Coagulase negative staphylococci (CoNS) have emerged as important pathogens causing hospital-acquired infections. In recent years, the clinical significance of CoNS species, other than *Staphylococcus epidermidis* has been recognized in many hospitals (1). Almost all methicillin resistant CoNS exhibit cross-resistance to other non-beta lactam antibiotics (2). In addition, studies have reported reduced glycopeptide susceptibility in CoNS (3).

*Staphylococcus haemolyticus* is the second most frequently isolated CoNS from clinical cases and is known for its propensity to exhibit multidrug resistance (MDR) (4).

Linezolid is an important alternative for the management of MDR-CoNS infections. Linezolid inhibits bacterial protein synthesis by binding to the peptidyl transferase center, interfering with the positioning of aminoacyl-tRNA on the ribosome (5). However, in recent years, linezolid resistance has increasingly been described in CoNS. Three different mechanisms of linezolid resistance have been described, including (i) mutation in the domain V of the 23S rRNA gene, (ii) acquisition of plasmid mediated *cfr* (chloramphenicol florfenicol resistance) gene, and (iii) mutation in the ribosomal L3/L4 protein (6–8). In India, the first case of linezolid resistant *S. haemolyticus* was reported in 2012 (9). Notably, the multiple mechanisms involved in linezolid resistance by *S. haemolyticus* were also documented (7,8).

Here, we report three linezolid resistant *S. haemolyticus*, VB5326, VB19458, and VB840, recovered from clinical specimens. Identification was performed using matrix-assisted laser desorption/ionization (MALDI-TOF MS) and the conventional coagulase test. Minimum inhibitory concentration (MIC) of linezolid was determined using the broth dilution method (BMD). A single bacterial colony of each strain grown on blood agar was used for genome sequencing. DNA was isolated from the pure cultures using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). Whole-genome shotgun sequencing was performed using an Ion Torrent PGM™ System (Life Technologies, Waltham, MA, USA) with a 400-bp chemistry kit. The raw data generated were assembled de novo using SPAdes Genome Assembler v.5.0.0.0 (10) embedded in the Torrent suite server v.5.0.4.

Additionally, MinION sequencing was performed on *cfr* positive strains (VB5326 and VB19458). A single contig of VB5326 and VB19458 complete chromosome generated from MinION sequencing, was error-corrected with Ion torrent reads (11). The raw data generated were assembled de novo using SPAdes Genome Assembler v.5.0.0.0 (10) embedded in the Torrent suite server v.5.0.4.
Rifampicin and Linezolid Resistant S. haemolyticus

Table 1. Genome characteristics, mechanism of linezolid and rifampicin resistance in S. haemolyticus isolated from clinical specimens.

| Genome feature | VB5326 | VB19458 | VB840 |
|----------------|--------|---------|-------|
| Linezolid MIC (µg/ml) | > 256 | > 256 | 8 |
| Gene bank accession no | CP045137.2 | CP045187.2 | QYYYK0000000 |
| Genome length (bp) | 2,699,292 | 2,699,210 | 2,464,353 |
| Genome coverage | 200X | 200X | 112X |
| No. of contigs | single | single | 145 |
| No. of predicted coding sequences (CDS) | 2,399 | 2,417 | 2,503 |
| No. of predicted rRNA | 62 | 62 | 55 |
| No. of predicted tRNA | 19 | 19 | 10 |
| SCCmec type | SCCmec V | SCCmec IV | SCCmec V |
| Sequence type | ST1 | ST3 | ST30 |
| Antimicrobial resistant genes | blaZ, mecA, aac(6')-aph(2''), ant (6)-la, aph(3')-III, erm (C), Isa (B), cfr, msr (A), mph (C), fuscB, dfpG | blaZ, mecA, aac(6')-aph(2''), ant (6)-la, aph(3')-III, cfr, fuscB, Isa (B), cfr, erm (C), dfpG | blaZ, mecA |
| Mutation in domain V of 23S rRNA gene | G2576T | G2576T | G2576T |
| rplC (L3) | R138V | R138V | - |
| rpoB | D471E, I527M, S532N | D471E, I527M, S532N | - |

with Pilon to reduce the base level errors in long read assembly. The genome sequence was annotated using PATRIC, the bacterial bioinformatics database and analysis resource (http://www.patricbrc.org) (12), and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). A comparative genomic analysis of VB5326 and VB19458 was performed against the reference genome of S. haemolyticus JCSC 1435 (accession no. AP006716.1), visualized using CGView Server.

