376396 R	NC_010079.1	USA300_TCH1516		
	1323236 R	NC_022222.1	6850	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1315892 R	NC_022226.1	CN1		
	1379143 R	NC_017340.1	04_02981		
	1399306 F	NC_021670.1	Bmb9393	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
43	1338943 R	NC_017340.1	04_02981		
44	1124668 F	NC_017340.1	04_02981		
	1121585 F	NC_010079.1	USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
45	559072 R	NC_017340.1	04_02981		
	504007 R	NC_007795.1	NCTC_8325		

					G; Tobramycin	
					Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
46	1675156 R	NC_017340.1	04_02981			
47	1187805 F 1078815 F	NC_017340.1 NC_022113.1	04_02981 55_2053		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1182930 F	NC_010079.1	USA300_TCH1516			
48	1356138 F	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
49	854815 R 953539 R	NC_007795.1 NC_017340.1	NCTC_8325 04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	948900 R	NC_010079.1	USA300_TCH1516			
50	2459738 F	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2364478 F	NC_022222.1	6850			

Table 4b: List of positions (corresponding to Table 3a, continued)

No.	best_pv	GenomeName	fasta_header	SNPPositioninGenome	gene
1	6,0168E-193	04_02981	gi 387149188 ref NC_017340.1	1958403 R	

		USA300_TCH1516	gi 161508266 ref NC_010079.1	2004910 R	
2	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	1641150 R	
3	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	978538 F	
4	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	705667 R	
5	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	1434811 R	
6	1,4167E-189	6850	gi 537441500 ref NC_022222.1	953696 R	rluA1
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1010027 R	
7	1,4167E-189	Bmb9393	gi 521210823 ref NC_021670.1	2101899 R	
		04_02981	gi 387149188 ref NC_017340.1	1972149 R	
8	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	208285 R	argC
		NCTC_8325	gi 88193823 ref NC_007795.1	161011 R	argC
9	1,4167E-189	11819_97	gi 385780298 ref NC_017351.1	2179136 R	
		04_02981	gi 387149188 ref NC_017340.1	2149064 R	
		08BA02176	gi 404477334 ref NC_018608.1	2107689 R	
10	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	2358535 F	
11	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	2023012 R	
12	1,4167E-189	NCTC_8325	gi 88193823 ref NC_007795.1	2777211 F	
		04_02981	gi 387149188 ref NC_017340.1	2779170 F	
13	1,4167E-189	08BA02176	gi 404477334 ref NC_018608.1	1801995 R	
		04_02981	gi 387149188 ref NC_017340.1	1790672 R	
14	3,0731E-189	04_02981	gi 387149188 ref NC_017340.1	1754561 F	
		NCTC_8325	gi 88193823 ref NC_007795.1	1691742 F	
15	3,1179E-189	04_02981	gi 387149188 ref NC_017340.1	1362060 F	
16	3,7988E-189	6850	gi 537441500 ref NC_022222.1	1242653 R	ribC
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1294527 R	
		04_02981	gi 387149188 ref NC_017340.1	1299554 R	
17	3,9683E-189	Bmb9393	gi 521210823 ref NC_021670.1	1252703 R	
		11819_97	gi 385780298 ref NC_017351.1	1520285 F	
		04_02981	gi 387149188 ref NC_017340.1	1523326 F	
18	3,9683E-189	04_02981	gi 387149188 ref NC_017340.1	1619285 R	

19	3,9683E-189	USA300_TCH1516 6850	gi 161508266 ref NC_010079.1	1661238 R	
		USA300_TCH1516 04_02981	gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	1875550 F 2006001 F 1959494 F	bcp
20	5,4236E-189	04_02981 NCTC_8325	gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	976788 F 878040 F	
21	7,5452E-189	04_02981 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	2590222 R 2637689 R	gntP
22	9,5271E-189	6850 04_02981 USA300_TCH1516	gi 537441500 ref NC_022222.1 gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	210528 R 267448 R 269814 R	
23	1,1093E-188	04_02981	gi 387149188 ref NC_017340.1	1814108 R	
24	3,3279E-188	MSSA476 ED133	gi 49484912 ref NC_002953.3 gi 384546269 ref NC_017337.1	170059 F 142263 F	
25	3,3279E-188	04_02981 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	534953 F 543821 F	
26	3,5518E-188	04_02981 08BA02176	gi 387149188 ref NC_017340.1 gi 404477334 ref NC_018608.1	517571 F 554542 F	
27	1,1492E-187	04_02981 11819_97	gi 387149188 ref NC_017340.1 gi 385780298 ref NC_017351.1	531649 R 531398 R	
28	1,1509E-187	NCTC_8325 04_02981	gi 88193823 ref NC_007795.1 gi 387149188 ref NC_017340.1	1050123 R 1147277 R	
29	9,0648E-187	USA300_TCH1516 M1 6850 08BA02176 CC45 Bmb9393	gi 161508266 ref NC_010079.1 gi 479328021 ref NC_021059.1 gi 537441500 ref NC_022222.1 gi 404477334 ref NC_018608.1 gi 514064966 ref NC_021554.1 gi 521210823 ref NC_021670.1	1881161 R 1871101 R 1759861 R 1855493 R 1858794 R 1964828 R	fmtB1
30	1,5807E-186	USA300_TCH1516 04_02981	gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	2268723 F 2221448 F	

31	3,6257E-174	M1 08BA02176 04_02981 NCTC_8325	gi 479328021 ref NC_021059.1 gi 404477334 ref NC_018608.1 gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	920768 F 956878 F 956978 F 858255 F	rocD rocD rocD rocD
32	3,9753E-174	VC40 6850 USA300_TCH1516	gi 379013365 ref NC_016912.1 gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1	2005634 F 2039052 F 2187801 F	rsbU
33	7,1007E-174	04_02981	gi 387149188 ref NC_017340.1	429303 F	
34	1,8906E-173	6850 04_02981 NCTC_8325	gi 537441500 ref NC_022222.1 gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	350202 F 402479 F 352104 F	
35	3,0713E-172	CN1 M1 6850 USA300_TCH1516 04_02981	gi 537459744 ref NC_022226.1 gi 479328021 ref NC_021059.1 gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	158073 F 193628 F 138357 F 196480 F 189192 F	
36	4,4332E-172	04_02981 NCTC_8325	gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	1121847 R 1024692 R	
37	4,4332E-172	USA300_TCH1516 04_02981	gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	2719339 R 2668764 R	
38	5,5156E-172	CN1	gi 537459744 ref NC_022226.1	1388095 R	
39	1,2003E-171	04_02981 08BA02176 NCTC_8325 USA300_TCH1516 M1	gi 387149188 ref NC_017340.1 gi 404477334 ref NC_018608.1 gi 88193823 ref NC_007795.1 gi 161508266 ref NC_010079.1 gi 479328021 ref NC_021059.1	1415365 R 1428821 R 1318646 R 1412563 R 1381147 R	femB
40	1,2003E-171	04_02981 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	1678734 R 1720315 R	
41	1,2491E-171	04_02981	gi 387149188 ref NC_017340.1	1928346 F	
42	2,3661E-171	USA300_TCH1516	gi 161508266 ref NC_010079.1	1376396 R	sbcC

