

RESEARCH ARTICLE

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Antimicrobial Resistance and Major Virulence Gene Detection in Methicillin Resistant *Staphylococcus aureus* in Humans and Livestock Animals of Assam: A North Eastern State of India

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Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is highly divergent antibiotic resistant bacteria earmarked as “High” in global pathogens’ priority list varying the severity and resistance geographically. Here, MRSA were screened using *mecA* gene with Cefoxitin and other 27 antibiotics of 19 classes using disc diffusion method from a highly humid climate of India. Multiple Antibiotic Resistance (MAR) index was calculated. Minimum Inhibitory Concentration (MIC) was determined against 11 classes of antibiotics. Detection of major virulence genes *tst-1* and *lukPV* were done. A total of 95.24% Hospital Associated (HA)-MRSA, 56.14% Community Associated (CA)-MRSA and 82.53 % Livestock Associated (LA)-MRSA were detected. Cefoxitin, Oxacillin, Ciprofloxacin, Fusidic acid and Ticarcillin-Clavulanic acid resistance was observed in more than 60% of HA-MRSA, CA-MRSA and LA-MRSA. Across the hosts, Mupirocin, Gentamicin, Linezolid, Co-trimoxazole, and Rifampicin were found effective. Vancomycin Intermediate *Staphylococcus aureus* (VISA) detected in CA-MRSA & LA-MRSA. Multidrug Resistant (MDR) was found very high but extensively drug-resistant (XDR) was detected moderately. No pan drug-resistant (PDR) was detected. Virulence gene *tst-1* and *lukPV* were detected in 7.69% and 32.69% MRSA isolates. The gene *tst-1* is reported for the first time in pre and post-caesarian samples from Gynaecology department in this region with high MDR. This study showed *S. aureus* and subsequent prevalence of MRSA is higher in this region than national data. 2nd generation Cephalosporins were found effective which is very encouraging due to their limited uses. Detection of *tst-1* in caesarian samples is a serious threat as neonatal transmission of MRSA from mother is reported.

Keywords: MRSA, OS-MRSA, BORSA, VISA, XDR, MDR, MAR Index

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Citation: Deka NK, Handique PJ, Borah P, et al. Antimicrobial Resistance and Major Virulence Gene Detection in Methicillin Resistant *Staphylococcus aureus* in Humans and Livestock Animals of Assam: A North Eastern State of India. *J Pure Appl Microbiol.* 2023;17(2):951-965. doi: 10.22207/JPAM.17.2.25

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INTRODUCTION

Drug resistant disease causes almost 700,000 deaths every year globally.¹ *Staphylococcus aureus* is classified as an important pathogen to be studied for Anti-Microbial Resistance (AMR). It is one of the 6 pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) tagged as Critical and High importance by WHO and eponymously called as “ESKAPE”.²⁻³ MRSA is classified under the “serious threat” category by CDC in their “Antibiotic resistance threats in the united states-2019” report. The anterior nares are the most frequent site for *S. aureus* colonization and nasal carriage of *S. aureus* is an important risk factor for bacteremia and subsequent death. Carriers have higher rates of nosocomial *S. aureus* bacteraemia than non-carriers and a large proportion of nosocomial *S. aureus* infections originates from the patients’ own flora.⁴ Currently, Oxacillin-resistant staphylococci are resistant to all available β -lactam antimicrobial agents except some newer Cephalosporins. Vancomycin has been widely used in the treatment of MRSA infection for the past two decades.⁵ The majority of MRSA strains have remained susceptible to Vancomycin at the current Minimum Inhibitory Concentration (MIC) susceptibility breakpoint designated by the Clinical Laboratory Standards Institute (CLSI).

Antimicrobial resistance in Asia Pacific region is literary a global problem being the fact that more than 70% of world population lives here. Asian countries have shown very high rates (> 50%) of occurrence of MRSA.⁶ India has a large burden of infectious diseases and is one among the largest consumers of antibiotics in the world. Resistance was mostly found against Penicillin, Mupirocin, and Ciprofloxacin and greater susceptibility was observed towards Gentamicin and Co-trimoxazole. Vancomycin intermediate *S. aureus* (VISA) strains were also found to be very minimal.⁷ Studies have shown that geographically North India and East India have a greater burden of HA-MRSA then West and South India.⁸ In the same perspective, North East India needed to be studied separately being geo-climatically different from the rest of India. On the other hand, a few earlier studies in

India detected higher frequency (5.4% –29.41%) of LA-MRSA in cattle and buffaloes with clinical mastitis⁹ and LA-MRSA is needed to be studied with a greater range of antibiotic classes.

