

Supplementary Material

Culture-independent Genotyping, Virulence and Antimicrobial Resistance Gene Identification of *Staphylococcus aureus* from Orthopaedic Implant-associated Infections

J. Christopher Noone^{1,2,*}, Fabienne Antunes Ferreira^{1,3} and Hege Vangstein Aamot^{1,4}

¹Department of Microbiology and Infection Control, Akershus University Hospital, 1478 Lørenskog, Norway;

²Faculty of Medicine, University of Oslo, 0316 Oslo, Norway

³Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brazil. Postcode 88040-900

⁴Department of Clinical Molecular Biology (EpiGen), Akershus University Hospital and University of Oslo, 1478 Lørenskog, Norway

*Correspondence: cnoo@ahus.no

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Table S1. Nanopore sequencing QC metrics, totals for each patient: meta* (shotgun metagenomic sequencing data), iso* (isolate WGS data).

Patient ID	Total reads	Total bases	Mean quality score	Mean read length (bp)
iso111	293910	1.9 Gb	13.55	6619
meta111	394935	1.1 Gb	12.46	2779
iso114	76076	186.8 Mb	12.21	2456
meta114	1740658	124.6 Mb	12.54	2811
iso128	137345	405.6 Mb	12.06	2963
meta128	37382	111.4 Mb	12.38	2980
iso139	205789	651.7 Mb	12.45	3166
meta139	41736	139.9 Mb	12.54	3351
iso140	53764	70.3 Mb	11.25	1307
meta140	2273296	9.8 Gb	12.87	4324
iso141	275640	1.2 Gb	12.58	4272
meta141	2744512	11.9 Gb	12.89	4335

Table S2. metaflye assembly metrics: meta* (shotgun metagenomic sequencing data), iso* (isolate WGS data).

Patient ID	Contig	Length	Depth of coverage
meta111			
	8	953233	272
	11	895294	225
	6	436786	232
	5	122429	233
	3	120684	247
	10	111219	238
	9	77748	260
	7	55734	155
	12	27619	48
	13	20696	1001
	2	742	10
meta114			
	2	1030116	995
	1	840535	945
	3	572122	961
	6	202434	912

5	112560	884
7	748	989

meta128

19	468070	38
18	341601	30
34	186963	30
30	176411	29
33	174906	35
3	130882	37
25	120045	36
1	111175	35
28	83346	28
32	79091	28
9	74636	31
17	68801	29
21	68765	30
29	61305	29
6	56539	31
20	52617	31
22	52172	42
31	50487	32

10	50329	32
16	48335	32
26	46236	39
27	43935	37
24	43016	32
35	38494	23
12	37503	30
11	33773	30
8	23277	25
5	21162	28
13	19307	24
7	17855	28
14	15465	21
15	13732	25
2	8036	25

meta139

12	1152988	48
9	976982	34
11	266425	35
8	193349	40
10	177700	39

14	43902	41
3	5297	40
6	2903	45
4	2722	66
7	1837	44
1	1259	23
5	870	204
2	837	16

meta140

4	1293595	969
3	824156	975
5	618099	966

meta141

12	692856	408
11	435165	271
3	254053	287
13	236318	345
16	219621	281
17	198927	310
18	195804	291
6	191249	272

15	124339	302
2	93434	327
7	64203	283
5	41675	360
9	36400	302
1	32786	365
8	25817	356
19	7124	168
4	7082	254
21	4017	293
20	2720	236
10	2393	367

