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Article

Genome Sequences of Three Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from Subclinical Mastitic Cows

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine milk poses a significant zoonotic and antimicrobial resistance threat. This study identifies three MRSA strains from subclinical mastitic (SCM) cow milk, carrying resistance genes to β -lactams, lincosamides, macrolides, streptogramin B, and aminoglycosides, underscoring the urgent need for improved hygiene and surveillance in dairy production.

Keywords: *Staphylococcus aureus*; MRSA; multidrug resistance; mastitic cow; antimicrobial resistance

Methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine milk poses a significant threat to both animal and public health due to its antimicrobial resistance and zoonotic potential (1). Its presence in raw and pasteurized milk increases the risk of clinical management and human transmission, especially among those in close contact with dairy animals or consuming unpasteurized products (2, 3). These concerns underscore the urgent need for improved hygiene practices and robust surveillance in the dairy industry to mitigate MRSA spread. Three subclinical mastitis (SCM) milk samples were collected from the Cumilla district, Bangladesh, and inoculated on 5% bovine blood agar followed by subculture of characteristic colony on Mannitol Salt Agar with incubation at 37 °C for 24 h. *Staphylococcus aureus* was confirmed by subsequent Gram staining, biochemical, and *nuc* gene specific PCR. Antimicrobial sensitivity profiling using twelve antimicrobials representing different classes including oxacillin and cefoxitin resulted in multidrug resistance. Genomic DNA was extracted from 2 to 3 isolated colony on 5% bovine Blood Agar using an AllPrep Bacterial DNA/RNA/Protein Kit (Qiagen, Hilden, Germany), and purity was assessed with a Nanodrop One (Thermo Fisher Scientific, MA, USA). DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, CA, USA). Genome sequencing was conducted on an Illumina NextSeq2000 platform generating 2 × 150 bp paired end reads. Raw data quality, including read quality, GC content, and adapter contamination, was assessed using FastQC v0.12.1

(4). Trim Galore v0.6.5dev (5) was employed to trim low-quality bases and adapter sequences, followed by normalization of read coverage with bbnorm v39.08 (6) to reduce redundancy. De novo genome assembly was performed using Unicycler v0.4.8 (7) with subsequent polishing using Pilon v1.24 (8) to enhance accuracy. Genome assembly quality was assessed using QUAST v5.2.0 (9), while Samtools v1.13 (10) was used for alignment file manipulation and indexing. The de novo genome assembly conducted through the BV-BRC (11) comprising the abovementioned tools yielded coverage ranging from 146× to 339×, with raw reads between 3,035,549 and 6,710,480. The assembled genomes were annotated using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (PGAP) v6.9 (12) and PATRIC (13) with the in-built RASTtk pipeline to identify the species, *Staphylococcus aureus*. Transport proteins were identified via TCDB (14), drug targets using DrugBank v6.0 (15), and additional analyses performed for CRISPR arrays and prophages using CRISPR CasFinder (16), plasmids using PlasmidFinder 2.1 (17) and integrative and conjugative elements using ICEFinder (18). All software parameters were set to default, unless specified otherwise. Detailed genomic characteristics are provided in Table 1.

Antimicrobial resistance genotypes were identified using ResFinder 4.6.0 (19). Acquired and chromosomal resistance genes were identified in all three MRSA strains. Methicilin resistant *mecA* gene and resistance to β-lactam antibiotics were present in all three strains. Additionally, resistance to lincosamide, macrolide, streptogramin b and aminoglycoside were found in BioB-3 and BioB-4 strains (Table 1). This study highlights the need for effective surveillance and control measures to prevent the spread of MRSA within dairy herds and to humans.

Table 1. Genomic features of the sequenced three *Staphylococcus aureus* strains isolated from subclinical mastitic cows in Cumilla, Bangladesh.