Assam, a state of India, is characterized by alternate cool and warm periods with high humidity. As per Thornthwaite’s classification, Assam falls in “pre-humid” (moisture index I_m 100 or more) and “humid” (moisture index I_m between 20 to 100) climate zone.¹⁰ Assam has a temperature variation of 8°C-10°C in winter to up to 36°C in summer with a high humidity of 80%-85%, in average yearly. This region, as a whole, excluding the high mountain barriers, enjoys 'Cwg' or humid mesothermal Gangetic type of climate as per Koppen’s classification.¹¹

High temperature and high relative humidity promotes sweat production and hydration of stratum corneum of the skin. These two factors provide suitable environmental conditions for maximized growth of *S. aureus* on skin, specifically during summer days.¹² Increasing humidity was found to be associated with greater prevalence of MRSA and with Triclosan effectiveness found to be very less in humid and wet conditions against *Staphylococcus aureus* infections.¹³

India’s Antimicrobial Resistance Surveillance and Research Network by ICMR has already recognized six pathogenic groups and staphylococci (MRSA) is one among them. ICMR has emphasized on generating regional data focusing on One Health approach. Still, community data, surveillance on animals and data on antibiotics used in different regions of the country are missing so far. Hence, ICMR has asked researchers to gather antibiotic resistance data from livestock and poultry.¹⁴

Panton Valentine Leukocidin (*Luk PV*) is a multicomponent protein which severely damage the cell membrane by forming a hetero oligomeric transmembrane pore.¹⁵ Toxic Shock Syndrome (TSS) is a rare disease caused by a potentially lethal toxin characterized by high fever, hypotension and subsequent multiple organ dysfunction. This toxin is encoded by *tst-1* (Toxic shock syndrome toxin) gene.¹⁶ Though earlier it was believed to be a disease of menstruating female, later it was also found in non-menstruating females and males.¹⁷ The toxin is hence divided into two distinct entities as “m-TSS” (menstruating Toxic Shock Syndrome)

and “non-mTSS”(non-menstruating Toxic Shock Syndrome).

Prevalence of MRSA in human and animals and their antibiotic resistance patterns are poorly studied in Assam with variable data reported in limited number of studies. In fact, AMR studies involving various antibiotics classes with a view to identify multidrug-resistant (MDR), extensively-drug resistant (XDR) and pandrug-resistant (PDR) among *Staphylococcus aureus* field isolates have not been reported so far from the North Eastern region of India.¹⁸ Prevalence of *tst-1* and *lukPV* are also reported less and due to the climatic and environmental differences, it is expected to be different from the national data.

In the present study, samples were collected from community, hospital wards (surgery and gynaecology) and livestock animals with a view to assess the MRSA prevalence as well as their antibiotic resistance patterns with respect to their virulence gene characterization.

MATERIALS AND METHODS

Collection

In the present study, skin swabs from anterior nares and surgical wounds were collected from patients admitted in the clinical wards of Gynaecology and Surgery departments of two tertiary care hospitals viz. Silchar Medical College

and Hospital and Gauhati Medical College and Hospital in Assam, India with preference to surgical wound samples, if available. Samples were collected during the summer season of 2019 and 2021. Wound swabs of under treatment animals from Veterinary Clinical Complex of College of Veterinary Sciences, Khanapara, Guwahati, Assam, domesticated animals, meat samples from poultry slaughterhouse and bovine milk samples from farm animals were selected for LA-MRSA isolation. Anterior nare swabs from communities and healthy human beings were collected as per CDC guidelines for CA-MRSA isolation. Except for slaughterhouse and milk samples, other samples were collected with sterile cotton swab (Hi-Media, PW009) in aseptic condition. Individual bovine milk samples were collected in sterile screw cap container (Hi-Media, PW126) directly from teats while milking and meat samples were aseptically collected from poultry slaughterhouses.

