

Plasmid-Encoded Transferable *mecB*-Mediated Methicillin Resistance in *Staphylococcus aureus*

Technical Appendix

Technical Appendix Table 1. Resistance profile of *S. aureus* UKM4229 determined by microarray and whole genome sequencing*

Gene	Description†	Result by			
		Microarray	WGS		
	UKM4229 genome		pSAWWU4229_1	pSAWWU4229_2	
Resistance genotype: methicillin					
<i>mecA</i>	Alternate penicillin binding protein 2, defining MRSA	-	-	-	-
<i>mecB</i>	Beta-lactam-inducible penicillin-binding protein	ND†	-	+	-
<i>mecC</i>	Novel <i>mecA</i> homologue, also associated with beta-lactam resistance	-	-	-	-
<i>mecD</i>	Beta-lactam-inducible penicillin-binding protein	ND	-	-	-
Resistance genotype: penicillinase					
<i>blaZ</i>	Beta-lactamase gene	+	-	+	+
<i>blaZ</i> -SCCmecXI	Beta-lactamase gene associated with SCCmec XI elements	-	-	-	-
<i>blaI</i>	Beta lactamase repressor (inhibitor)	+	-	-	+
<i>blaR</i>	Beta-lactamase regulatory protein	+	-	-	+
Resistance genotype: MLS antibiotics					
<i>erm(A)</i>	rRNA methyltransferase associated with macrolide/lincosamide resistance	-	-	-	-
<i>erm(B)</i>	rRNA methyltransferase associated with macrolide/lincosamide resistance	+	-	+	-
<i>erm(C)</i>	rRNA methyltransferase associated with macrolide/lincosamide resistance	-	-	-	-
<i>lnu(A)</i>	Lincosaminide nucleotidyltransferase (= <i>linA</i>)	-	-	-	-
<i>msr(A)</i>	Macrolide efflux pump	-	-	-	-
<i>mef(A)</i>	Macrolide efflux protein A	-	-	-	-
<i>mph(C)</i>	Macrolide phosphotransferase II (= <i>mpbBM</i>)	-	-	-	-
<i>vat(A)</i>	Virginiamycin A acetyltransferase	-	-	-	-
<i>vat(B)</i>	Acetyltransferase inactivating streptogramin A	-	-	-	-
<i>vga(A)</i>	ABC transporter conferring resistance to streptogramin A and related compounds	-	-	-	-
<i>vga(A)</i> (BM 3327)	<i>vga(A)</i> allele from strain BM 3327	-	-	-	-
<i>vgB(A)</i>	Virginiamycin B hydrolase (= <i>vgb</i>)	-	-	-	-
Resistance genotype: aminoglycosides					
<i>aacA-aphD</i>	Aminoglycoside adenylyl-/phosphotransferase (gentamicin, tobramycin)	+	-	+	-
<i>aadD</i>	Aminoglycoside adenylyltransferase (neo-/ kanamycin, tobramycin)	-	-	-	-
<i>aphA3</i>	Aminoglycoside phosphotransferase (neo-/ kanamycin)	+	-	+	-
Resistance genotype: miscellaneous genes					
<i>sat</i>	Streptothricin acetyltransferase	+	-	+	-
<i>dfrS1</i>	Dihydrofolate reductase mediating trimethoprim resistance (= <i>dfrA</i>)	-	-	-	-
<i>fusB</i>	Fusidic acid resistance gene (= <i>far1</i>)	-	-	-	-
<i>fusC</i>	Fusidic acid resistance gene (= Q6GD50)	-	-	-	-
<i>mupA</i>	Isoleucyl-tRNA synthetase associated with mupirocin resistance (= <i>mupR</i>)	-	-	-	-
<i>tet(K)</i>	Tetracycline efflux protein	-	-	-	-
<i>tet(M)</i>	Ribosomal protection protein associated with tetracycline resistance	-	-	-	-
<i>tetS</i>	Tetracycline resistance protein TetS	ND	-	+	-
<i>cat</i> (total)	Chloramphenicol acetyltransferase	-	-	-	-
<i>cfr</i>	23S rRNA methyltransferase (phenicols, lincosamides, oxazolidinones, pleuromutilins, streptogramin A)	-	-	-	-
<i>fexA</i>	Chloramphenicol/florfenicol exporter	-	-	-	-
<i>fosB</i>	Metallothiol transferase	-	-	-	-