iso111

1	2803888	995
2	41435	646

iso114

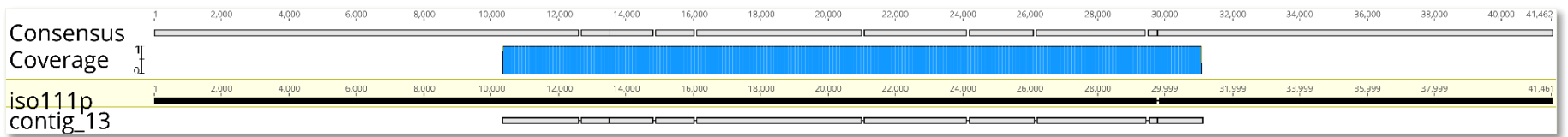
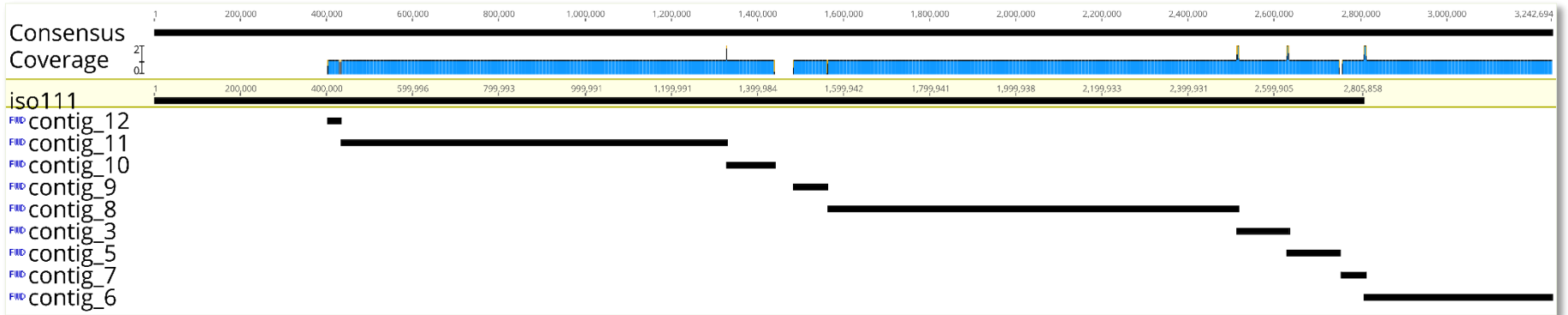
1	2770789	64
2	3117	757