		6850 CN1	gi 537441500 ref NC_022222.1	1323236 R	
		04_02981	gi 537459744 ref NC_022226.1	1315892 R	
		Bmb9393	gi 387149188 ref NC_017340.1	1379143 R	
			gi 521210823 ref NC_021670.1	1399306 F	
43	2,3661E-171	04_02981	gi 387149188 ref NC_017340.1	1338943 R	
44	5,3408E-171	04_02981	gi 387149188 ref NC_017340.1	1124668 F	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1121585 F	
45	1,0222E-170	04_02981	gi 387149188 ref NC_017340.1	559072 R	
		NCTC_8325	gi 88193823 ref NC_007795.1	504007 R	
46	1,0498E-170	04_02981	gi 387149188 ref NC_017340.1	1675156 R	
47	2,283E-170	04_02981	gi 387149188 ref NC_017340.1	1187805 F	
		55_2053	gi 532358222 ref NC_022113.1	1078815 F	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1182930 F	
48	2,3666E-170	04_02981	gi 387149188 ref NC_017340.1	1356138 F	
49	4,1086E-170	NCTC_8325	gi 88193823 ref NC_007795.1	854815 R	
		04_02981	gi 387149188 ref NC_017340.1	953539 R	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	948900 R	prsa1
50	4,2262E-169	04_02981	gi 387149188 ref NC_017340.1	2459738 F	
		6850	gi 537441500 ref NC_022222.1	2364478 F	

Table 4c: List of positions (corresponding to Table 3a, continued)

No.	AminoAcids	Codons	GenomeGI	Protein_GI
1	A_T	ACA_GCA	387149188	446792191
	A_T	ACA_GCA	161508266	161510080
2	E_K	AAA_GAA	387149188	446032753
3	I_M	ATC_ATG	387149188	446312722
4	E_K	AAG_GAG	387149188	446725640

5	A_S	GCA_TCA	387149188	446180863
6	L_P	CCT_CTT	537441500	537465549
	L_P	CCT_CTT	161508266	161509205
7	M_V	ATG_GTG	521210823	752533903
	M_V	ATG_GTG	387149188	446753128
8	K_T	AAA_ACA	387149188	446556386
	K_T	AAA_ACA	88193823	88193961
9	M_V	ATG_GTG	385780298	446324804
	M_V	ATG_GTG	387149188	446324797
	M_V	ATG_GTG	404477334	446324791
10	K_N	AAA_AAC	387149188	445930822
11	E_V	GAA_GTA	387149188	446943955
12	F_L	TTG_TTT	88193823	88196623
	F_L	TTG_TTT	387149188	446800117
13	Q_R	CAG_CGG	404477334	446795417
	Q_R	CAG_CGG	387149188	446795407
14	L_V	GTA_TTA	387149188	446028277
	L_V	GTA_TTA	88193823	88195494
15	I_N	AAT_ATT	387149188	447178207
16	I_T	ACC_ATC	537441500	537465687
	I_T	ACC_ATC	161508266	161509438
	I_T	ACC_ATC	387149188	446786934
17	L_S	TCA_TTA	521210823	521258120
	L_S	TCA_TTA	385780298	446060496
	L_S	TCA_TTA	387149188	446060495
18	N_S	AAT_AGT	387149188	446940596
	N_S	AAT_AGT	161508266	161509778
19	D_Y	GAT_TAT	537441500	537465893
	D_Y	GAT_TAT	161508266	161510081
	D_Y	GAT_TAT	387149188	446862272

20	F_L F_L	TTA_TTT TTA_TTT	387149188 88193823	447047252 88194665
21	K_T K_T	AAA_ACA AAA_ACA	387149188 161508266	446403560 161510698
22	A_V A_V A_V	GCA_GTA GCA_GTA GCA_GTA	537441500 387149188 161508266	537465126 447077358 161508491
23	A_V	GCG_GTG	387149188	446784840
24	G_R G_R	AGA_GGA AGA_GGA	49484912 384546269	487756815 446093782
25	F_L F_L	TTA_TTT TTA_TTT	387149188 161508266	446874184 161508745
26	K_R K_R	AAA_AGA AAA_AGA	387149188 404477334	446973880 446973883
27	N_T N_T	AAT_ACT AAT_ACT	387149188 385780298	446076361 446076373
28	F_L F_L	TTA_TTT TTA_TTT	88193823 387149188	88194836 446593607
29	K_T K_T K_T K_T K_T K_T	AAG_ACG AAG_ACG AAG_ACG AAG_ACG AAG_ACG AAG_ACG	161508266 479328021 537441500 404477334 514064966 521210823	161509974 505394769 537465850 446973259 514074897 521258173
30	L_S L_S	TCA_TTA TCA_TTA	161508266 387149188	161510359 446293068
31	E_K E_K E_K E_K	AAA_GAA AAA_GAA AAA_GAA AAA_GAA	479328021 404477334 387149188 88193823	505394709 446089469 446089454 88194651

32	I_V	ATA_GTA	379013365	487720346
	I_V	ATA_GTA	537441500	537465949
	I_V	ATA_GTA	161508266	161510279
33	G_V	GGA_GTA	387149188	446343556
34	K_T	AAA_ACA	537441500	537465192
	K_T	AAA_ACA	387149188	446129782
	K_T	AAA_ACA	88193823	88194138
35	I_V	ATT_GTT	537459744	537467717
	I_V	ATT_GTT	479328021	505394663
	I_V	ATT_GTT	537441500	537465062
	I_V	ATT_GTT	161508266	161508437
	I_V	ATT_GTT	387149188	446513509
36	I_T	ACA_ATA	387149188	446104798
	I_T	ACA_ATA	88193823	88194808
37	P_T	ACA_CCA	161508266	161510779
	P_T	ACA_CCA	387149188	446083969
38	C_Y	TAT_TGT	537459744	686312170
39	L_S	TCA_TTA	387149188	446595763
	L_S	TCA_TTA	404477334	446595752
	L_S	TCA_TTA	88193823	88195101
	L_S	TCA_TTA	161508266	161509542
	L_S	TCA_TTA	479328021	505394733
40	I_L	ATT_CTT	387149188	446059917
	I_L	ATT_CTT	161508266	161509840
41	A_G	GCA_GGA	387149188	446506832
42	I_T	ACT_ATT	161508266	161509514
	I_T	ACT_ATT	537441500	537465718
	I_T	ACT_ATT	537459744	537467986
	I_T	ACT_ATT	387149188	446725826
	I_T	ACT_ATT	521210823	521258127