Gene	Description†	Result by			
		Microarray	WGS		
	UKM4229 genome		pSAWWU4229_1	pSAWWU4229_2	
<i>fosB</i> (plasmid)		-	-	-	-
Resistance genotype: efflux systems					
<i>qacA</i>	Quaternary ammonium compound/multidrug efflux protein A	-	-	-	-
<i>qacC</i> (total)	Quaternary ammonium compound/multidrug efflux protein C	-	-	-	-
<i>sdrM</i>	Multidrug efflux pump	+	+	-	-
Resistance genotype: glycopeptides					
<i>vanA</i>	Vancomycin resistance gene	-	-	-	-
<i>vanB</i>	Vancomycin resistance gene from enterococci and <i>Clostridium</i>	-	-	-	-
<i>vanZ</i>	Teicoplanin resistance gene from enterococci	-	-	-	-

*+, positive; -, negative; ND, not determined; WGS, whole genome sequencing
†Description as provided by the manufacturer (*S. aureus* Genotyping Kit 2.0 manual, Alere Technologies GmbH, Jena, Germany), except for ND cases

Technical Appendix Table 2. Virulence profile of *S. aureus* UKM4229 determined by microarray and whole genome sequencing*

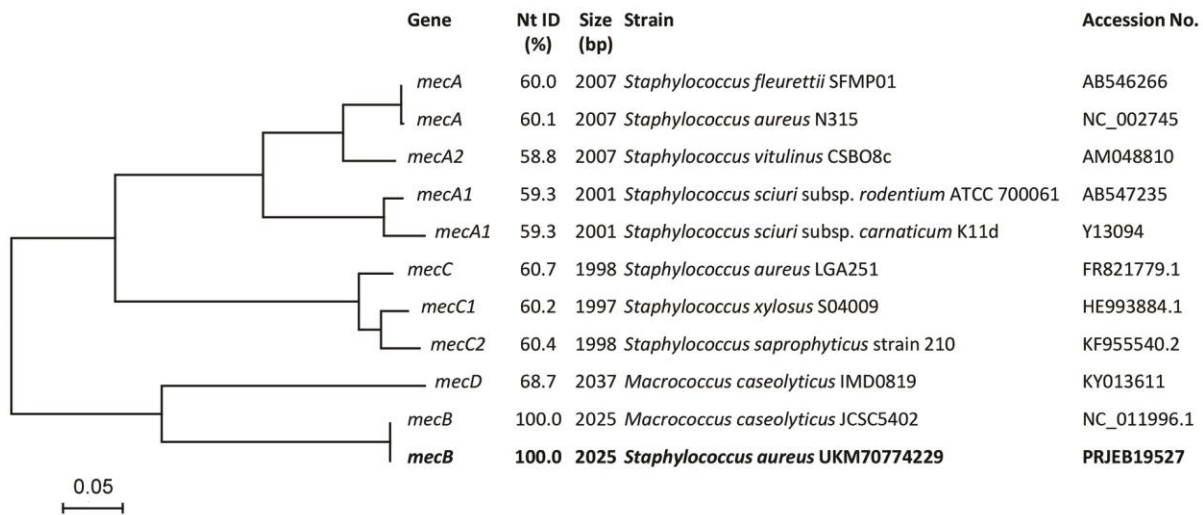
Gene	Description†	Result by			
		Microarray	WGS		
	UKM4229 genome		pSAWWU4229_1	pSAWWU4229_2	
Virulence: enterotoxins					
<i>sea</i>	Enterotoxin A (= <i>entA</i>)	-	-	-	-
<i>sea</i> (320E)	Enterotoxin A, allele from strain 320E	-	-	-	-
<i>seb</i>	Enterotoxin B (= <i>entB</i>)	-	-	-	-
<i>sec</i>	Enterotoxin C (= <i>entC</i>)	-	-	-	-
<i>sed</i>	Enterotoxin D (= <i>entD</i>)	-	-	-	-
<i>see</i>	Enterotoxin E (= <i>entE</i>)	-	-	-	-
<i>seg</i>	Enterotoxin G (= <i>entG</i>)	-	-	-	-
<i>seh</i>	Enterotoxin H (= <i>entH</i>)	-	-	-	-
<i>sei</i>	Enterotoxin I (= <i>entI</i>)	-	-	-	-
<i>sej</i> / <i>selj</i>	Enterotoxin J (= <i>entJ</i>)	-	-	-	-
<i>sek</i> / <i>selk</i>	Enterotoxin K (= <i>entK</i>)	-	-	-	-
<i>sel</i> / <i>sell</i>	Enterotoxin L (= <i>entL</i>)	-	-	-	-
