Methicillin-resistant *Staphylococcus haemolyticus* ST25 isolated from carriage samples in uMgungundlovu district, South Africa

To the Editor: *Staphylococcus haemolyticus* is the second most frequent causative agent of staphylococcal infections among coagulase-negative staphylococci (CoNS).[1,2] This staphylococcal species has an important ability to acquire multiresistance against various antibiotics.[1,2] In fact, methicillin-resistant *S. haemolyticus* (MRSH) has been reported worldwide in clinical cases, notably in bloodstream infections. Methicillin resistance results from the recombinase-mediated insertion of staphylococcal cassette chromosome mec (*SCC*mec), the mobile genetic element carrying *mecA*, at the 3′ end of a chromosomal open reading frame designated as *orfX*. The *mec*A gene encodes for complete resistance to the beta-lactam family and various levels of co-resistance to other antibiotics. It has been postulated that CoNS, including *S. haemolyticus*, could act as reservoirs of resistance genes harboured on mobile genetic elements including the *SCC*mec, plasmids, prophages, transposons and pathogenicity islands that enable the horizontal transmission of resistance genes in staphylococcal species.[3,4] Considering its great ability and adaptability to survive in hospitals, especially on medical devices, *S. haemolyticus* is particularly involved in nosocomial infections.[1,2]

The isolates presented herein formed part of a bigger study investigating the molecular epidemiology of antibiotics in clinical and carriage samples from patients admitted to a rural district hospital and an urban tertiary hospital in the uMgungundlovu District in South Africa (SA). The isolates were identified using biochemical tests, and Rosco Diagnostica (Taastrup, Denmark) was used to screen for methicillin resistance and vancomycin resistance. Antimicrobial susceptibility testing was performed by broth microdilution against a panel of antibiotics consisting of cefoxitin, amikacin, gentamicin, tobramycin, ciprofloxacin, moxifloxacin, erythromycin, clindamycin, fusidic acid, linezolid, tetracycline, doxycycline, teicoplanin, vancomycin, tigecycline, trimethoprim and nitrofurantoin. The European Committee on Antimicrobial Susceptibility testing (EUCAST) breakpoints[5] were used for interpretation of the results, and *S. aureus* ATCC 29213 was used as the control.

Whole-genome sequencing (WGS) analysis was performed on an Illumina MiSeq platform (Illumina Inc., USA) with 100× coverage. CLC Genomics Workbench version 10 (CLC, Bio-QIAGEN, Denmark) and SPAdes[6] version 3.5 were used for *de novo* assembly. The bacterial analysis pipeline of GoSeqIt tools was also used to annotate and identify known acquired antibiotic-resistant genes via ResFinder,[7] virulence factors using VirulenceFinder[8] and mobile genetic elements through PlasmidFinder.[9] The multi-locus sequence type was also determined from WGS data using eight housekeeping genes (*gapa*, *infb*, *mdh*, *pgi*, *phoe*, *rpob* and *tonb*).

The two MRSH, G808N1B1 (GenBank accession number PGWX00000000) and A109N1B1 (GenBank accession number PGWY00000000), originated from carriage samples of patients admitted to tertiary and district hospitals, respectively. Both isolates were ascribed to the sequence type (ST) 25 and showed multidrug resistance (Table 1). WGS confirmed this resistance profile by identifying *blaZ* and *mec*A together with several resistant determinants to non-beta-lactam antibiotics, notably to aminoglycosides (*ant*(6)-Ia, *aac*(6′)-*aph*(2″), *aph*(3′)-III), fusidic acid (*fus*B), MLS ((*msr*(A), *mph*(C), *vga*(A)) and teicoplanin (*Tca*S, *Tca*B) (Table 1). The multidrug and toxin extrusion (MATE) transport protein, *mep*A, encoding for resistance to tigecycline, the multidrug exporter *Acr*B and *mex*E from the resistance-nodulation-cell division family, the MDR efflux pump (*qac*A) and small MDR family (*qac*) were additionally detected (Table 1). Additionally, a plasmid (RepA(VRSAp)) was identified in the tertiary hospital. It is noteworthy to mention that none of the *S. haemolyticus* carried virulence factors.

To the best of our knowledge, this is the first report of MRSH ST25 isolated from carriage samples in SA. The identification of these MRSH ST25 in two different levels of care demonstrate their probable presence in public hospitals in uMgungundlovu District. Taking into consideration that *S. haemolyticus* is an opportunistic pathogen that is rather difficult to eradicate owing to its bacterial resistance, routine screening of patients and appropriate implementation of infection control measures should be strictly implemented in these settings. The observed resistance to last-resort antibiotics is of concern, necessitating antibiotic stewardship. Genomic data on these bacteria could provide better understanding of their evolution and guide interventions for their containment.

Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, South Africa (ref. no. BF512/16, sub-study of BCA444/16). Permission to conduct the research was also granted by the provincial Department of Health, uMgungundlovu District and hospital managers.

Table 1. Antimicrobial susceptibility to selected antibiotics in individual MRSH isolates

Isolate name	Minimum inhibitory concentrations (mg/L)													Antibiotic resistance genes
	Cefoxitin	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Moxifloxacin	Erythromycin	Clindamycin	Linezolid	Teicoplanin	Vancomycin	Tetracycline	Doxycycline	Tigecycline	Fusidic acid	Trimethoprim	Nitrofurantoin	
A109N1B1	≥512	32	256	128	≥512	32	256	2	16	64	16	8	4	16	32	≥512	64	*mecA*, *blaZ*, *aac*(6′)-*aph*(2″), *aac*(6′)-Ia, *ant*(6)-Ia, *norA*, *msr*(A), *mph*(C), *dfrG*, *mepA*, *qac*, *bacA*, MATE, *AcrB*, *TcaA*, *TcaB*
G808N1B1	32	2	1	2	8	0.5	1	1	32	64	8	16	1	2	64	≥512	32	*mecA*, *blaZ*, *aac*(6′)-*aph*(2″), *aac*(6′)-Ia, *aph*(3′)-IIIa, *ant*(6)-Ia, *norA*, *fusB*, *msr*(A), *mph*(C), *vga*(A), *dfrG*, *mepA*, *qac*, *qacA*, *mexE*, *fusB*, MATE, *AcrB*, *TcaA*, *TcaB*

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