All swab samples were collected in sterile Cary Blair Transport Media w/o Charcoal (Sisco Research Lab, CM012) and processed within 48 hours of collection. All milk and meat samples were processed immediately after collection.

Isolation

The collected samples were inoculated in Peptone Water broth (Hi-Media, M614) followed by Mannitol Salt Agar (Hi-Media, MH118).

Table 1. Primer pairs used for amplification of different genes of *Staphylococcus aureus*

No.	Gene name	Primer details size	Amplicon temp	Annealing	Ref.
1.	<i>aroA</i>	5'-AAGGGCGAAATAGAAGTGCCG-3' 5'-ATTGGTTGAAGCATTGGTGT-3'	872 bp	52	Self-designed
2.	<i>coa</i>	5'-ACCACAAGGTAAGTCAACG-3' 5'-TCTTGGTCTCGCTTCATATCC-3'	211 bp	58	19
3.	<i>mecA</i>	5'-AGAAGATGGTATGTGGAAGTTAG-3' 5'-ATGTATGTGCGATTGTATTGC-3'	584 bp	57	21
4.	<i>nuc</i>	5'-AAGCGATTGATGGTGATACGGTT-3' 5'-CAAGCCTTGACGAACTAAAGC-3'	278 bp	50	19
5.	<i>16s RNA</i>	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-GGTACCTTGTACGACTT-3'	1513 bp	47	20
6.	<i>tst-1</i>	5'-TTATCGTAAGCCCTTTGTTG-3' 5'-TAAAGGTAGTTCTATTGGAGTAGG-3'	398 bp	60	21
7.	<i>lukPV</i>	5'-ATCATTAGGTAATGTCTGGA CATGATCCA-3' 5'-GCATCAASTGTATTGGATAG CAAAAGC-3'	433 bp	55	24

Suspected isolates were confirmed on Baird Parker Agar (Hi-Media, M043) with concentrated egg yolk solution (Hi-Media, FD045) and Potassium Tellurite (Hi-Media, FD047) as per manufacturer's instructions. Coagulase positivity were confirmed with Coagulase plasma (Hi-Media, FD248) as per manufacturer's instructions.

DNA isolation

Genomic DNA was isolated from overnight grown pure cultures of *S. aureus* using Presto™ Mini gDNA bacteria kit (Geneid, GBB300). Isolated DNA was quantified spectrophotometrically (Picodrop, Pico100). Average 300-500ng/μl DNA was obtained. Isolated DNA was immediately preserved at -20°C.

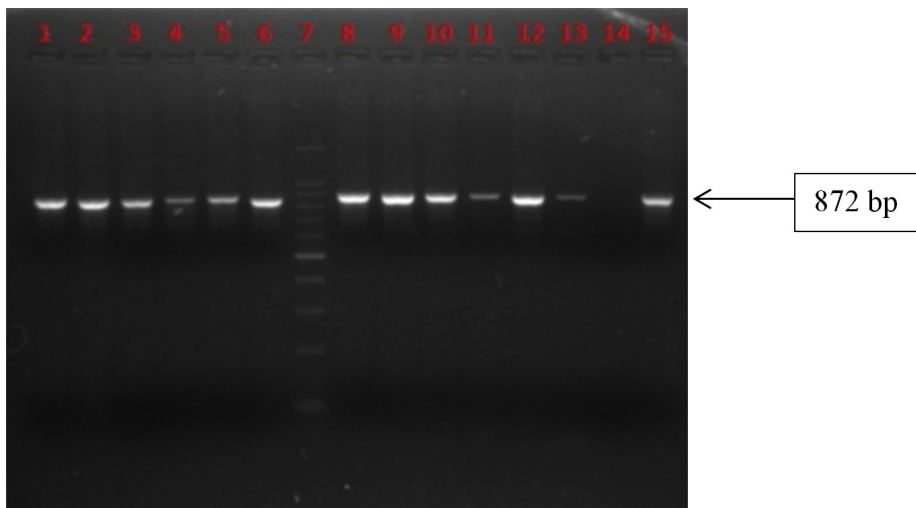


Figure 1. Amplification of *aroA* gene of *Staphylococcus aureus* by PCR
Lane 1-6 & 8-13: Isolates; Lane 7: 100 bp ladder; Lane 14: Negative control; Lane 15: Positive control (MTCC 96)