<i>selm</i>	Enterotoxin-like gene/protein M (= <i>sem</i> , <i>entM</i>)	-	-	-	-
<i>seln</i> (consensus)	Enterotoxin-like gene/protein N (= <i>sen</i> , <i>entN</i>), consensus probe	-	-	-	-
<i>selo</i>	Enterotoxin-like gene/protein O (= <i>seo</i> , <i>entO</i>)	-	-	-	-
<i>selp</i> / <i>sea</i> (N315)	Enterotoxin A, allele from strain N315 (= enterotoxin P, <i>entP</i>)	+	+	-	-
<i>egc</i>	Enterotoxin gene cluster, consisting of <i>seg</i> , <i>sei</i> , <i>selm</i> , <i>seln</i> , <i>selo</i> , <i>selu</i>	-	-	-	-
<i>seq</i> / <i>selq</i>	Enterotoxin Q (= <i>entQ</i>)	-	-	-	-
<i>ser</i>	Enterotoxin R (= <i>entR</i>)	-	-	-	-
<i>selu</i>	Enterotoxin-like gene/protein U (= <i>seu</i> , <i>entU</i>)	-	-	-	-
Virulence: hemolysin gamma and leukocidins					
<i>lukF</i> / <i>hlgB</i>	Hemolysin gamma / leukocidin, component B (F)	+	+	-	-
<i>lukS</i> / <i>hlgC</i>	Hemolysin gamma / leukocidin, component C (S)	+	+	-	-
<i>lukS</i> (ST22+ST45)	Hemolysin gamma / leukocidin, component C (S), allele from ST22 and ST45	+	+	-	-
<i>hlgA</i>	Hemolysin gamma, component A	+	+	-	-
<i>lukF-PV</i>	Panton Valentine leukocidin F component	-	-	-	-
<i>lukS-PV</i>	Panton Valentine leukocidin S component	-	-	-	-
<i>lukF-PV</i> (P83)	F component of leukocidin from ruminants	-	-	-	-
<i>lukM</i>	S component of leukocidin from ruminants	-	-	-	-
<i>lukD</i>	Leukocidin D component	+	+	-	-
<i>lukE</i>	Leukocidin E component	+	+	-	-
<i>lukX</i>	Leukocidin/hemolysin toxin family protein (= <i>lukA</i> or <i>lukG</i>)	+	+	-	-
<i>lukY</i>	Leukocidin/hemolysin toxin family protein (= <i>lukB</i> or <i>lukH</i>)	+	+	-	-
<i>lukY</i> (ST30+ST45)	Leukocidin/hemolysin toxin family protein (= <i>lukB</i> or <i>lukH</i>),	-	-	-	-