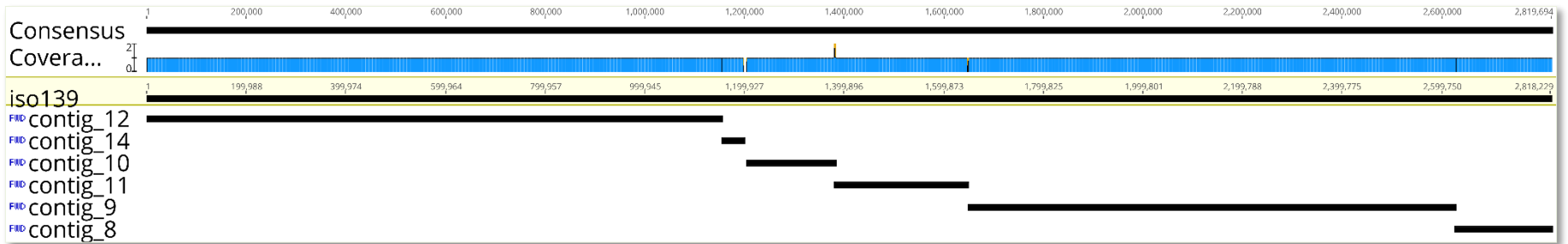
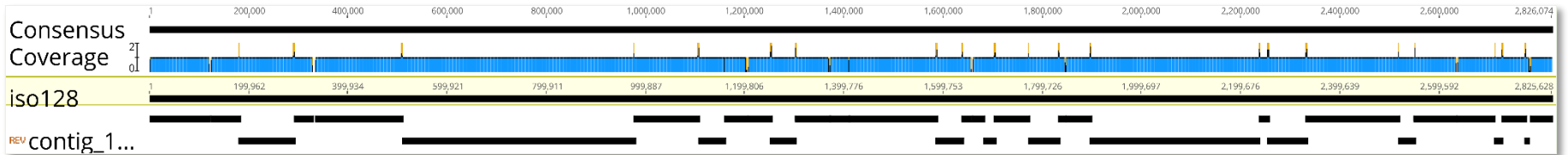
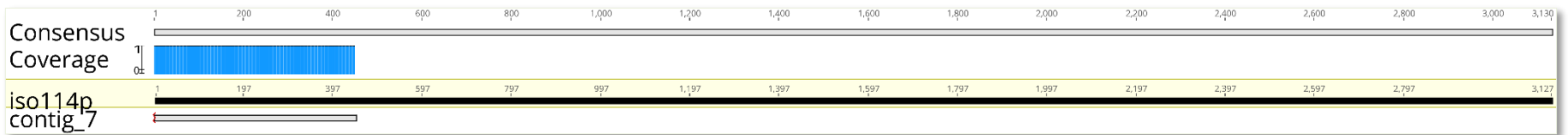
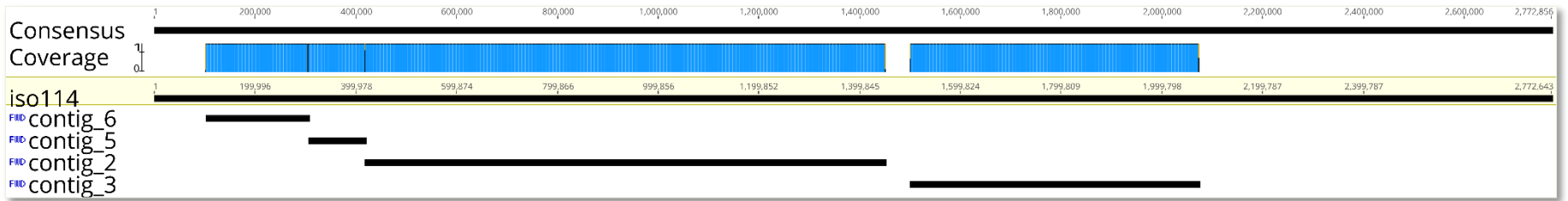
iso128

1	2823596	142
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iso139

	1	2816259	231
<hr/>			
iso140			
	1	2736340	22
<hr/>			
iso141			
	1	2883453	402
<hr/>			