Downstream analysis was performed using the Centre for Genomic Epidemiology (CGE) server (http://www.cbs.dtu.dk/services) and PATRIC. From the CGE server, staphylococcal cassette chromosome (SCC) mec type was identified using SCC mec finder (https://cge.cbs.dtu.dk/services/SCCmecFinder); resistance gene profile was analyzed using ResFinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder/); sequence types (ST) were determined in the allele order of mec, cfxE, cfr, msr, fusB, dfrG, and vbG. Two isolates (VB5326 and VB19458) harbored multiple antimicrobial resistant genes and showed similar multidrug resistant phenotype, exhibiting resistance to cefoxitin, gentamicin, trimethoprim-sulfamethoxazole, rifampicin, erythromycin, clindamycin, chloramphenicol, and linezolid. All the three strains were susceptible to tetracycline, minocycline, vancomycin (1 µg/ml), and teicoplanin (4 µg/ml). The blaZ gene was identified in the Tn552 transposons. A plasmid, rep15, was identified in both VB5326 and VB19458 and was integrated into the chromosome. None of the isolates harbored sel and pvl genes, and the biofilm-associated genes ica, a, b, c, d, e, and f.

All the three isolates were found with the mutation G2576T, at the domain V of the 23S rRNA. In addition, two isolates (VB5326 and VB19458) carried a copy of the cfr gene on the chromosome (Fig. 1). Notably, a novel mutation, R138V, identified in rplC (L3) was predicted with a SIFT score of < 0.01, which potentially affects the function of protein. Two isolates (VB5326 and VB19458) were resistant to rifampicin with an MIC of > 32 µg/ml, which carried mutations D471E, I527M, and S532N in the rpoB gene. A similar genetic environment was observed in the two cfr-positive isolates shown in Fig. 2. The genome sequences were deposited at the DDBJ/ENA/GenBank under the accession numbers mentioned in Table 1.

Linezolid resistant S. haemolyticus has been documented in the clinical isolates and outbreaks in healthcare settings (14). Resistance to linezolid was primarily reported with a mutation, G2576T, in the 23S rRNA. Successively, several mutations including T2500A, G2603T, C2534T, T2504A, G2447T, G2215A, and G2631T have been reported in the 23S rRNA of linezolid-resistant strains (15). S. haemolyticus carrying the cfr gene, which encodes methyltransferase that
catalyzes the post-transcriptional methylation of the nucleotide A2503 in the 23S rRNA, has been described in a clinical isolate (6). Seven cfr-carrying plasmids, pSCFS1, pSCFS3, pSCFS6, pBS-01, pSS-01, pSS-02, and pSS-03, have been described previously (16). In addition, insertion sequences, including IS21-558, IS1216, IS256, ISEnfa4, and IS26, located in close proximity to cfr, may contribute to the dissemination of linezolid resistance (15).

Few cases of linezolid-resistant CoNS have been reported from India (7–9,17,18). The clonal spread of linezolid-resistant S. haemolyticus harboring cfr gene and mutation in 23S rRNA have also been reported (7,8,17). We studied the linezolid resistance mechanism in S. haemolyticus isolated from a clinical specimen. Consistently, this study showed the presence of multiple mechanisms of linezolid resistance in S. haemolyticus. These multiple resistance mechanisms could contribute to high-level linezolid resistance than G2576T mutation in 23S rRNA alone. S. haemolyticus belonging to ST1 and ST3 were reported as the predominant STs in India (19). Our observation of the cfr gene in these predominant STs is worrisome. Increasing clinical significance of S. haemolyticus is causing invasive infections and resistance to linezolid, which greatly limits the management of MDR infections.

In conclusion, this is the first report on the draft genome sequence of linezolid resistant S. haemolyticus from India. Our study shows the presence of cfr-mediated linezolid resistance in S. haemolyticus. This emphasizes the need for continuous surveillance, judicious use of antibiotics, and effective infection control strategy to prevent the emergence of resistance.

Conflict of interest None to declare.

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Rifampicin and Linezolid Resistant *S. haemolyticus*

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