Gene	Description†	Result by			
		Microarray	WGS		
			UKM4229 genome	pSAWWU4229_1	pSAWWU4229_2
allele from ST30 and ST45					
Virulence: hemolysins					
<i>hla / hly</i>	Hemolysin alpha	+	+	-	-
<i>hlb</i>	Hemolysin beta	+	+	-	-
undisrupted <i>hlb</i>	Hemolysin beta without phage insertion	-	-	-	-
<i>hld</i>	Hemolysin delta	ND	+	-	-
Virulence: hlb-converting phage					
<i>sak</i>	Staphylokinase	+	+	-	-
<i>chp</i>	Chemotaxis-inhibiting protein (CHIPS)	-	-	-	-
<i>scn</i>	Staphylococcal complement inhibitor	+	+	-	-
Virulence: exfoliative toxins					
<i>etA</i>	Exfoliative toxin serotype A	-	-	-	-
<i>etB</i>	Exfoliative toxin serotype B	-	-	-	-
<i>etD</i>	Exfoliative toxin D	-	-	-	-
Virulence: epidermal cell differentiation inhibitors					
<i>edinA</i>	Epidermal cell differentiation inhibitor	-	-	-	-
<i>edinB</i>	Epidermal cell differentiation inhibitor B	-	-	-	-
<i>edinC</i>	Epidermal cell differentiation inhibitor C	-	-	-	-
Virulence: ACME locus					
<i>ACME cluster</i>	Arginine catabolic mobile element	-	-	-	-
<i>arcA-SCC</i>	ACME-locus: arginine deiminase	-	-	-	-
<i>arcB-SCC</i>	ACME-locus: ornithincarbamoyltransferase	-	-	-	-
<i>arcC-SCC</i>	ACME-locus: carbamatkinase	-	-	-	-
<i>arcD-SCC</i>	ACME-locus: arginine/ornithine-antiporter	-	-	-	-
Virulence: proteases					
<i>aur</i> (consensus)	Aureolysin	+	+	-	-
<i>aur</i> (other than MRSA252)		+	+	-	-
<i>aur</i> (MRSA252)		-	-	-	-
<i>spIA</i>	Serinprotease A	+	+	-	-
<i>spIB</i>	Serinprotease B	+	+	-	-
<i>spIC</i>	Serinprotease C	ND	+	-	-
<i>spIE</i>	Serinprotease E	+	+	-	-
<i>spIF</i>	Serinprotease F	ND	+	-	-
<i>sspA</i>	Glutamylendopeptidase	+	+	-	-
<i>sspB</i>	Staphopain B, protease	+	+	-	-
<i>sspP</i> (consensus)	Staphopain A (staphylopain A), protease	+	+	-	-
Capsule- and biofilm-associated genes					
<i>cap 1</i> (total)	Capsule type 1	-	-	-	-
<i>cap 5</i> (total)	Capsule type 5	-	-	-	-
<i>cap 8</i> (total)	Capsule type 8	+	+	-	-
<i>capH8</i>	Capsular polysaccharide synthesis enzyme	+	+	-	-
<i>capI8</i>	Capsular polysaccharide biosynthesis protein	+	+	-	-
<i>capJ8</i>	O-antigen polymerase	+	+	-	-
<i>capK8</i>	Capsular polysaccharide biosynthesis protein	+	+	-	-
<i>icaA</i>	Intercellular adhesion protein A	+	+	-	-
<i>icaC</i>	Intercellular adhesion protein C	+	+	-	-
<i>icaD</i>	Biofilm PIA synthesis protein D	+	+	-	-
<i>bap</i>	Surface protein involved in biofilm formation	-	-	-	-
Adhesion factors/MSCRAMM genes					
<i>bbp</i> (total)	Bone sialoprotein-binding protein	+	+	-	-
<i>clfA</i> (total)	Clumping factor A	+	+	-	-
<i>clfB</i> (total)	Clumping factor B	+	+	-	-
<i>cna</i>	Collagen-binding adhesin	-	-	-	-
<i>ebh</i> (consensus)	Cell wall associated fibronectin-binding protein	+	+	-	-
<i>ebpS</i> (total)	Cell surface elastin binding protein	+	+	-	-
<i>eno</i>	Enolase	+	+	-	-
<i>fib</i>	Fibrinogen binding protein (19 kDa)	+	+	-	-
<i>fnbA</i> (total)	Fibronectin-binding protein A	+	+	-	-

