Supplementary Table 1: Gene and chromosomal mutation presence/absence as predicted by Genefinder, Mykrobe, Typewriter (n=1379)

Gene	Type (resistance gene, mutation or other)	Absence/Presence for Genefinder, MyKrobe, Typewriter							Discordant across programs (n, %)
		AAA	PPP	PAA	APA	PPA	PAP	APP	
aac(6')-aph(2")	Gentamicin, gene	1300	76	0	2	1	0	0	3 (0.2%)
aadD or aph(4)-Ia	Kanamycin, gene	1291	86	0	2	0	0	0	2 (0.1%)
aadE or aph(6)-Ia	Streptomycin, gene	1328	21	0	28	2	0	0	**30 (2.2%)**
ant9-Ia	Spectinomycin, gene	1227	145	0	4	3	0	0	7 (0.5%)
ant9-1b	Spectinomycin, gene	1379	0	0	0	0	0	0	0 (0.0%)
aph(2")-Ic	Gentamicin, gene	1379	0	0	0	0	0	0	0 (0.0%)
aphA3 or aph(3')-III	Kanamycin, gene	1327	51	0	0	1	0	0	1 (0.1%)
blaZ	Penicillin, gene	222	1130	0	19	7	0	1	**27 (2.0%)**
cat	Chloramphenicol, gene	1379	0	0	0	0	0	0	0 (0.0%)
cfr	Chloramphenicol & Linezolid, gene	1376	2	0	0	1	0	0	1 (0.1%)
dfrA	Trimethoprim, gene	1341	34	0	3	0	0	1	4 (0.3%)
dfrC	Trimethoprim, gene	1341	1	0	0	3	0	34	**37 (2.7%)**
dfrD	Trimethoprim, gene	1379	0	0	0	0	0	0	0 (0.0%)
dfrG	Trimethoprim, gene	1325	53	0	1	0	0	0	1 (0.1%)
dfrK	Trimethoprim, gene	1373	6	0	0	0	0	0	0 (0.0%)
ermA	Erythromycin, clindamycin, gene	1229	144	0	1	5	0	0	6 (0.4%)
ermB	Erythromycin, clindamycin, gene	1375	3	0	1	0	0	0	1 (0.1%)
ermC	Erythromycin, clindamycin, gene	1191	176	0	10	2	0	0	12 (0.9%)

Gene	Type (resistance gene, mutation or other)	Absence/Presence for Genefinder, MyKrobe, Typewriter							Discordant across programs (n, %)
		AAA	PPP	PAA	APA	PPA	PAP	APP	
ermT	Erythromycin, clindamycin, gene	1359	20	0	0	0	0	0	0 (0.0%)
ermY	Erythromycin, clindamycin, gene	1379	0	0	0	0	0	0	0 (0.0%)
fusB	Fusidic acid, gene	1355	22	0	2	0	0	0	2 (0.1%)
fusC	Fusidic acid, gene	1302	74	0	1	2	0	0	3 (0.2%)
lsaB	Lincosamide, gene	1379	0	0	0	0	0	0	0 (0.0%)
lnuA	Clindamycin, gene	1364	9	6	0	0	0	0	6 (0.4%)
lnuB	Clindamycin, gene	1378	1	0	0	0	0	0	0 (0.0%)
mecA	Methicillin, penicillin, gene	999	377	0	1	2	0	0	3 (0.2%)
mecC	Methicillin, penicillin, gene	1362	17	0	0	0	0	0	0 (0.0%)
mphC	Erythromycin, gene	1361	17	0	1	0	0	0	1 (0.1%)
msrA	Erythromycin, gene	1360	18	0	1	0	0	0	1 (0.1%)
mupA	Mupirocin, gene	1362	15	2	0	0	0	0	2 (0.1%)
mupB	Mupirocin, gene	1379	0	0	0	0	0	0	0 (0.0%)
qacA	Antiseptic, gene	1333	44	0	1	1	0	0	2 (0.1%)
qacB	Antiseptic, gene	1333	0	0	2	0	0	44	**46 (3.3%)**
qacC	Antiseptic, gene	1319	50	0	7	2	0	1	10 (0.7%)
sat4	Spectinomycin, gene	1327	49	0	2	1	0	0	3 (0.2%)
str	Streptomycin, gene	1375	4	0	0	0	0	0	0 (0.0%)
tetK	Tetracycline, gene	1272	99	0	4	4	0	0	8 (0.6%)
tetL	Tetracycline, gene	1377	2	0	0	0	0	0	0 (0.0%)
tetM	Tetracycline, gene	1339	38	0	1	1	0	0	2 (0.1%)
tetO	Tetracycline, gene	1379	0	0	0	0	0	0	0 (0.0%)
vanA	Vancomycin, gene	1379	0	0	0	0	0	0	0 (0.0%)
vanB	Vancomycin, gene	1379	0	0	0	0	0	0	0 (0.0%)
vanC	Vancomycin, gene	1379	0	0	0	0	0	0	0 (0.0%)
vgaA	Lincosamide, gene	1378	1	0	0	0	0	0	0 (0.0%)
vgaB	Lincosamide, gene	1379	0	0	0	0	0	0	0 (0.0%)