Strain name	BioB-2	BioB-3	BioB-4
Information title			
Species	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>
Genome submission ID	SUB14894378	SUB14894378	SUB14894378
Bioproject ID	PRJNA1190104	PRJNA1190104	PRJNA1190104
BioSample accession number	SAMN45025667	SAMN45025668	SAMN45025669
SRA accession number	SRS23333889	SRS23333890	SRS23333891
Genome assembly accession number	GCA_047604675.1	GCA_047604515.1	GCA_047604495.1
GenBank accession number	IBJLRB000000000	IBJLRA000000000	IBJLQZ000000000
FastQC Phred score	34	34	34
Raw reads	6,710,480	4,498,755	3,035,549
Coverage	339x	219x	146x
Contig count	31	21	41
Coarse consistency (%)	100	100	100
Fine consistency (%)	99.7	99.7	99.7
Completeness (%)	100	100	100
Contamination (%)	0	0	0
Genome size (bp)	2,761,071	2,782,581	2,797,289
Contigs N50 (bp)	219,219	329,157	137,483
Contigs L50	5	4	5
Largest contig (bp)	402,935	536,524	380,235
Guanine–cytosine content (%)	32.75696	32.72947	32.637028
Genes (Total), PGAP	2,763	2,791	2,799
CDS (PGAP; PATRIC)	2,641; 2,606	2,668; 2,624	2,634; 2,670
tRNA	55	55	57
rRNA	4	4	4
CDS ratio	0.9438366	0.9430094	0.9544956

Hypothetical CDS	510	507	568
Hypothetical CDS ratio	0.2943208	0.29115853	0.31722847
PLFAM CDS	2,540	2,557	0
PLFAM CDS ratio	0.9746738	0.97446644	-
Hypothetical proteins	513	507	570
Proteins with functional assignments	2,093	2,115	2,100
Proteins with EC number assignments	735	737	740
Proteins with GO assignments	607	609	613
Proteins with pathway assignments	532	533	536
Proteins with subsystem assignments	975	973	974
Proteins with PATRIC genus-specific family (PLfam) assignments	2,540	2,557	0
Proteins with PATRIC cross-genus family (PGfam) assignments	2,573	2,593	2,629
Proteins with FIGfam assignments	0	0	0
Transporter (TCDB)	98	99	100
Drug target (DrugBank)	33	36	34
Antibiotic resistance genes (PATRIC)	39	40	40
ResFinder 4.6.0 (acquired; <i>str</i> , <i>mecA</i> ; 23S, <i>dfrB</i> , <i>fusA</i> , <i>grrA</i> , <i>grrB</i> , <i>gyrA</i> , <i>ileS</i> , <i>pbp2</i> , <i>pbp4</i> , <i>rpoB</i> , <i>pbp4-promoter</i>)	<i>mecA</i> , <i>blaZ</i> , <i>erm(C)</i> ; 23S, <i>dfrB</i> , <i>fusA</i> , <i>grrA</i> , <i>grrB</i> , <i>gyrA</i> , <i>ileS</i> , <i>pbp2</i> , <i>pbp4</i> , <i>rpoB</i> , <i>pbp4-promoter</i>	<i>mecA</i> , <i>blaZ</i> ; 23S, <i>dfrB</i> , <i>fusA</i> , <i>grrA</i> , <i>grrB</i> , <i>gyrA</i> , <i>ileS</i> , <i>pbp2</i> , <i>pbp4</i> , <i>rpoB</i> , <i>pbp4-promoter</i>	<i>mecA</i> , <i>blaZ</i> ; 23S, <i>dfrB</i> , <i>fusA</i> , <i>grrA</i> , <i>grrB</i> , <i>gyrA</i> , <i>ileS</i> , <i>pbp2</i> , <i>pbp4</i> , <i>rpoB</i> , <i>pbp4-promoter</i>
AMR phenotype class	β-lactam	β-lactam, lincosamide, macrolide, streptogramin b	β-lactam, aminoglycoside
Plasmid finder 2.1	rep7a (contig23: 2056..2895)	rep5a (contig13: 16621..17481), rep16(contig13: 18772..19515), rep10(contig18: 1881..2356)	repUS5(contig21: 33881..35455), rep21(contig21: 4961..5956)
Integrative and conjugative elements (ICEs)	1(2745519..2754895, 9377 bp)	1(2111899..2114925, 3027 bp)	2 (2759741..2794168, 34428bp; 1058307..1163904, 105598 bp)
No. of clustered interspaced short repeats (CRISPR) (number of cas clusters) (name of cas genes)	4(1) (cas4_Type IA)	6(1) (cas4_Type IA)	8(1) (cas3_Type I)

Data Availability: The accession numbers for the genomes of the three *Staphylococcus aureus* strains are shown in Table 1. All genomes are available under BioProject accession number [PRJNA1190104](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1190104).

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