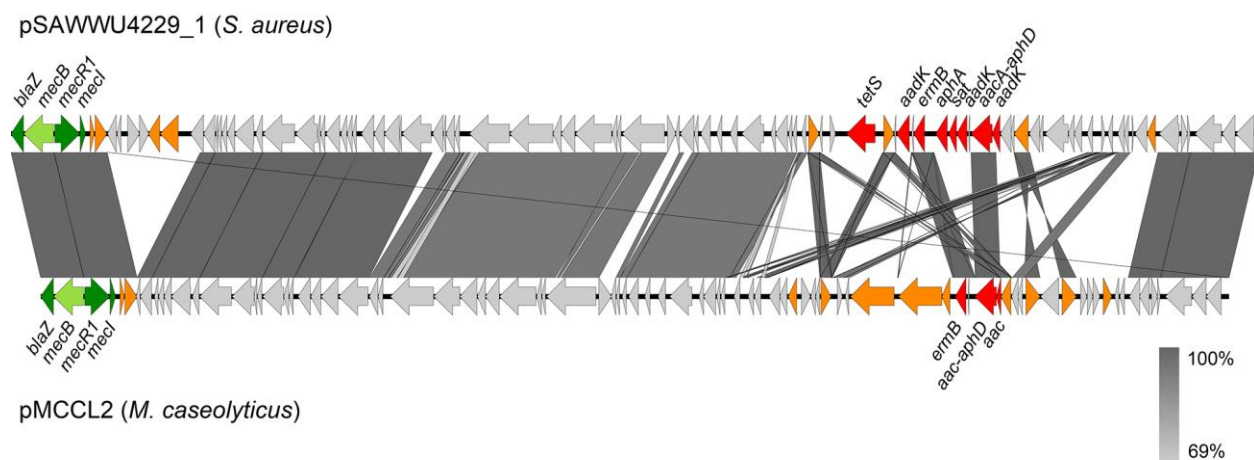
Gene	Description†	Result by			
		Microarray	WGS		
			UKM4229 genome	pSAWWU4229_1	pSAWWU4229_2
<i>fnbB</i> (total)	Fibronectin-binding protein B	+	+	-	-
<i>map</i> (total)	Major histocompatibility complex class II analog protein (= Extracellular adherence protein, <i>eap</i>)	+	+	-	-
<i>sasG</i> (total)	<i>Staphylococcus aureus</i> surface protein G	-	-	-	-
<i>sdrC</i> (total)	Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein C	+	+	-	-
<i>sdrD</i> (total)	Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein D	+	+	-	-
<i>sdrE</i>	Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein E	ND	+	-	-
<i>sdrF</i>	Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein F	ND	-	+	-
<i>vwb</i> (total)	Van Willebrand factor binding protein	+	+	-	-

*+, positive; -, negative; ND, not determined; MSCRAMM, microbial surface components recognizing adhesive matrix molecules; WGS, whole genome sequencing.

†Description as provided by the manufacturer (*S. aureus* Genotyping Kit 2.0 manual, Alere Technologies GmbH, Jena, Germany), except for ND cases



Technical Appendix Figure 1. Phylogenetic relationships of *mec* genes conferring methicillin resistance and overview of characteristic features. Evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (1) in MEGA7 (2). Nucleotide sequences were aligned using MUSCLE (3). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Nucleotide identity (nt ID) between the *mecB* gene in strain UKM4229 and other *mec* genes was determined by sequence alignment using Clustal OMEGA (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).



Technical Appendix Figure 2. Structural comparison of pSAWWU4229_1 and pMCCL2 (*M. caseolyticus* JCSC5402; NC_011996.1) performed by Easyfig software (49). Gray areas represent regions with nucleotide sequence similarities ranging between 69% and 100%. The *mec*-complex is colored in green, antibiotic resistance genes in red, and transposase/integrase genes in orange.

References

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