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

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Contents

Table S1. Nanopore sequencing QC metrics

Table S2. metaflye assembly metrics

Figure S1. Alignment of the assembled metagenomic assemblies with each's respective isolate

Table S3. VirulenceFinder results

Table S4. Results of resistance phenotype (AST) and resistance genes detected.

Patient	Total	Total	Mean quality	Mean read length
ID	reads	bases	score	(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 S1. Nanopore sequencing QC metrics, totals for each patient: meta*(shotgun metagenomic sequencing data), iso* (isolate WGS data).

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

iso139

	1	2816259	231	
iso140				
_	1	2736340	22	
iso141				
	1	2883453	402	

C	1	200,000	400	0,000	600,000	800,000	1,000,0	00 1,	200,000	1,400,000	1,600,00	0 1,80	0,000	2,000,000	2,200,000	2,400,00	0 2,6	00,000	2,800,000	3,000,000	3,242,694
Consensus Coverage	I																I				
iso111 Feo contig_12 Feo contig_11 Feo contig_9 Feo contig_9 Feo contig_3 Feo contig_3 Feo contig_5 Feo contig_7 Feo contig_6	1	280,000	400	••••	599 ₁ 996	799,993	993,95	91 1.	199,991	1,399,984	1,599,94	2 1,79	9,941	1,999,938	2,199,933	2,399,03	1 2.5	99,305	2,805,858		
Consensus Coverage	1 	2,000	4,000	6,000	8,000	10,000	12,000 ——————————————————————————————————	14,000	16,000	18,000	20,000	22,000 H	24,000	26,000	28,000	30,000	32,000	34,000	36,000	38,000	40,000 41,462
iso111p contig_13	1	2,000	4,000	6,000	8,000	10,000	12,000 H	14,000	16,000 H	18,000	20,000	22,000	24,000 H	26,000 H	28,000	29,999	31,999	33,999	35,999	37,999	41,461

Consensus	1 200,00	00 400,000	600,000	800,000	1,000,000	1,200,000	1,400,000	1,600,000	1,800,000	2,000,000	2,200,000	2,400,00	0 2,60 <u>0</u> ,0	2,772,856
Coverage														
iso114 For contig_6 For contig_5 For contig_2 For contig_3	, 1 134,37	90 339 ¹ 318	299/874	, 33 ¹ 900		1,19,852	1,399,845	1,599,824	1'\43'903	1,434,748	2,199,787	2,399,78	/	2,772,643
c	1 200	400	600	800	1,000 1,200	0 1,400	1,600	1,800	2,000	2,200	2,400	2,600	2,800	3,000 3,130
Consensus Coverage d														
iso114p contig_7	1 197 E	397	597	797	997 1,193	7 1,397	1,597	1.797	1,997	2,197	2,397	2,597	2,797	3,127
C	1 200,000	400,000	600,000	800 _/ 000	1,000,000	1,200,000	1,400,000	1,600,000	1,800,000	2,000,000	2,200,000	2,400,000	2,600,000	2,826,074
Coverage ²												1		
iso128	1 199,962	2 399,934	599,921	799,911	999,887	1,199,806	1,399,776	1,599,753	1,799,726	1,999,697	2,199,676	2,399,639	2,599,592	2,825,628
[⊯] contig_1		_										_	_	•••
Consonsus	1 200,000	400,000	600,000	800,000	1,000,000	1,200,000	1,400,000	1,600,000	1,800,000	2,000,000	2,200,000	2,400,000	2,600,000	2,819,694
							l	1						
iso139 For contig_12 For contig_14 For contig_10 For contig_11 For contig_9 For contig_8	1 199,988	399,974	599,964	799,957	999,945	1,199,927	1,399,896	1,599,873	1,799,825	1,999,801	2,199,788	2,399,775	2,599,750	2,818,229

Consonsus	1	200 ₁ 000	400,000	600 ₁ 000	800,000	1,000,000	1,200,000	1,400,000	1,600,000	1,800,000	2,000,0	00	2,200,000	2,400,000	2,600,000	2,759,848
Covera																
iso140	1	199,939	399,869	599,821	799,754	999 ₁ 687	1,199,644	1,399,597	1,599,554	1,799,463	1,999,4	31	2,199,361	2,399,285	2,599,101	2,738,414
™ contig_3 ™ contig_4 ™ contig_5					_							_				
c	1	200 ₁ 000	400 ₁ 000	600,000 8	00,000 1,000,1	1,200,000	1,400,000	1,600,000	1,800,000	2,000,000	2.200,000	2,400,000	2,600,000	2,800.000	3,000,00	0 3,148,636
Consensus Coverage ²																
iso141	1	125,263	325,257	525,248 7	25,243 925,2	43 1,125,242	1,325,239	1,525,232	1,725,215	1,925,214	2,125,210	2,325,202	2,525,198	2,725,193	2,885,401	
™ contig_2																
™ contig_17			_													
contig_9				-												
contig_6					_											
contig_4																
™contig_ <u>1</u> 6																
© contig_/																
™ contig_8										-						
™contig_13																
contig_12															_	
contig_1														•		

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/).

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

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	AST (phenotype)	ResFinder_iso	ResFinder_meta	EPI2ME_iso	EPI2ME_meta
ID		(% similarity)	(% similarity)	(% similarity)	(% similarity)
111	Find: S. aureus 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)	blaZ (100)	blaZ (100)	blaZ (91.9) tet38 (94) sav1886 (93.6) mepA (93.4) mgrA (94.1) arlS (94) mepR (94.2) arlR (93.8)	blaZ (92) tet38 (93.6) sav1886 (93.3) mepA (93.9) mgrA (94.3) arlS (94) mepR (94.9)

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

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

	Find: S. aureus Penicillin (R)	blaZ (100)	blaZ (100)	blaZ (91.8)	blaZ (91.5)
141	Penicillin (R) Ciprofloxacin (S) Erythromycin (S) Fusidic acid (S) Gentamicin (S) Clindamycin (S) Linezolid (S) Oxacillin (S) Rifampicin (S) Tetracycline (S)	blaZ (100)	bla2 (100)	bla2 (91.8) tet38 (93.1) sav1866 (93.3) mepA (93.1) mgrA (94.3) arlS (93) arlR (93.2) mepR (93.5)	bla2 (91.5) tet38 (92.9) sav1866 (93.2) mepA (92.6) mgrA (94.1) arlS (92.9) arlR (93.4) mepR (93.6)
	Trimethoprim/sulfamethoxazole (S)				