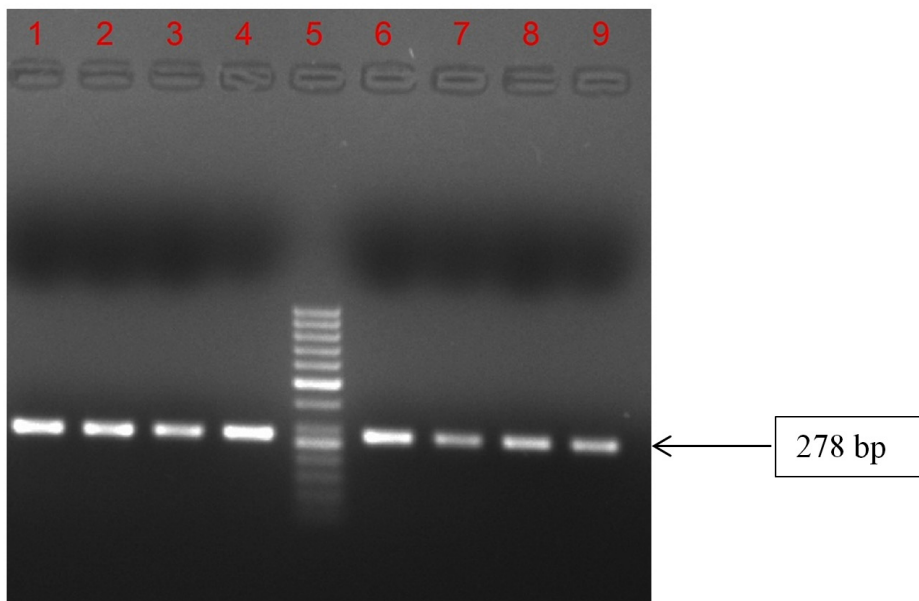


Figure 2. Amplification of *nuc* gene of *S. aureus*
Lane 1-4, 6-8: Isolates; Lane 5: 50 bp ladder

Molecular Confirmation

All suspected *Staphylococcus aureus* isolates were screened for *aroA* (Figure 1), *nuc* (Figure 2) and *coa* (Figure 3) genes for confirmation.

The primer set for *aroA* was designed in-house. A 50 µl PCR reaction mixture consisting of 1 µl each of 10 µM forward and reverse primers, 25µl of 2X Dream Taq PCR master mix (Thermo Scientific, K1071), 21 µl of nuclease free water (Thermo Scientific, AM9914G) and 2µl template DNA was prepared and PCR conditions were standardized at 95°C for 5 minutes followed by 35

cycles of 95°C for 1 minute, 52°C for 45 seconds and 72°C for 1 minute, and then 72°C for 5 minutes for final extension and 4°C for forever.

Primers and PCR conditions for *nuc* and *coa* gene (Table 1) were taken as reported.¹⁹ Annealing temperature for *nuc* was slightly modified using MTCC96 as control for standardization.

10 randomly selected isolates of *S. aureus* were subjected to *16s RNA* amplification following reported protocol²⁰ with slight modification of annealing temperature (Table 1). Gel products were purified with NucleoSpin® Gel and PCR Clean Up kit (Takara, 740609) and outsourced for

Table 2. MIC distribution percentage details of HA-MRSA isolates

Antibiotics	MIC (µg/ml) distribution percentage of 20 HA-MRSA isolates													
	<0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
FC			100											
RIF	100													
TET					65	35								
TMP					10	10	5				75			
VAN						90	10							
OXA				10			10	30	10	15	5	20		
LNZ							20	70	10					
TEI					60	40								
ERY					20	45	5		30					
PEN					10	15	15			10	50			
CLI			5	65	30									
FOX							15	15	40	30				