43	C_G	GGT_TGT	387149188	445990753
44	I_L	ATA_TTA	387149188	446710589
	I_L	ATA_TTA	161508266	161509291
45	A_V	GCC_GTC	387149188	446804811
	A_V	GCC_GTC	88193823	88194284
46	D_N	AAT_GAT	387149188	446305320
47	I_T	ACA_ATA	387149188	446462960
	I_T	ACA_ATA	532358222	532479591
	I_T	ACA_ATA	161508266	161509351
48	E_Q	CAA_GAA	387149188	446764090
49	A_V	GCA_GTA	88193823	88194648
	A_V	GCA_GTA	387149188	445957208
	A_V	GCA_GTA	161508266	161509155
50	H_Y	CAT_TAT	387149188	446198905
	H_Y	CAT_TAT	537441500	537466083

Table 4d: List of positions (corresponding to Table 3a, continued)

No.	best_pheno	best_pheno_class	num_amino-glycoside	num_fluoro-quinolone	num_lactam	num_lincosamide	num_macrolide	num_tetra-cycline
1	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
2	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
3	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
4	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
5	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
6	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
7	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
8	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
9	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0

10	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
11	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
12	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
13	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
14	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
15	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
16	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
17	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
18	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
19	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
20	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
21	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
22	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
23	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
24	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
25	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
26	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
27	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
28	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
29	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
30	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
31	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
32	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
33	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
34	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
35	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
36	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
37	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
38	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
39	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
40	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
41	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0

42	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
43	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
44	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
45	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
46	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
47	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
48	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
49	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
50	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0

Table 4e: List of positions (corresponding to Table 3b)

No.	position	reference genome	genome name	sign_phenos	sign_phenos_class
1	1958403 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
2	1641150 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
3	978538 F	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
4	705667 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide

				G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
5	1434811 R	NC_017340.1	04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
6	953696 R	NC_022222.1	6850	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
7	1010027 R	NC_010079.1	USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2101899 R	NC_021670.1	Bmb9393	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1972149 R	NC_017340.1	04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
8	208285 R	NC_017340.1	04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	161011 R	NC_007795.1	NCTC_8325	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
9	2179136 R	NC_017351.1	11819_97	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2149064 R	NC_017340.1	04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2107689 R	NC_018608.1	08BA02176	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
10	2358535 F	NC_017340.1	04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
11	2023012 R	NC_017340.1	04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; G; Tobramycin			aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide

12	2777211 F	NC_007795.1			Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	ne; lactam; lincosamide; macrolide
	2779170 F	NC_017340.1		NCTC_8325	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
13	1801995 R	NC_018608.1		08BA02176	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1790672 R	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
14	1754561 F	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1691742 F	NC_007795.1		NCTC_8325	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
15	1362060 F	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
16	1242653 R	NC_022222.1		6850	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1294527 R	NC_010079.1		USA300_TCH1516	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1299554 R	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
17	1252703 R	NC_021670.1		Bmb9393	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1520285 F	NC_017351.1		11819_97	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1523326 F	NC_017340.1		04_02981	Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide

18	1619285 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
19	1661238 R 1875550 F 2006001 F 1959494 F	NC_010079.1 NC_022222.1 NC_010079.1 NC_017340.1	USA300_TCH1516 6850 USA300_TCH1516 04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
20	976788 F 878040 F	NC_017340.1 NC_007795.1	04_02981 NCTC_8325	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
21	2590222 R 2637689 R	NC_017340.1 NC_010079.1	04_02981 USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
22	210528 R 267448 R 269814 R	NC_022222.1 NC_017340.1 NC_010079.1	6850 04_02981 USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
23	1814108 R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
24	170059 F 142263 F	NC_002953.3 NC_017337.1	MSSA476 ED133	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin;	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide

					Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	mid; macrolide
25	534953 F	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
26	543821 F	NC_010079.1	USA300_TCH1516		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	517571 F	NC_017340.1	04_02981			
	554542 F	NC_018608.1	08BA02176			
27	531649 R	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	531398 R	NC_017351.1	11819_97			
28	1050123 R	NC_007795.1	NCTC_8325		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1147277 R	NC_017340.1	04_02981			
29	1881161 R	NC_010079.1	USA300_TCH1516		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1871101 R	NC_021059.1	M1			
	1759861 R	NC_022222.1	6850			
	1855493 R	NC_018608.1	08BA02176			
	1858794 R	NC_021554.1	CC45			
	1964828 R	NC_021670.1	Bmb9393			
30	2268723 F	NC_010079.1	USA300_TCH1516		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2221448 F	NC_017340.1	04_02981			

					G; Tobramycin	
31	920768 F	NC_021059.1	M1	08BA02176 04_02981 NCTC_8325	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	956878 F	NC_018608.1				
	956978 F	NC_017340.1				
	858255 F	NC_007795.1				
32	2005634 F	NC_016912.1	VC40	6850 USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	2039052 F	NC_022222.1	6850			
	2187801 F	NC_010079.1				
33	429303 F	NC_017340.1	04_02981		Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	350202 F	NC_022222.1	6850		Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
34	402479 F	NC_017340.1	04_02981		Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	352104 F	NC_007795.1	NCTC_8325		Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
35	158073 F	NC_022226.1	CN1	6850 USA300_TCH1516 04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	193628 F	NC_021059.1	M1			
	138357 F	NC_022222.1	6850			
	196480 F	NC_010079.1				
	189192 F	NC_017340.1				
36	1121847 R	NC_017340.1	04_02981		Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	1024692 R	NC_007795.1	NCTC_8325		Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	2719339 R	NC_010079.1	USA300_TCH1516		Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide

	2668764	R	NC_017340.1	04_02981	Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	fluoroquinolone;lactam; lincosamide;macrolide
38	1388095	R	NC_022226.1	CN1	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
39	1415365	R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	1428821	R	NC_018608.1	08BA02176		
	1318646	R	NC_007795.1	NCTC_8325		
	1412563	R	NC_010079.1	USA300_TCH1516		
	1381147	R	NC_021059.1	M1		
40	1678734	R	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	1720315	R	NC_010079.1	USA300_TCH1516		
41	1928346	F	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
42	1376396	R	NC_010079.1	USA300_TCH1516	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	1323236	R	NC_022222.1	6850		
	1315892	R	NC_022226.1	CN1		
	1379143	R	NC_017340.1	04_02981		
	1399306	F	NC_021670.1	Bmb9393		
43	1124668	F	NC_017340.1	04_02981	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone;lactam; lincosamide;macrolide
	1121585	F	NC_010079.1	USA300_TCH1516		

					Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	ne; lactam; lincosamide; macrolide
44	559072 R	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	504007 R	NC_007795.1	NCTC_8325		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
45	1675156 R	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
46	1187805 F 1078815 F	NC_017340.1 NC_022113.1	04_02981 55_2053		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	1182930 F	NC_010079.1	USA300_TCH1516		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
47	1356138 F	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
48	854815 R 953539 R	NC_007795.1 NC_017340.1	NCTC_8325 04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	948900 R	NC_010079.1	USA300_TCH1516		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
49	2459738 F	NC_017340.1	04_02981		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide
	2364478 F	NC_022222.1	6850		Ampicillin/Sulbactam; Cefepime; Cefotaxim; Cefuroxim; Ciprofloxacin; Clindamycin; Erythromycin; Imipenem; Levofloxacin; Moxifloxacin; Oxacillin; Penicillin G; Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide; macrolide

50	1812380 R 1775835 R 1714993 R	NC_010079.1 NC_017340.1 NC_007795.1	USA300_TCH1516 04_02981 NCTC_8325	Ampicillin/Sulbactam;Cefepime;Cefotaxim; Cefuroxim;Ciprofloxacin;Clindamycin; Erythromycin;Imipenem;Levofloxacin; Moxifloxacin;Oxacillin;Penicillin G;Tobramycin	aminoglycoside; fluoroquinolone; lactam; lincosamide;macrolide
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Table 4f: List of positions (corresponding to Table 3b, continued)

No.	best_pv	GenomeName	fasta_header	SNPPositioninGenome	gene
1	6,0168E-193	04_02981 USA300_TCH1516	gi 387149188 ref NC_017340.1 gi 161508266 ref NC_010079.1	1958403 R 2004910 R	
2	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	1641150 R	
3	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	978538 F	
4	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	705667 R	
5	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	1434811 R	
6	1,4167E-189	6850 USA300_TCH1516	gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1	953696 R 1010027 R	rluA1
7	1,4167E-189	Bmb9393 04_02981	gi 521210823 ref NC_021670.1 gi 387149188 ref NC_017340.1	2101899 R 1972149 R	
8	1,4167E-189	04_02981 NCTC_8325	gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	208285 R 161011 R	argC argC
9	1,4167E-189	11819_97 04_02981	gi 385780298 ref NC_017351.1 gi 387149188 ref NC_017340.1	2179136 R 2149064 R	
10	1,4167E-189	08BA02176 04_02981	gi 404477334 ref NC_018608.1 gi 387149188 ref NC_017340.1	2107689 R 2358535 F	
11	1,4167E-189	04_02981	gi 387149188 ref NC_017340.1	2023012 R	
12	1,4167E-189	NCTC_8325 04_02981	gi 88193823 ref NC_007795.1 gi 387149188 ref NC_017340.1	2777211 F 2779170 F	
13	1,4167E-189	08BA02176	gi 404477334 ref NC_018608.1	1801995 R	

14	3,0731E-189	04_02981	gi 387149188 ref NC_017340.1	1790672 R	
		04_02981	gi 387149188 ref NC_017340.1	1754561 F	
		NCTC_8325	gi 88193823 ref NC_007795.1	1691742 F	
15	3,1179E-189	04_02981	gi 387149188 ref NC_017340.1	1362060 F	
		6850	gi 537441500 ref NC_022222.1	1242653 R	ribC
16	3,7988E-189	USA300_TCH1516	gi 161508266 ref NC_010079.1	1294527 R	
		04_02981	gi 387149188 ref NC_017340.1	1299554 R	
17	3,9683E-189	Bmb9393	gi 521210823 ref NC_021670.1	1252703 R	
		11819_97	gi 385780298 ref NC_017351.1	1520285 F	
		04_02981	gi 387149188 ref NC_017340.1	1523326 F	
18	3,9683E-189	04_02981	gi 387149188 ref NC_017340.1	1619285 R	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1661238 R	
19	3,9683E-189	6850	gi 537441500 ref NC_022222.1	1875550 F	bcp
		USA300_TCH1516	gi 161508266 ref NC_010079.1	2006001 F	
		04_02981	gi 387149188 ref NC_017340.1	1959494 F	
20	5,4236E-189	04_02981	gi 387149188 ref NC_017340.1	976788 F	
		NCTC_8325	gi 88193823 ref NC_007795.1	878040 F	
21	7,5452E-189	04_02981	gi 387149188 ref NC_017340.1	2590222 R	gntP
		USA300_TCH1516	gi 161508266 ref NC_010079.1	2637689 R	
22	9,5271E-189	6850	gi 537441500 ref NC_022222.1	210528 R	
		04_02981	gi 387149188 ref NC_017340.1	267448 R	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	269814 R	
23	1,1093E-188	04_02981	gi 387149188 ref NC_017340.1	1814108 R	
24	3,3279E-188	MSSA476	gi 49484912 ref NC_002953.3	170059 F	
		ED133	gi 384546269 ref NC_017337.1	142263 F	
25	3,3279E-188	04_02981	gi 387149188 ref NC_017340.1	534953 F	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	543821 F	
26	3,5518E-188	04_02981	gi 387149188 ref NC_017340.1	517571 F	
		08BA02176	gi 404477334 ref NC_018608.1	554542 F	
27	1,1492E-187	04_02981	gi 387149188 ref NC_017340.1	531649 R	