Gene	Type (resistance gene, mutation or other)	Absence/Presence for Genefinder, MyKrobe, Typewriter							Discordant across programs (n, %)
		AAA	PPP	PAA	APA	PPA	PAP	APP	
vgbA	Lincosamide, gene	1379	0	0	0	0	0	0	0 (0.0%)
dfrB	Mutations, see Supplementary Table 3*	1297	81	0	0	0	1	0	1 (0.1%)
fusA	Mutations, see Supplementary Table 3*	1320	57	0	0	2	0	0	2 (0.1%)
*grlA/gyrA***	Mutations, see Supplementary Table 3*	1072	304	0	1	0	2	0	3 (0.2%)
rpoB	Mutations, see Supplementary Table 3*	1354	7	0	1	0	1	0	**2 (0.1%)**
arcA	Virulence gene	1372	6	0	1	0	0	0	1 (0.1%)
arcB	Virulence gene	1373	0	0	0	0	0	6	6 (0.4%)
arcC	Virulence gene	1373	0	0	0	0	0	6	6 (0.4%)
arcD	Virulence gene	1373	0	0	0	0	0	6	6 (0.4%)
sak	Virulence gene	306	1,065	0	3	5	0	0	8 (0.6%)
sasx	Virulence gene	1364	0	0	1	0	0	14	**15 (1.1%)**
chp	Virulence gene	449	883	0	1	46	0	0	**47 (3.4%)**
eta	Virulence gene	1338	40	0	1	0	0	0	1 (0.1%)
etb	Virulence gene	1372	5	0	1	1	0	0	2 (0.1%)
etd	Virulence gene	1320	57	0	1	1	0	0	2 (0.1%)
lukM	Virulence gene	1370	9	0	0	0	0	0	0 (0.0%)
lukF-P83	Virulence gene	1370	3	0	0	0	0	6	6 (0.4%)
lukpvf	Virulence gene	1266	111	0	2	0	0	0	2 (0.1%)
lukpvs	Virulence gene	1266	109	0	3	1	0	0	4 (0.3%)
scn	Virulence gene	144	1,217	0	3	15	0	0	**17 (1.2%)**
sea	Virulence gene	1080	295	0	1	3	0	0	4 (0.3%)
seb	Virulence gene	1267	109	0	1	2	0	0	3 (0.2%)
sec	Virulence gene	1149	225	0	1	4	0	0	5 (0.4%)
sed	Virulence gene	1313	66	0	0	0	0	0	0 (0.0%)

Gene	Type (resistance gene, mutation or other)	Absence/Presence for Genefinder, MyKrobe, Typewriter							Discordant across programs (n, %)
		AAA	PPP	PAA	APA	PPA	PAP	APP	
see	Virulence gene	1375	4	0	0	0	0	0	0 (0.0%)
seg	Virulence gene	600	753	0	6	20	0	0	**26 (1.9%)**
seh	Virulence gene	1241	134	0	2	2	0	0	4 (0.3%)
sei	Virulence gene	596	741	0	3	33	0	6	**36 (2.6%)**
sej	Virulence gene	1310	69	0	0	0	0	0	0 (0.0%)
selr	Virulence gene	1312	67	0	0	0	0	0	0 (0.0%)
sep	Virulence gene	1268	108	0	0	2	0	1	3 (0.2%)
seu	Virulence gene	595	725	0	2	57	0	0	**59 (4.3%)**
tsst1	Virulence gene	1119	255	0	1	4	0	0	5 (0.4%)
ccrA	ccr	1009	365	0	2	3	0	0	5 (0.4%)
ccrB	ccr	1010	336	9	1	22	0	1	**23 (1.7%)**
ccrC(a)	ccr	1338	23	0	8	1	0	9	9 (0.7%)
ccrC(b)	ccr	1333	2	0	22	0	0	22	**44 (3.2%)**
ccrC(c)	ccr	1338	18	0	16	1	0	6	**23 (1.7%)**
Total (% of 114457 predictions)		102780 (89.8%)	11050 (9.7%)	17 (0.01%)	179 (0.2%)	263 (0.2%)	4 (<0.01%)	164 (0.1%)	627 (0.5%)

* for chromosomal mutations, “A” means no resistance-conferring mutations in Table 2 were identified. “P” means one or more were identified. Identical mutations were identified by all three. In all other cases, “P” means the gene was detected and “A” means it was absent.

