

Novel Observations in 11 Heteroresistant Vancomycin-Intermediate Methicillin-Resistant *Staphylococcus aureus* Strains from South India

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We report here the draft genome sequences of 11 heteroresistant vancomycin-intermediate *Staphylococcus aureus* (hVISA) strains from bloodstream infection. All strains harbor mutations in *vraSR*, *graSR*, *walkR*, and/or *tcaRAB* and are often implicated as the frequently mutated candidate genes in hVISA phenotypes.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause serious community- and nosocomial-acquired infection. The use of vancomycin in the treatment of MRSA infection is challenged by the emergence of heteroresistant vancomycin-intermediate *S. aureus* (hVISA) and vancomycin-intermediate *S. aureus* (VISA). Several studies have reported the increasing frequency of hVISA/VISA-associated treatment failures in invasive infections (1, 2).

Here, we present the draft genome sequences of 11 hVISA strains isolated from bloodstream infections. All the isolates were found to have vancomycin MICs of 1 to 1.5 $\mu\text{g/ml}$. All these hVISA strains were confirmed with a population analysis profile-area under curve (PAP-AUC) method with the following PAP-AUC ratios: VB988, 1.03; VB9939, 0.92; VB16578, 0.96; VB20017, 1.0; VB44094, 0.97; VB44746, 1.33; VB46389, 1.03; VB35316, 0.96; BA43011, 1.28; *Staphylococcus aureus* strain 2016, 1.02; and VB1490, 1.0.

DNA isolation from pure cultures was performed using QIAamp DNA minikit (Qiagen, Germany). Whole-genome shotgun sequencing was performed using the Ion Torrent PGM system (Life Technologies, Inc.) with 400-bp chemistry. The raw data generated were assembled *de novo* using the assembler SPAdes version 5.0.0.0 embedded in Torrent suite server version 5.0.4. The genome sequence was annotated using PATRIC, the bacterial bioinformatics database and analysis resource (<http://www.patricbrc.org>) (3), and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/) (4). Downstream analysis was performed using the Center for Genomic Epidemiology (CGE) server (<http://www.cbs.dtu.dk/services>) and PATRIC. The resistance gene profile was analyzed using ResFinder 2.1 from the CGE server (<https://cge.cbs.dtu.dk/services/ResFinder/>) (5). The CRISPR finder (<http://crispr.u-psud.fr/Server/>) was used to detect and identify clustered regularly interspaced short palindromic repeat (CRISPR) and spacer sequences in the genome. The sequence type was determined for all the isolates in the allele order of *arcc*, *aroe*,

glpf, *gmk*, *pta*, *tpi*, and *yqil* by comparing the sequences with *S. aureus* database maintained at the MLST website (<http://saureus.mlst.net/>).

The annotated genome size of MRSA isolates ranged from ~2.7 to ~2.9 Mb, with coverages of 33 \times to 88 \times (Table 1). The number of coding DNA sequences (CDSs) per genome ranged from 2,603 to 3,011. Genome annotation by PATRIC predicted a total of 41 to 60 tRNAs and five to 10 rRNAs in the sequenced isolates (Table 1). All the isolates were found to harbor various toxin and antimicrobial resistance genes. Further, none of the isolates were found to have CRISPR regions.

Infection with hVISA/VISA has been associated with high vancomycin MIC and poor clinical outcome. The most frequently mutated two-component system (TCS) determinants are *vraSR*, *graSR*, and *walkR*, and the *rpoB* gene (6). This chromosomal mutation leads to the upregulation of peptidoglycan biosynthesis and cell wall thickening and further prevents vancomycin from reaching its target (7). Multiple nonsynonymous mutations were seen in the *vraSR*, *graSR*, and *walkR* TCSs of all sequenced isolates. In addition, a mutation was seen in a teicoplanin resistance-associated (*tcaRAB*) operon. A mutation in the *rpoB* gene was not observed in any of the sequenced isolates.

Taken together, comparative genomic analysis of these sequenced isolates revealed preferential clustering of single nucleotide polymorphisms (SNPs) in hVISA candidate genes with high diversity across the loci of *vraSR*, *graSR*, *walkR*, and *tcaRAB*.

Accession number(s). The draft genome sequences have been deposited in DDBJ/ENA/GenBank under the accession numbers as provided in Table 1.