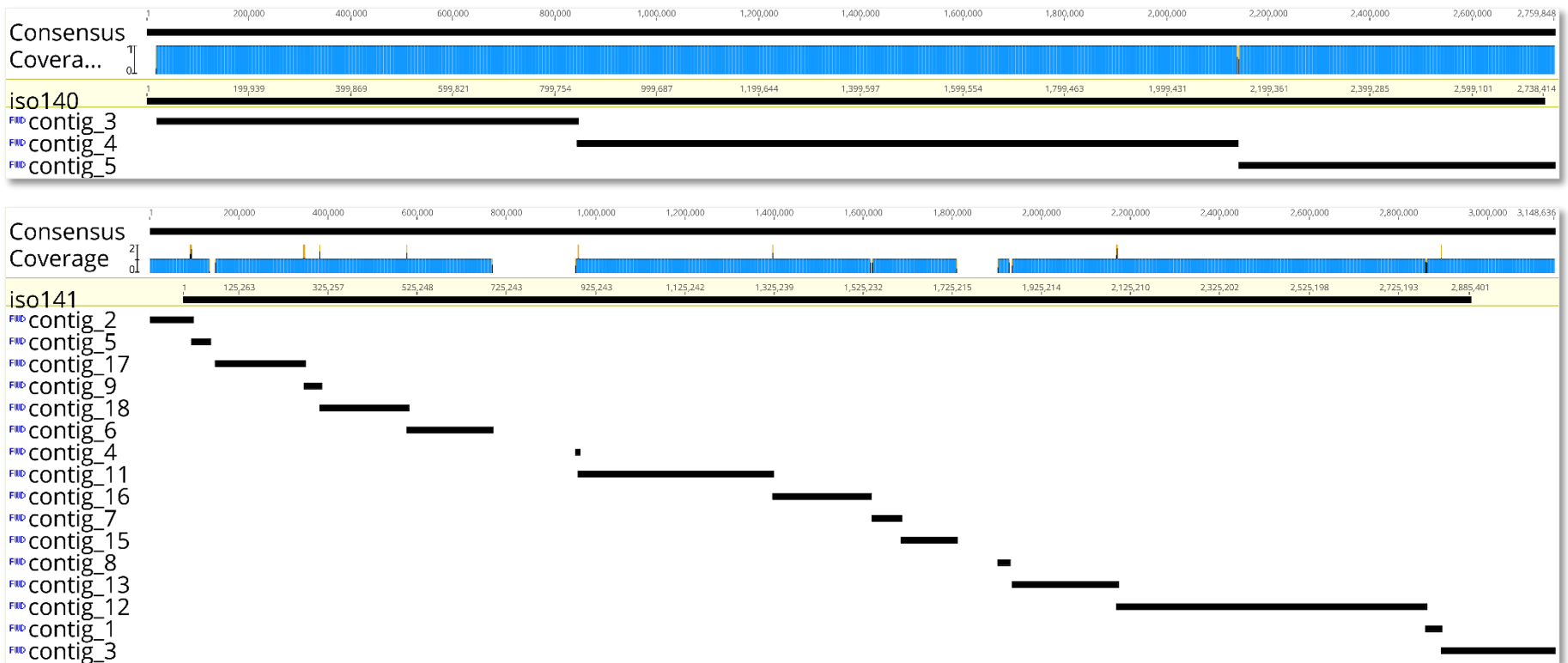


Figure S1. Alignment of the assembled metagenomic assemblies with each's respective isolate. The assembled metagenomic contigs are shown here aligned to each respective isolate's chromosome (iso*). Isolate nanopore WGS assemblies (pale yellow bar) were set as the reference genome for each patient's respective metagenomic assembly (contig*). Two isolates revealed the presence of plasmids (IDs iso111p, iso114p). The blue bar reflects the area of the isolate genome covered by the metagenomic

contigs. The alignment of ID 128 has been collapsed for easier viewing. Metagenomic contigs overhanging the ends of the isolate reference are due to the circular genome of *S. aureus*. Figure rendered in Geneious v.2021.0.3.

Table S3. VirulenceFinder results. *S. aureus* virulence genes detected in shotgun metagenomic sequencing data (meta*) and isolate WGS data (iso*) (<https://cge.cbs.dtu.dk/services/VirulenceFinder/>).

Patient 111		Patient 114		Patient 128		Patient 139		Patient 140		Patient 141	
iso111	meta111	iso114	meta114	iso128	meta128	iso139	meta139	iso140	meta140	iso141	meta141
<i>aur</i>	<i>Aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>
								<i>eta</i>	<i>eta</i>		
<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>
<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>
<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>
<i>lukD</i>	<i>lukD</i>							<i>lukD</i>	<i>lukD</i>		
<i>lukE</i>	<i>lukE</i>							<i>lukE</i>	<i>lukE</i>		
		<i>sak</i>	<i>sak</i>	<i>sak</i>	<i>sak</i>	<i>sak</i>	<i>sak</i>			<i>sak</i>	<i>sak</i>
<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>
										<i>sea</i>	<i>sea</i>
		<i>seg</i>	<i>seg</i>	<i>seg</i>	<i>seg</i>	<i>seg</i>	<i>seg</i>			<i>seg</i>	<i>seg</i>
		<i>sei</i>	<i>sei</i>	<i>sei</i>	<i>sei</i>	<i>sei</i>	<i>sei</i>			<i>sei</i>	<i>sei</i>
		<i>sem</i>	<i>sem</i>	<i>sem</i>	<i>sem</i>	<i>sem</i>	<i>sem</i>			<i>sem</i>	<i>sem</i>
		<i>sen</i>	<i>sen</i>	<i>sen</i>	<i>sen</i>	<i>sen</i>	<i>sen</i>			<i>sen</i>	<i>sen</i>
		<i>seo</i>	<i>seo</i>	<i>seo</i>	<i>seo</i>	<i>seo</i>	<i>seo</i>			<i>seo</i>	<i>seo</i>
		<i>seu</i>	<i>seu</i>	<i>seu</i>	<i>seu</i>	<i>seu</i>	<i>seu</i>			<i>seu</i>	<i>seu</i>
<i>splA</i>	<i>splA</i>							<i>splA</i>	<i>splA</i>		
<i>splB</i>	<i>splB</i>							<i>splB</i>	<i>splB</i>		
<i>splE</i>	<i>splE</i>			<i>splE</i>	<i>splE</i>	<i>splE</i>	<i>splE</i>	<i>splE</i>	<i>splE</i>	<i>splE</i>	<i>splE</i>
		<i>tst</i>	<i>tst</i>	<i>tst</i>	<i>tst</i>	<i>tst</i>	<i>tst</i>			<i>tst</i>	<i>tst</i>