** *gyrA/grlA* mutational pattern combined for analysis since both relate to ciprofloxacin resistance

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8 **Supplementary Table 2: Method agreement or disagreement according to sample set and type of determinant**

Set	Type of determinant	Disagreement between the three methods	Agreement between all three methods	Total
PHE	Resistance gene	161 (0.9%)	17,871 (99.1%)	18,032 (100%)
	Mutations in resistance genes	3 (0.2%)	1,565 (99.8%)	1,568 (100%)
	CCR	83 (4.2%)	1,877 (95.8%)	1,960 (100%)
	Virulence factors	102 (0.9%)	10,874 (99.1%)	10,976 (100%)
	Total	349 (1.1%)	32,187 (98.9%)	32,536 (100%)
Oxford validation	Resistance gene	19 (0.1%)	22,337 (99.9%)	22,356 (100%)
	Mutations in resistance genes	1 (<0.1%)	1,943 (>99.9%)	1,944 (100%)
	CCR	10 (0.4%)	2,420 (99.6%)	2,430 (100%)
	Virulence factors	15 (0.1%)	13,593 (99.6%)	13,608 (100%)
	Total	45 (0.1%)	40,293 (99.9%)	40,338 (100%)
Oxford derivation	Resistance gene	41 (0.2%)	23,005 (99.8%)	23,046 (100%)
	Mutations in resistance genes	4 (0.2%)	2,000 (99.8%)	2,004 (100%)

	CCR	30 (1.2%)	2,475 (98.8%)	2,505 (100%)
	Virulence factors	158 (1.1%)	13,870 (98.9%)	14,028 (100%)
	Total	233 (0.6%)	41,350 (99.4%)	41,583 (100%)
Total		627 (0.5%)	113,830 (99.5%)	114,457 (100%)
All	Resistance gene	221 (0.3%)	63,213 (99.7%)	63,434 (100%)
	Mutations in resistance genes	8 (0.1%)	5,508 (99.9%)	5,516 (100%)
	CCR	123 (1.8%)	6,772 (98.2%)	6,895 (100%)
	Virulence factors	275 (0.7%)	38,337 (99.3%)	38,612 (100%)

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Supplementary Table 3: Sensitivity and specificity of antimicrobial resistance predictions using each method in each dataset

Set	Method	Sensitivity	Specificity
Oxford validation	Genefinder	0.977 (0.966-0.985)	0.997 (0.994-0.998)
	Mykrobe	0.978 (0.967-0.986)	0.994 (0.991-0.996)
	Typewriter	0.975 (0.963 -0.984)	0.998 (0.995 -0.999)
Oxford derivation	Genefinder	0.973 (0.958-0.983)	0.991 (0.988-0.994)
	Mykrobe	0.973 (0.958 – 0.983)	0.991 (0.987-0.994)
	Typewriter	0.970 (0.955-0.981)	0.992 (0.988-0.994)
Colindale	Genefinder	0.954 (0.940-0.965)	0.981 (0.976-0.986)
	Mykrobe	0.954 (0.955-0.981)	0.979 (0.974-0.984)
	Typewriter	0.949 (0.935-0.961)	0.984 (0.979-0.988)

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15 **Supplementary Table 4: Chromosomal mutations**

Gene	Antibiotic	Present in at least one WGS within this study and resistance-determinant	Present in at least one WGS within this study, but not a resistance-determinant
dfrB	Trimethoprim	H150R, L21V*, H31N, L41F, F99S, F99Y,	F99X
fusA	Fusidic acid	A67T*, V90I, P404Q, P404L, P406L, G452S, H457Y, H457Q, L461K, L461S,	A71X; A160X, P161X; T326I, D373G, D373X, A376T, P404?, P406?, L456X, L461X, L461F, E468V, C473X, T656S
gyrA	Ciprofloxacin	S84L, S84A	S84V, S84X, E88X
grlA	Ciprofloxacin	I45M*, S80F, S80Y, E84G*, E84K*	
rpoB	Rifampicin	S464P, Q468R, D471Y, A477D,	

H481N, H481Y

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17 * mutation only used to predict resistance if occurred in combination with other mutations, see Gordon NC, Price JR, Cole K, Everitt R, Morgan
18 M, Finney J, Kearns AM, Pichon B, Young B, Wilson DJ, Llewelyn MJ, Paul J, Peto TE, Crook DW, Walker AS, Golubchik T. 2014. Prediction of
19 *Staphylococcus aureus* antimicrobial resistance by whole-genome sequencing. J Clin Microbiol 52:1182-9 Supplementary Table S1 for details of
20 combinations. Note: only including resistance-conferring mutations from Gordon *et al* that were observed in one or more study isolates. “X”
21 means no call made at this position.