28	1,1509E-187	11819_97 NCTC_8325 04_02981	gi 385780298 ref NC_017351.1 gi 88193823 ref NC_007795.1 gi 387149188 ref NC_017340.1	531398 R 1050123 R 1147277 R	
29	9,0648E-187	USA300_TCH1516 M1 6850 08BA02176 CC45 Bmb9393	gi 161508266 ref NC_010079.1 gi 479328021 ref NC_021059.1 gi 537441500 ref NC_022222.1 gi 404477334 ref NC_018608.1 gi 514064966 ref NC_021554.1 gi 521210823 ref NC_021670.1	1881161 R 1871101 R 1759861 R 1855493 R 1858794 R 1964828 R	fmtB1
30	1,5807E-186	USA300_TCH1516 04_02981	gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	2268723 F 2221448 F	
31	3,6257E-174	M1 08BA02176 04_02981 NCTC_8325	gi 479328021 ref NC_021059.1 gi 404477334 ref NC_018608.1 gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	920768 F 956878 F 956978 F 858255 F	rocD rocD rocD rocD
32	3,9753E-174	VC40 6850 USA300_TCH1516	gi 379013365 ref NC_016912.1 gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1	2005634 F 2039052 F 2187801 F	rsbU
33	7,1007E-174	04_02981	gi 387149188 ref NC_017340.1	429303 F	
34	1,8906E-173	6850 04_02981 NCTC_8325	gi 537441500 ref NC_022222.1 gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	350202 F 402479 F 352104 F	
35	3,0713E-172	CN1 M1 6850 USA300_TCH1516 04_02981	gi 537459744 ref NC_022226.1 gi 479328021 ref NC_021059.1 gi 537441500 ref NC_022222.1 gi 161508266 ref NC_010079.1 gi 387149188 ref NC_017340.1	158073 F 193628 F 138357 F 196480 F 189192 F	
36	4,4332E-172	04_02981 NCTC_8325	gi 387149188 ref NC_017340.1 gi 88193823 ref NC_007795.1	1121847 R 1024692 R	
37	4,4332E-172	USA300_TCH1516	gi 161508266 ref NC_010079.1	2719339 R	

38	5,5156E-172	04_02981	gi 387149188 ref NC_017340.1	2668764 R	
39	1,2003E-171	CN1	gi 537459744 ref NC_022226.1	1388095 R	
		04_02981	gi 387149188 ref NC_017340.1	1415365 R	femB
		08BA02176	gi 404477334 ref NC_018608.1	1428821 R	
		NCTC_8325	gi 88193823 ref NC_007795.1	1318646 R	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1412563 R	
		M1	gi 479328021 ref NC_021059.1	1381147 R	
40	1,2003E-171	04_02981	gi 387149188 ref NC_017340.1	1678734 R	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1720315 R	
41	1,2491E-171	04_02981	gi 387149188 ref NC_017340.1	1928346 F	
42	2,3661E-171	USA300_TCH1516	gi 161508266 ref NC_010079.1	1376396 R	sbcC
		6850	gi 537441500 ref NC_022222.1	1323236 R	
		CN1	gi 537459744 ref NC_022226.1	1315892 R	
		04_02981	gi 387149188 ref NC_017340.1	1379143 R	
		Bmb9393	gi 521210823 ref NC_021670.1	1399306 F	
43	5,3408E-171	04_02981	gi 387149188 ref NC_017340.1	1124668 F	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1121585 F	
44	1,0222E-170	04_02981	gi 387149188 ref NC_017340.1	559072 R	
		NCTC_8325	gi 88193823 ref NC_007795.1	504007 R	
45	1,0498E-170	04_02981	gi 387149188 ref NC_017340.1	1675156 R	
46	2,283E-170	04_02981	gi 387149188 ref NC_017340.1	1187805 F	
		55_2053	gi 532358222 ref NC_022113.1	1078815 F	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	1182930 F	
47	2,3666E-170	04_02981	gi 387149188 ref NC_017340.1	1356138 F	
48	4,1086E-170	NCTC_8325	gi 88193823 ref NC_007795.1	854815 R	prsA1
		04_02981	gi 387149188 ref NC_017340.1	953539 R	
		USA300_TCH1516	gi 161508266 ref NC_010079.1	948900 R	
49	4,2262E-169	04_02981	gi 387149188 ref NC_017340.1	2459738 F	
		6850	gi 537441500 ref NC_022222.1	2364478 F	
50	1,3444E-168	USA300_TCH1516	gi 161508266 ref NC_010079.1	1812380 R	dnaE2

	04_02981	gi 387149188 ref NC_017340.1	1775835 R
	NCTC_8325	gi 88193823 ref NC_007795.1	1714993 R

Table 4g: List of positions (corresponding to Table 3b, continued)

No.	AminoAcids	Codons	GenomeGI	Protein_GI
1	A_T	ACA_GCA	387149188	446792191
	A_T	ACA_GCA	161508266	161510080
2	E_K	AAA_GAA	387149188	446032753
3	I_M	ATC_ATG	387149188	446312722
4	E_K	AAG_GAG	387149188	446725640
5	A_S	GCA_TCA	387149188	446180863
6	L_P	CCT_CTT	537441500	537465549
	L_P	CCT_CTT	161508266	161509205
7	M_V	ATG_GTG	521210823	752533903
	M_V	ATG_GTG	387149188	446753128
8	K_T	AAA_ACA	387149188	446556386
	K_T	AAA_ACA	88193823	88193961
9	M_V	ATG_GTG	385780298	446324804
	M_V	ATG_GTG	387149188	446324797
	M_V	ATG_GTG	404477334	446324791
10	K_N	AAA_AAC	387149188	445930822
11	E_V	GAA_GTA	387149188	446943955
12	F_L	TTG_TTT	88193823	88196623
	F_L	TTG_TTT	387149188	446800117
13	Q_R	CAG_CGG	404477334	446795417
	Q_R	CAG_CGG	387149188	446795407
14	L_V	GTA_TTA	387149188	446028277
	L_V	GTA_TTA	88193823	88195494
15	I_N	AAT_ATT	387149188	447178207

16	I_T I_T I_T	ACC_ATC ACC_ATC ACC_ATC	537441500 161508266 387149188	537465687 161509438 446786934
17	L_S L_S L_S	TCA_TTA TCA_TTA TCA_TTA	521210823 385780298 387149188	521258120 446060496 446060495
18	N_S N_S	AAT_AGT AAT_AGT	387149188 161508266	446940596 161509778
19	D_Y D_Y D_Y	GAT_TAT GAT_TAT GAT_TAT	537441500 161508266 387149188	537465893 161510081 446862272
20	F_L F_L	TTA_TTT TTA_TTT	387149188 88193823	447047252 88194665
21	K_T K_T	AAA_ACA AAA_ACA	387149188 161508266	446403560 161510698
22	A_V A_V A_V	GCA_GTA GCA_GTA GCA_GTA	537441500 387149188 161508266	537465126 447077358 161508491
23	A_V	GCG_GTG	387149188	446784840
24	G_R G_R	AGA_GGA AGA_GGA	49484912 384546269	487756815 446093782
25	F_L F_L	TTA_TTT TTA_TTT	387149188 161508266	446874184 161508745
26	K_R K_R	AAA_AGA AAA_AGA	387149188 404477334	446973880 446973883
27	N_T N_T	AAT_ACT AAT_ACT	387149188 385780298	446076361 446076373
28	F_L F_L	TTA_TTT TTA_TTT	88193823 387149188	88194836 446593607
29	K_T	AAG_ACG	161508266	161509974