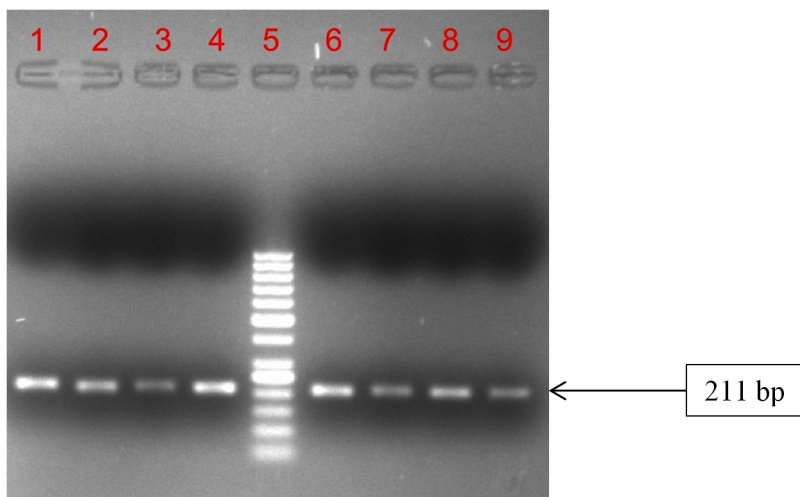


Figure 3. Amplification of *coa* gene of *S. aureus* by PCR
Lane 1-4, 6-8: Isolates; Lane 5: 50 bp ladder.

Table 3. MIC distribution percentage details of CA-MRSA isolates

Antibiotics	MIC ($\mu\text{g/ml}$) distribution percentage of 32 CA-MRSA isolates													
	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
FC		3.1	50.0	6.3	6.3	3.1	3.1	6.3	15.6	6.3				
RIF	56.3								43.8					
TET				28.1	59.4	9.4						3.1		
TMP							21.9	9.4	3.1		65.6			
VAN				3.1	15.6	40.6	21.9	6.3	12.5					
OXA			6.3	28.1	28.1	18.8		6.3		3.1	3.1		3.1	3.1
LNZ					3.1	9.4	40.6	34.4	12.5					
TEI					34.4	37.5	15.6	9.4	3.1					
ERY				9.4	9.4	18.8	18.8	9.4	15.6		9.4			9.4
PEN			3.1		3.1	9.4	9.4	28.1	12.5	15.6	18.8			
CLI	9.4	6.3	21.9	46.9	15.6									
FOX						28.1	43.8	18.8	6.3	3.1				

Table 4. MIC distribution percentage details of LA-MRSA isolates

Antibiotics	MIC ($\mu\text{g/ml}$) distribution percentage of 52 LA-MRSA isolates													
	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
FC		17.3	51.9	9.6	11.5	9.6								
RIF	92.3						1.9	3.8	1.9					
TET			3.8	17.3	40.4	28.8	1.9				1.9	5.8		
TMP					15.4	23.1	21.2	7.7			32.7			
VAN					9.6	25.0	38.5	19.2	7.7					
OXA			5.8	9.6	19.2	11.5	19.2	3.8	11.5	7.7	3.8			7.7
LNZ						13.5	50.0	30.8	5.8					
TEI					7.7	48.1	26.9	17.3						
ERY				3.8	23.1	21.2	25.0	3.8	5.8	9.6				7.7
PEN	3.8	1.9		11.5	19.2	7.7	15.4	7.7	19.2		13.5			
CLI			3.8	46.2	32.7	7.7	5.8				3.8			
FOX					1.9	23.1	23.1	21.2	19.2	5.8				5.8

Sanger Sequencing. Nucleotide BLAST (blastn) was performed with obtained sequences.

MRSA confirmation

Methicillin resistance was screened phenotypically with Oxacillin and Cefoxitin by disc diffusion method and genotypically with the screening of *mecA* gene (Figure 4). Bacterial turbidity of 0.5 McFarland constant was achieved with log phased growth of *S. aureus* isolates. The isolates were smeared on sterile Muller Hinton Agar plate using sterile swab (Hi-Media, PW003). Antibiotic discs were placed aseptically and first incubated at 35°C for 18 hours followed by another

6 hours at the same temperature to screen hetero-resistance. Zone of inhibition was measured for Cefoxitin and Oxacillin discs after 18 and 24 hours, respectively.

Reported primers and PCR conditions²¹ were used for *mecA* detection but it was found that MTCC96 is *mecA* negative. Hence, first three phenotypically Oxacillin and Cefoxitin resistant field samples were directly subjected to PCR amplification using the reported *mecA* primer and conditions. Amplified products were purified and sequenced to check the efficiency of the selected primer set to detect the presence of *mecA* gene. Nucleotide BLAST (<https://blast.ncbi.nlm>

nih.gov/Blast.cgi?LINK_LOC=blasthome&PAGE_TYPE=BlastSearch&PROGRAM=blastn) was performed with the obtained sequences.