Table S4. Results of resistance phenotype (AST) and resistance genes detected. Abbreviations are as follows: AST (antimicrobial susceptibility testing), ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>), *iso (data from WGS *S. aureus* isolate sequencing), *meta (data from shotgun metagenomic sequencing), R (resistant), I (susceptible, increased exposure), S (susceptible).

Patient ID	AST (phenotype)	ResFinder_iso (% similarity)	ResFinder_meta (% similarity)	EPI2ME_iso (% similarity)	EPI2ME_meta (% similarity)
111	Find: <i>S. aureus</i> Penicillin (R) Ciprofloxacin (S) Erythromycin (S) Fusidic acid (S) Gentamicin (S) Clindamycin (S) Chloramphenicol (S) Linezolid (S) Oxacillin (S) Rifampicin (S) Tetracycline (S) Trimethoprim/sulfamethoxazole (S)	<i>blaZ</i> (100)	<i>blaZ</i> (100)	<i>blaZ</i> (91.9) <i>tet38</i> (94) <i>sav1886</i> (93.6) <i>mepA</i> (93.4) <i>mgrA</i> (94.1) <i>arlS</i> (94) <i>mepR</i> (94.2) <i>arlR</i> (93.8)	<i>blaZ</i> (92) <i>tet38</i> (93.6) <i>sav1886</i> (93.3) <i>mepA</i> (93.9) <i>mgrA</i> (94.3) <i>arlS</i> (94) <i>mepR</i> (94.9)

114	<p>Find: <i>S. aureus</i> Penicillin (R) Ciprofloxacin (S) Erythromycin (S) Fusidic acid (S) Gentamicin (S) Clindamycin (S) Linezolid (S) Oxacillin (S) Rifampicin (S) Tetracycline (S) Trimethoprim/sulfamethoxazole (S)</p>	<i>blaZ</i> (100)	<i>blaZ</i> (99.89)	<p><i>blaZ</i> (91.5) <i>tet38</i> (93.8) <i>sav1886</i> (93.1) <i>mgrA</i> (94.5) <i>arlS</i> (93.2) <i>arlR</i> (93.2) <i>mepR</i> (93.3) <i>mepA</i> (93.8)</p>	<p><i>blaZ</i> (91) <i>tet38</i> (93.5) <i>sav1866</i> (93.4) <i>mgrA</i> (94) <i>arlS</i> (93.5) <i>arlR</i> (93.7) <i>mepR</i> (93)</p>
128	<p>Find: <i>S. aureus</i> Penicillin (R) Tetracycline (R) Ciprofloxacin (I) Erythromycin (S) Fusidic acid (S) Gentamicin (S) Clindamycin (S) Linezolid (S) Oxacillin (S) Rifampicin (S) Trimethoprim/sulfamethoxazole (S)</p>	<p><i>blaZ</i> (100) <i>tetK</i> (100)</p>	<p><i>blaZ</i> (99.88) <i>tetK</i> (100)</p>	<p><i>blaZ</i> (91.2) <i>tetK</i> (93.9) <i>sav1866</i> (93.7) <i>tet38</i> (92.9) <i>mepR</i> (94.3) <i>mgrA</i> (94) <i>mepA</i> (92.5) <i>arlS</i> (92.6) <i>arlR</i> (93.1)</p>	<p><i>blaZ</i> (89.5) <i>tetK</i> (93.5) <i>sav1866</i> (93.1) <i>tet38</i> (92.4) <i>mepR</i> (93.5) <i>mgrA</i> (93.8) <i>mepA</i> (92.2) <i>arlS</i> (92.1) <i>arlR</i> (93.4)</p>