	K_T	AAG_ACG	479328021	505394769
	K_T	AAG_ACG	537441500	537465850
	K_T	AAG_ACG	404477334	446973259
	K_T	AAG_ACG	514064966	514074897
	K_T	AAG_ACG	521210823	521258173
30	L_S	TCA_TTA	161508266	161510359
	L_S	TCA_TTA	387149188	446293068
31	E_K	AAA_GAA	479328021	505394709
	E_K	AAA_GAA	404477334	446089469
	E_K	AAA_GAA	387149188	446089454
	E_K	AAA_GAA	88193823	88194651
32	I_V	ATA_GTA	379013365	487720346
	I_V	ATA_GTA	537441500	537465949
	I_V	ATA_GTA	161508266	161510279
33	G_V	GGA_GTA	387149188	446343556
34	K_T	AAA_ACA	537441500	537465192
	K_T	AAA_ACA	387149188	446129782
	K_T	AAA_ACA	88193823	88194138
35	I_V	ATT_GTT	537459744	537467717
	I_V	ATT_GTT	479328021	505394663
	I_V	ATT_GTT	537441500	537465062
	I_V	ATT_GTT	161508266	161508437
	I_V	ATT_GTT	387149188	446513509
36	I_T	ACA_ATA	387149188	446104798
	I_T	ACA_ATA	88193823	88194808
37	P_T	ACA_CCA	161508266	161510779
	P_T	ACA_CCA	387149188	446083969
38	C_Y	TAT_TGT	537459744	686312170
39	L_S	TCA_TTA	387149188	446595763
	L_S	TCA_TTA	404477334	446595752

	L_S	TCA_TTA	88193823	88195101
	L_S	TCA_TTA	161508266	161509542
	L_S	TCA_TTA	479328021	505394733
40	I_L	ATT_CTT	387149188	446059917
	I_L	ATT_CTT	161508266	161509840
41	A_G	GCA_GGA	387149188	446506832
42	I_T	ACT_ATT	161508266	161509514
	I_T	ACT_ATT	537441500	537465718
	I_T	ACT_ATT	537459744	537467986
	I_T	ACT_ATT	387149188	446725826
	I_T	ACT_ATT	521210823	521258127
43	I_L	ATA_TTA	387149188	446710589
	I_L	ATA_TTA	161508266	161509291
44	A_V	GCC_GTC	387149188	446804811
	A_V	GCC_GTC	88193823	88194284
45	D_N	AAT_GAT	387149188	446305320
46	I_T	ACA_ATA	387149188	446462960
	I_T	ACA_ATA	532358222	532479591
	I_T	ACA_ATA	161508266	161509351
47	E_Q	CAA_GAA	387149188	446764090
48	A_V	GCA_GTA	88193823	88194648
	A_V	GCA_GTA	387149188	445957208
	A_V	GCA_GTA	161508266	161509155
49	H_Y	CAT_TAT	387149188	446198905
	H_Y	CAT_TAT	537441500	537466083
50	D_E	GAA_GAT	161508266	161509916
	D_E	GAA_GAT	387149188	446149063
	D_E	GAA_GAT	88193823	88195511

Table 4h: List of positions (corresponding to Table 3b, continued)

No.	best_pheno	best_pheno_class	num_amino-glycoside	num_fluoro-quinolone	num_lactam	num_lincosamide	num_macrolide	num_tetra-cycline
1	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
2	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
3	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
4	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
5	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
6	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
7	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
8	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
9	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
10	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
11	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
12	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
13	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
14	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
15	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
16	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
17	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
18	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
19	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
20	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
21	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
22	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
23	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
24	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
25	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
26	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
27	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
28	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0

29	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
30	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
31	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
32	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
33	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
34	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
35	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
36	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
37	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
38	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
39	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
40	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
41	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
42	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
43	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
44	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
45	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
46	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
47	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
48	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
49	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0
50	Moxifloxacin	fluoroquinolone	1	3	7	1	1	0

In Tables 4a-h, the annotations are as above in Example 1 for Tables 2a and 2b, with the following extra annotations in the columns:

best_pheno: Phenotype (drug) with smallest adjusted p-value

5 best_pheno_class: drug class of best drug (if phenotypes are drugs)

best_pv: adj. p-value of best phenotype calculated using Fishers exact test and adjusted by FDR (Benjamini Hochberg method (Benjamini Hochberg, 1995))

10 sign_phenos: names of all phenotypes with significant adj. p-value separated by ";"

sign_phenos_class: drug classes of all significant drugs (if phenotypes are drugs)

15 Further, in Tables 4a-h, all SNP are again non-synonymous (1=yes, 0=no), and the SNPs lie within a coding region (are "OnProtein")

20 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
25 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
30 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.

5

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.

10

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.

15

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.

20

The current approach enables

25

- a. Identification and validation of markers for genetic identification and susceptibility/resistance testing within one diagnostic test

30

- 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

5

Claims

1. A method of determining an antimicrobial drug, e.g. anti-
biotic, resistance profile for a microorganism, particu-
5 larly a bacterial microorganism, comprising:
obtaining or providing a first data set of gene sequences
of a plurality of clinical isolates of the microorganism;
wherein at least a part of the gene sequences of the
first data set are assembled;
10 analyzing the gene sequences of the first data set for
genetic variants to obtain a third data set of genetic
variants;
providing a second data set of antimicrobial drug, e.g.
antibiotic, resistance and/or susceptibility of the plu-
15 rality of clinical isolates of the microorganism;
correlating the third data set with the second data set
and statistically analyzing the correlation; and
determining the genetic sites in the genome of the micro-
organism with antimicrobial drug, e.g. antibiotic, re-
20 sistance.
2. The method of claim 1, wherein the genetic variants in
the gene sequences of the first data set are single nu-
cleotide polymorphisms (SNPs).
- 25 3. The method of claim 2, wherein the SNPs are detected
alignment-free.
4. The method of claim 2 or 3, wherein the SNPs are annotat-
30 ed to a pan-genome of the microorganism and/or annotated
to one or more reference genomes.

5. The method of any one of the preceding claims, wherein the microorganism is a Staphylococcus species, particularly Staphylococcus aureus, optionally wherein the antimicrobial drug is methicillin.

5

6. A diagnostic method of determining an infection of a patient with a microorganism, particularly a bacterial microorganism potentially resistant to antimicrobial drug treatment, comprising the steps of:

10

a) obtaining or providing a sample containing or suspected of containing a microorganism, particularly a bacterial microorganism, from the patient;

15

b) determining the presence of at least one genetic variant in at least one position of at least one genetic sequence of the microorganism, particularly bacterial microorganism, as determined by the method of any one of claims 1 to 5, wherein the presence of said at least one genetic variant is indicative of an infection with an antimicrobial drug resistant microorganism in said patient.