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TABLE 1 Genome characteristics of hVISA isolates from bloodstream infection

Isolate ID	Accession no.	Draft genome size (Mbp)	No. of CDSs	No. of contigs	No. of tRNAs	No. of rRNAs	Coverage (×)	Virulence genes ^a	Resistance genes	ST/SCCmec/spa type ^b
VB9882	MLQI00000000	2.77	2,692	132	60	10	79	<i>aur, scn, sak, seq, sen, seu, sei, sem, seo, seg, hlb, hlgB, hlgC, hlgA, lukS-PV, lukF-PV</i>	<i>aadD, acc(6')-aph(2''), mecA, blaZ, norA</i>	2371/II/t6827
VB9939	MLQK00000000	2.83	2,803	437	48	7	33	<i>aur, scn, seq, sem, seg, seo, sec3, sel, hlb, hlgB, hlgC, hlgA, lukS-PV, lukF-PV, sen, seu, sea/sep</i>	<i>ant(6)-la, aph(3')-III, acc(6')-aph(2''), spc, mecA, blaZ, norA, mph(C), msr(A), dfrG</i>	772/V/t657
VB16578	MLQD00000000	2.79	2,667	143	59	9	61	<i>aur, scn, seq, sen, seu, sei, sem, seo, seg, sec3, sel, hlb, hlgB, hlgC, hlgA, lukS-PV, lukF-PV</i>	<i>ant(6)-la, aph(3')-III, acc(6')-aph(2''), spc, mecA, blaZ, norA, mph(C), msr(A), dfrG</i>	772/V
VB20017	MLQE00000000	2.85	2,916	420	41	5	34	<i>aur, scn, sak, seq, sen, seu, sei, sem, seo, seg, hlb, hlgB, hlgC, hlgA, lukS-PV, lukF-PV</i>	<i>aadD, acc(6')-aph(2''), mecA, blaZ, norA, ermC</i>	2371/VI t6827
VB44094	MLQH00000000	2.77	2,683	110	57	10	79	<i>aur, scn, sak, seq, sen, seu, sei, sem, seo, hlb, hlgB, hlgC, hlgA, lukS-PV, lukF-PV</i>	<i>acc(6')-aph(2''), mecA, blaZ, norA, ermC</i>	22/IVc/t474
VB44746	MLQF00000000	2.84	2,818	265	50	8	41	<i>splA, splE, aur, scn, sak, lukD, luke, hlb, hlgB, hlgC, hlgA, eta</i>	<i>blaZ, norA, ermC</i>	1290/IVh/t131
VB46389	MLQG00000000	2.80	2,700	235	50	7	42	<i>aur, scn, sea/sep, seg, seq, sen, seu, sei, sem, seo, seg, sec3, sel, hlb, hlgB, hlgC, hlgA, lukS-PV, lukF-PV</i>	<i>ant(6)-la, aph(3')-III, acc(6')-aph(2''), mecA, blaZ, mph(C), msrA, norA, dfrG</i>	772/V/t458
VB35316	MLQC00000000	2.77	2,638	88	59	9	85	<i>splB, splA, splE, aur, scn, sak, lukD, luke, seg, sen, seu, sei, sem, seo, hlb, hlgB, hlgC, hlgA</i>	<i>acc(6')-aph(2''), mecA, blaZ, norA, dfrG</i>	72/III/V/t2473
VB43011	MLQJ00000000	2.77	2,604	67	59	8	76	<i>splB, splA, splE, aur, scn, sak, sea/sep, seb, seq, sek, seh, lukD, luke, hlb, hlgB, hlgC, hlgA</i>	<i>ant(6)-la, aph(3')-III, blaZ, mphC, msrA, norA,</i>	1/V/t127
<i>Staphylococcus aureus</i> strain 2016	MLQA00000000	2.72	2,603	85	60	9	88	<i>splA, splE, aur, scn, sak, lukD, luke, hlb, hlgB, hlgC, hlgA</i>	<i>blaZ, norA</i>	580/II/t4615
VB1490	MLQB00000000	2.97	3,011	143	58	8	64	<i>splB, splA, splE, aur, scn, sak, seq, sek, lukD, luke, hlb, hlgB, hlgC, hlgA</i>	<i>ant(6)-la, aph(3')-III, acc(6')-aph(2''), spc, mecA, blaZ, norA, ermA, tet(M)</i>	239/III/V/t037

^a PV, Pantone-Valentine.^b ST, sequence type; SCCmec, staphylococcal cassette chromosome mec element.

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