139	<p>Find: <i>S. aureus</i> Penicillin (R) Ciprofloxacin (S) Erythromycin (S) Fusidic acid (S) Gentamicin (S) Clindamycin (S) Linezolid (S) Oxacillin (S) Rifampicin (S) Tetracycline (S) Trimethoprim/sulfamethoxazole (S)</p>	<i>blaZ</i> (100)	<i>blaZ</i> (99.88)	<i>blaZ</i> (91.2) <i>tet38</i> (93.2) <i>mepA</i> (92.7) <i>sav1866</i> (93.6) <i>arlS</i> (93.2) <i>mepR</i> (93.2) <i>arlR</i> (93.7) <i>mgrA</i> (94.2)	<i>blaZ</i> (91.3) <i>tet38</i> (93.2) <i>mepA</i> (92.6) <i>sav1866</i> (92.9) <i>arlS</i> (93) <i>mepR</i> (96.2) <i>arlR</i> (94.4)
140	<p>Find: <i>S. aureus</i> Penicillin (R) Ciprofloxacin (S) Erythromycin (S) Fusidic acid (S) Gentamicin (S) Clindamycin (S) Linezolid (S) Oxacillin (S) Rifampicin (S) Tetracycline (S) Trimethoprim/sulfamethoxazole (S)</p>	<i>blaZ</i> (97.55)	<i>blaZ</i> (100)	<i>blaZ</i> (90.6) <i>tet38</i> (94.0) <i>sav1866</i> (94) <i>mepA</i> (95) <i>mgrA</i> (93.8) <i>arlS</i> (94.2) <i>arlR</i> (93.9) <i>mepR</i> (91.6)	<i>blaZ</i> (91.2) <i>tet38</i> (94.1) <i>sav1866</i> (93.7) <i>mepA</i> (93.5) <i>mgrA</i> (94.4) <i>arlS</i> (94.2) <i>arlR</i> (93.4) <i>mepR</i> (94.8) <i>vgaA</i> (91.9)

141	Find: <i>S. aureus</i> Penicillin (R) Ciprofloxacin (S) Erythromycin (S) Fusidic acid (S) Gentamicin (S) Clindamycin (S) Linezolid (S) Oxacillin (S) Rifampicin (S) Tetracycline (S) Trimethoprim/sulfamethoxazole (S)	<i>blaZ</i> (100)	<i>blaZ</i> (100)	<i>blaZ</i> (91.8) <i>tet38</i> (93.1) <i>sav1866</i> (93.3) <i>mepA</i> (93.1) <i>mgrA</i> (94.3) <i>arlS</i> (93) <i>arlR</i> (93.2) <i>mepR</i> (93.5)	<i>blaZ</i> (91.5) <i>tet38</i> (92.9) <i>sav1866</i> (93.2) <i>mepA</i> (92.6) <i>mgrA</i> (94.1) <i>arlS</i> (92.9) <i>arlR</i> (93.4) <i>mepR</i> (93.6)
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