Details of Antibiotics used for disc diffusion and MIC test were provided in Supplementary Table 2 and Supplementary Table 3, respectively. Interpretation was done according to CLSI guideline.²²

Multi Drug Resistance (MDR), Extensive Drug Resistance (XDR) and Pan Drug Resistance (PDR) were interpreted as defined²³ and reported accordingly.

Virulence gene characterization

All *mecA* positive isolates were screened for *lukPV*²⁴ (Figure 5) and *tst-1*²¹ (Figure 6) gene

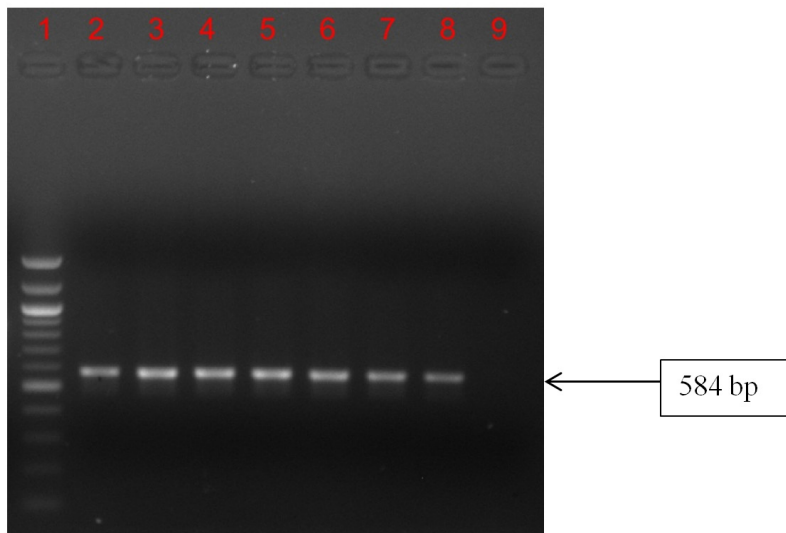


Figure 4. Amplification of *mecA* gene of MRSA isolates
Lane 1: 100 bp ladder; Lane 2-7: Isolates; Lane 8: Positive control; Lane 9: Negative control

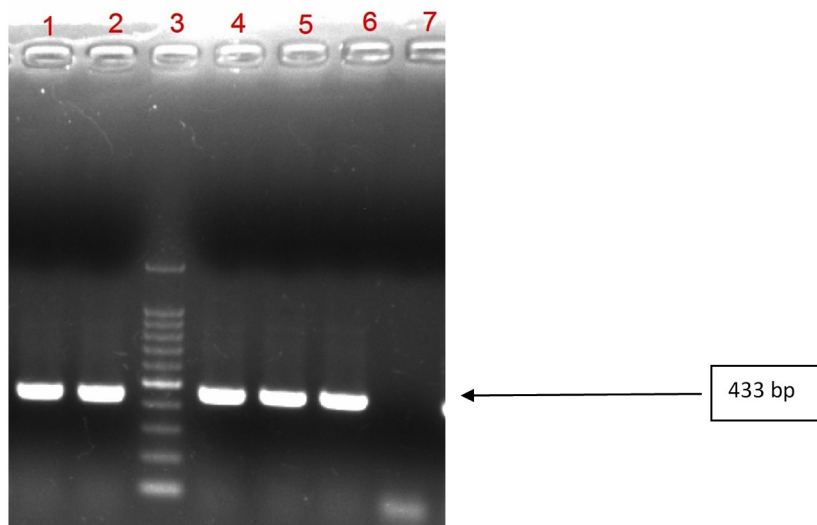


Figure 5. Amplification of *lukPV* gene of MRSA isolates
Lane 1,2,4,5: Isolates; Lane 3 : 100 bp ladder; Lane 6: Positive control (MTCC 96); Lane 7: Negative control

using reported primers. Details are provided in Table 1.