20

7. A method of selecting a treatment of a patient suffering from an infection with a potentially resistant microorganism, particularly bacterial microorganism, comprising the steps of:

25

a) obtaining or providing a sample containing or suspected of containing a microorganism, particularly a bacterial microorganism, from the patient;

30

b) determining the presence of at least one genetic variant in at least one position of at least one genetic sequence of the microorganism, particularly bacterial microorganism, as determined by the method of any one of claims 1 to 5, wherein the presence of said at least one

genetic variant 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 the infection with the microorganism, particularly the bacterial microorganism.

8. A method of acquiring, respectively determining, an antimicrobial drug, e.g. antibiotic, resistance profile for a clinical isolate of a microorganism, particularly a bacterial microorganism, comprising:

obtaining or providing at least one gene sequence of the clinical isolate of the microorganism, particularly the bacterial microorganism; and

determining the presence of genetic variants in the at least one gene sequence of the clinical isolate of the microorganism, particularly bacterial microorganism, as determined by the method of any one of claims 1 to 5.

9. Computer program product comprising computer executable instructions which, when executed, perform a method according to any one of claims 1 to 8.

10. A diagnostic method of determining an infection of a patient with a Staphylococcus species, particularly Staphylococcus aureus, 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 Staphylococcus species, particularly Staphylococcus aureus, from the patient;

- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1,
5 wherein the presence of said at least two genetic variations is indicative of an infection with an antimicrobial drug, e.g. antibiotic, resistant *Staphylococcus*, particularly *Staphylococcus aureus*, strain in said patient, wherein for some positions more than one position in
10 different reference genomes is annotated.
11. A method of selecting a treatment of a patient suffering from an infection with a potentially resistant *Staphylococcus* species, particularly *Staphylococcus aureus*, comprising the steps of:
- 15 a) obtaining or providing a sample containing or suspected of containing at least one *Staphylococcus*, particularly *Staphylococcus aureus*, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Table 1,
20 wherein the presence of said at least two genetic variations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for some positions more than one position in different reference genomes is annotated;
- 25 c) identifying said at least one or more antimicrobial, e.g. antibiotic, drugs; and
- 30 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 *Staphylococcus*, particularly *Staphylococcus aureus*, infection.

12. The method of claim 10 or 11, wherein the method involves determining the resistance of the *Staphylococcus* species, particularly *Staphylococcus aureus*, to one or more antimicrobial, e.g. antibiotic, drugs.
13. The method of one or more of claims 10 to 12, wherein the antimicrobial drug, e.g. antibiotic drug, is selected from the group consisting of β -lactams, β -lactam inhibitors, quinolones and derivatives thereof, e.g. fluoroquinolones, aminoglycosides, glycopeptides, lincosamides, macrolides, nitrofuranes, oxazolidinones polyketides, respectively tetracyclines, and folate synthesis inhibitors, e.g. benzene derived/sulfonamide antibiotics, particularly from the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Methicillin, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin, particularly wherein the antimicrobial drug is Methicillin.
14. The method of one or more of claims 10 to 13, wherein the resistance of *Staphylococcus aureus* against 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 17, 18, 19, 20 or more antibiotic drugs is determined.

15. The method of one or more of claims 10 to 14, wherein determining the nucleic acid sequence information or the presence of a genetic variation comprises determining a partial or entire sequence of the genome of the Staphylococcus species, particularly Staphylococcus aureus, wherein said partial or entire sequence of the genome comprises at least one of the positions with the genetic variation.

16. The method of one or more of the claims 10 to 15, wherein determining the nucleic acid sequence information or the presence of a genetic variation comprises using a next generation sequencing or high throughput sequencing method, preferably wherein a partial or entire genome sequence of the Staphylococcus, particularly Staphylococcus aureus, strain is determined by using a next generation sequencing or high throughput sequencing method.

17. A diagnostic method of determining an infection of a patient with a Staphylococcus species, particularly Staphylococcus aureus, 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 Staphylococcus, particularly Staphylococcus aureus, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b, wherein the presence of said at least two genetic variations is indicative of an infection with an

antimicrobial drug, e.g. antibiotic, resistant Staphylococcus, particularly Staphylococcus aureus, strain in said patient, wherein for some positions more than one position in different reference genomes is annotated.

5

18. A method of selecting a treatment of a patient suffering from an infection with a potentially resistant Staphylococcus species, particularly Staphylococcus aureus, comprising the steps of:

- 10 a) obtaining or providing a sample containing or suspected of containing at least one Staphylococcus, particularly Staphylococcus aureus, strain from the patient;
- b) determining the presence of at least one genetic variation in at least two positions from the group of positions annotated with Nos. 1 - 50 with regard to the reference genomes with the genome names given in Tables 3a and/or 3b, wherein the presence of said at least two genetic variations is indicative of a resistance to one or more antimicrobial, e.g. antibiotic, drugs, wherein for
15 some positions more than one position in different reference genomes is annotated;
- 20 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
25 being suitable for the treatment of a Staphylococcus, particularly Staphylococcus aureus, infection.

19. The method of claim 17 or 18, wherein the method involves
30 determining the resistance of a Staphylococcus species, particularly Staphylococcus aureus, to one or more antimicrobial, e.g. antibiotic, drugs.

20. The method of one or more of claims 17 to 19, wherein the antimicrobial drug, e.g. antibiotic drug, is selected from the group consisting of β -lactams, β -lactam inhibitors, quinolones and derivatives thereof, e.g.

5 fluoroquinolones, aminoglycosides, glycopeptides, lincosamides, macrolides, nitrofuranes, oxazolidinones polyketides, respectively tetracyclines, and folate synthesis inhibitors, e.g. benzene derived/sulfonamide antibiotics, particularly from the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam,

10 Azithromycin, Cefalothin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Methicillin, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G,

15 Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin, particularly from the group consisting of Amoxicillin/Clavulanate, Ampicillin, Ampicillin/Sulbactam, Azithromycin, Cefalothin, Cefazolin, Cefepime,

20 Cefotaxime, Cefoxitin, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Ertapenem, Erythromycin, Fosfomycin, Fusidic acid, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Penicillin G,

25 Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, and Vancomycin.

30

21. The method of one or more of claims 17 to 20, wherein the resistance of *Staphylococcus aureus* against 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 17, 18, 19, 20 or more antibiotic drugs is determined.

5

22. The method of one or more of claims 17 to 21, wherein determining the nucleic acid sequence information or the presence of a genetic variation comprises determining a partial or entire sequence of the genome of the *Staphylococcus*, particularly *Staphylococcus aureus*, wherein said partial or entire sequence of the genome comprises at least one of the positions with the genetic variation.

