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] 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 (SCCmec), the mobile genetic element carrying mecA, at the 3’ end of a chromosomal open reading frame designated as orfX. The mecA 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 SCCmec, 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 ceftoxitin, 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 100x coverage. CLC Genomics Workbench version 10 (CLC, Bio-)

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

<table>
<thead>
<tr>
<th>Isolate name</th>
<th>Minimum inhibitory concentrations (mg/L)</th>
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<tr>
<td>A109N1B1</td>
<td>256 32 32 128 256 2 16 16 8 8 16 16 16</td>
</tr>
<tr>
<td>G808N1B1</td>
<td>32 2 1 0.5 1 1 32 64 8 16 64 32 32</td>
</tr>
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</table>

Genotyping was performed on an Illumina MiSeq platform (Illumina Inc., USA) with 100x coverage. CLC Genomics Workbench version 10 (CLC, Bio-)

Nitrofurantoin
Trimethoprim
Fusidic acid
Tigecycline
Doxycycline
Tetracycline
Vancomycin
Teicoplanin
Linezolid
Clindamycin
Erythromycin
Moxifloxacin
Ciprofloxacin
Tobramycin
Gentamicin
Amikacin
Cefoxitin

QIAGEN, Denmark) and SPAdes[6] version 3.5 were used for de novo assembly. The bacterial analysis pipeline of GoSeqlt tools was also used to annotate and identify known acquired antibiotic-resistant genes via ResFinder, virulence factors using VirulenceFinder[7] and mobile genetic elements through PlasmidFinder.[8] The multi-locus sequence type was also determined from WGS data using eight housekeeping genes (gapA, infB, mdh, pgI, phoE, rpoB and tetM).

The two MRSH, G808N1B1 (GenBank accession number PGW000000000) and A109N1B1 (GenBank accession number PGWY000000000), 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 mecA together with several resistant determinants to non-beta-lactam antibiotics, notably to aminoglycosides (ant(6)-Ia, aac(6’)-aph(2′)), aac(3’)-Ia, MLS (tmsrA), fusID (fusB), MLS (tmsrA), mepA, vanB (van), mcr1, Tca (TcaS, TcaB) (Table 1). The multidrug and toxin extrusion (MATE) transport protein, mepA, encoding for resistance to tigecycline, the multidrug exporter AcrB and mexE from the resistance-nodulation-cell division family, the MDR efflux pump (qacA) and small MDR family (qac) were additionally detected (Table 1). Additionally, a plasmid (RepA(VRSPapp)) 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. 16-12-16, sub-study of BCA444/16). Permission to conduct the research was also granted by the provincial Department of Health, uMgungundlovu District and hospital managers.

References

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Author contributions. RCF co-conceptualised the study, undertook sample collection, laboratory and statistical analyses, prepared tables and figures, interpreted results and drafted the manuscript. LF undertook sample collection and laboratory analyses and vetted the results. MA contributed to bioinformatics analyses. AI undertook whole-genome sequencing. SYE co-conceptualised the study, undertook vetting of the results and critically reviewed the manuscript. All authors read and approve the manuscript.

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