10

23. The method of one or more of the claims 17 to 22, wherein determining the nucleic acid sequence information or the presence of a genetic variation comprises using a next generation sequencing or high throughput sequencing method, preferably wherein a partial or entire genome sequence of the *Staphylococcus*, particularly *Staphylococcus aureus* strain is determined by using a next generation sequencing or high throughput sequencing method.

15

20

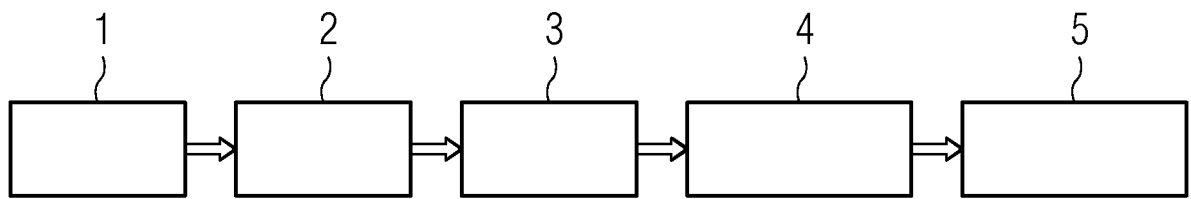


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2015/068188

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/068188

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/068188A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BENJAMIN P. HOWDEN ET AL: "Evolution of Multidrug Resistance during Staphylococcus aureus Infection Involves Mutation of the Essential Two Component Regulator WalKR", PLOS PATHOGENS, vol. 7, no. 11, 10 November 2011 (2011-11-10), pages 1-15, XP055169475, DOI: 10.1371/journal.ppat.1002359 summary p. e10022360 col. 1, p. e10022360 col. 2 "Results" tab. 1 and 2, and p. e10022372 " material and methods" ----- -/--	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 November 2015

Date of mailing of the international search report

19/04/2016

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Lapopin, Laurence

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/068188

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YU GONGXIN: "GenHtr: a tool for comparative assessment of genetic heterogeneity in microbial genomes generated by massive short-read sequencing", BMC BIOINFORMATICS, BIOMED CENTRAL, LONDON, GB, vol. 11, no. 1, 12 October 2010 (2010-10-12), page 508, XP021071835, ISSN: 1471-2105, DOI: 10.1186/1471-2105-11-508 abstract, p. 3 col. 1 last para., p. 3 col. 1 to p. 5 col. " Methods" -----	1-9
X	B. P. HOWDEN ET AL: "Genomic Analysis Reveals a Point Mutation in the Two-Component Sensor Gene graS That Leads to Intermediate Vancomycin Resistance in Clinical Staphylococcus aureus", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 52, no. 10, 21 July 2008 (2008-07-21), pages 3755-3762, XP055228904, US ISSN: 0066-4804, DOI: 10.1128/AAC.01613-07 abstract, tab. 1 and tab. 3 -----	1-9
X	A. D. KENNEDY ET AL: "Epidemic community-associated methicillin-resistant Staphylococcus aureus: Recent clonal expansion and diversification", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 105, no. 4, 29 January 2008 (2008-01-29), pages 1327-1332, XP055228900, US ISSN: 0027-8424, DOI: 10.1073/pnas.0710217105 abstract, tab. 1 -----	1-9
X	MICHAL WOZNIAK ET AL: "An approach to identifying drug resistance associated mutations in bacterial strains", BMC GENOMICS, BIOMED CENTRAL LTD, LONDON, UK, vol. 13, no. Suppl 7, 7 December 2012 (2012-12-07), page S23, XP021127984, ISSN: 1471-2164, DOI: 10.1186/1471-2164-13-S7-S23 abstract, p. s24 col. 1 and p. s24 col. 2 "Material and methods" to p. s24 col. 2. ----- -/--	1-9

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/068188

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012/027302 A2 (UNIV CALIFORNIA [US]; TESSARAE INC [US]; LYNCH SUSAN V [US]; BRODIE EO) 1 March 2012 (2012-03-01) abstract and claims 6 and 13 -----	1-9
A	US 7 135 283 B1 (NERENBERG MICHAEL [US] ET AL) 14 November 2006 (2006-11-14) col. 4, l. 31-38, col. 5 l. 22-64 and claims 1-3 -----	1-9
A	M.W. SYRMIS ET AL: "Comparison of a multiplexed MassARRAY system with real-time allele-specific PCR technology for genotyping of methicillin-resistant Staphylococcus aureus", CLINICAL MICROBIOLOGY AND INFECTION., vol. 17, no. 12, 1 December 2011 (2011-12-01), pages 1804-1810, XP055229274, United Kingdom, Switzerland ISSN: 1198-743X, DOI: 10.1111/j.1469-0691.2011.03521.x abstract -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2015/068188

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012027302 A2	01-03-2012	US 2013157876 A1 WO 2012027302 A2	20-06-2013 01-03-2012
US 7135283 B1	14-11-2006	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-9

concerns a method for determining genetic sites (resistance profile) associated with antimicrobial resistance in a microorganism by whole genome sequencing and comparative analysis of the sequences. The invention concerns as well method for diagnosing, selecting a treatment, and a method detecting an infection by a anti-microbial resistant microorganism by detecting the presence of at least one variation identified by comparative whole genome sequence analysis, and concerns as well a computer for executing said method.

2. claims: 10-16(partially)

concerns a method for diagnosing an antimicrobial resistant *Staphylococcus aureus* and a method for selecting a treatment by determining the presence of at least one mutation in at least two positions listed in tab. 1, wherein the first position is 534953F in combination with at least one other positions listed in tab. 1.

3-52. claims: 10-16(partially)

concerns a method for diagnosing an antimicrobial resistant *Staphylococcus aureus* and a method for selecting a treatment by determining the presence of at least one mutation in at least two positions listed in tab. 1, wherein the first position 543821F to 1675156R in combination with at least one other positions listed in tab. 1.

53. claims: 17-23(partially)

concern a method for diagnosing an antimicrobial resistant *Staphylococcus aureus* and a method for selecting a treatment by determining the presence of at least one mutation in at least two gene positions as listed in tab. 3a and/or 3b, wherein the first position is 1958403R, respectively in combination with at least one other of the positions listed in tab. 3a and/or 3b.

54-103. claims: 17-23(partially)

concern a method for diagnosing an antimicrobial resistant *Staphylococcus aureus* and a method for selecting a treatment by determining the presence of at least one mutation in at least two gene positions as listed in tab. 3a and/or 3b, wherein the first position is 20049910R to 2364478F and/or

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1714993R, respectively in combination with at least one
other of the positions listed in tab. 3a and/or 3b.