Statistical analysis

Chi-Square Test (χ^2) was performed to find the significance level of *Staphylococcus aureus* isolation from different sources. A value of $p < 0.01$ was accepted as statistically significant. MAR (Multiple Antibiotic Resistance) index was calculated as described.²⁵ A MAR index > 0.2 signifies the high risk contamination where several antibiotics are used for treatment or growth promotion, A MAR index < 0.2 indicates the use of less antibiotics. MAR index =1 indicates that isolates were resistant against all antibiotics screened.

RESULTS

A total of 611 samples were screened which included 154 hospital associated, 253 community associated and 204 livestock associated samples. Sequence similarity search by blastn with *16s RNA* sequences of the isolates clearly identified the coagulase-positive isolates as *Staphylococcus aureus* with $> 99\%$ similarity with identical isolates in the public database.

Details of sample isolation are provided in Supplementary Table 1. The Chi-square statistics of the three different types of sample source was

14.776. The p value was 0.000619. The result was found to be significant at $p < 0.01$.

Blastn result with obtained *mecA* sequence showed $> 99\%$ similarity with identical sequences of *S. aureus* in GenBank database. One of these sequences was used as positive control for further screening of other Methicillin resistant *S. aureus* (MRSA) isolates.

Depending upon phenotypic test and presence of *mecA* gene, the rate of occurrence of HA-MRSA was 95.24% (20/21) followed by 56.14%

Table 5. MAR indexing against antibiotics with respective no of isolates

No.	No. of Antibiotics resistant (a)	No. of Sample	No. of Antibiotics screened (b)	MAR index (a/b)
1.	2	2	28	0.071
2.	4	7	28	0.143
3.	5	7	28	0.179
4.	6	11	28	0.214
5.	7	15	28	0.250
6.	8	8	28	0.286
7.	9	12	28	0.321
8.	10	9	28	0.357
9.	11	13	28	0.393
10.	12	4	28	0.429
11.	13	8	28	0.464
12.	14	3	28	0.500
13.	15	3	28	0.536
14.	17	1	28	0.607
15.	23	1	28	0.821

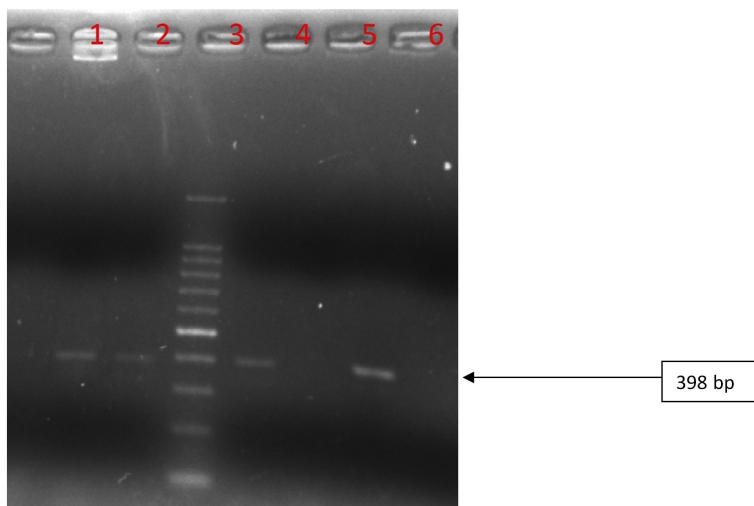


Figure 6. Amplification of *tst-1* gene of MRSA isolates
Lane 1,2,4: Isolates; Lane 3: 100 bp ladder; Lane 5: Negative control; Lane 6: Positive control

(32/57) CA-MRSA and 82.53% (52/63) LA-MRSA. The variation in the rate of occurrence was found to be significant ($p < 0.01$).

MRSA isolates resistant against different antibiotics are provided in Supplementary Table 4 & Figure 7.

All HA-MRSA isolates were Multi Drug Resistant (MDR) and 20% among them were Extensive Drug Resistant (XDR) with resistance against 10 to 11 antimicrobial classes.

MIC study revealed that except for Fusidic Acid and Rifampicin, other 10 antibiotics mostly fell within the MIC range of 0.5-16 μ g/ml. The Glycopeptides tested, viz. Vancomycin and Teicoplanin had MIC value between 1-2 μ g/ml and 0.5-1 μ g/ml, respectively. (Table 2).

It was found that 93.75% CA-MRSA were MDR and 10% among them were XDR with resistance shown against 10 to 17 antimicrobial classes.

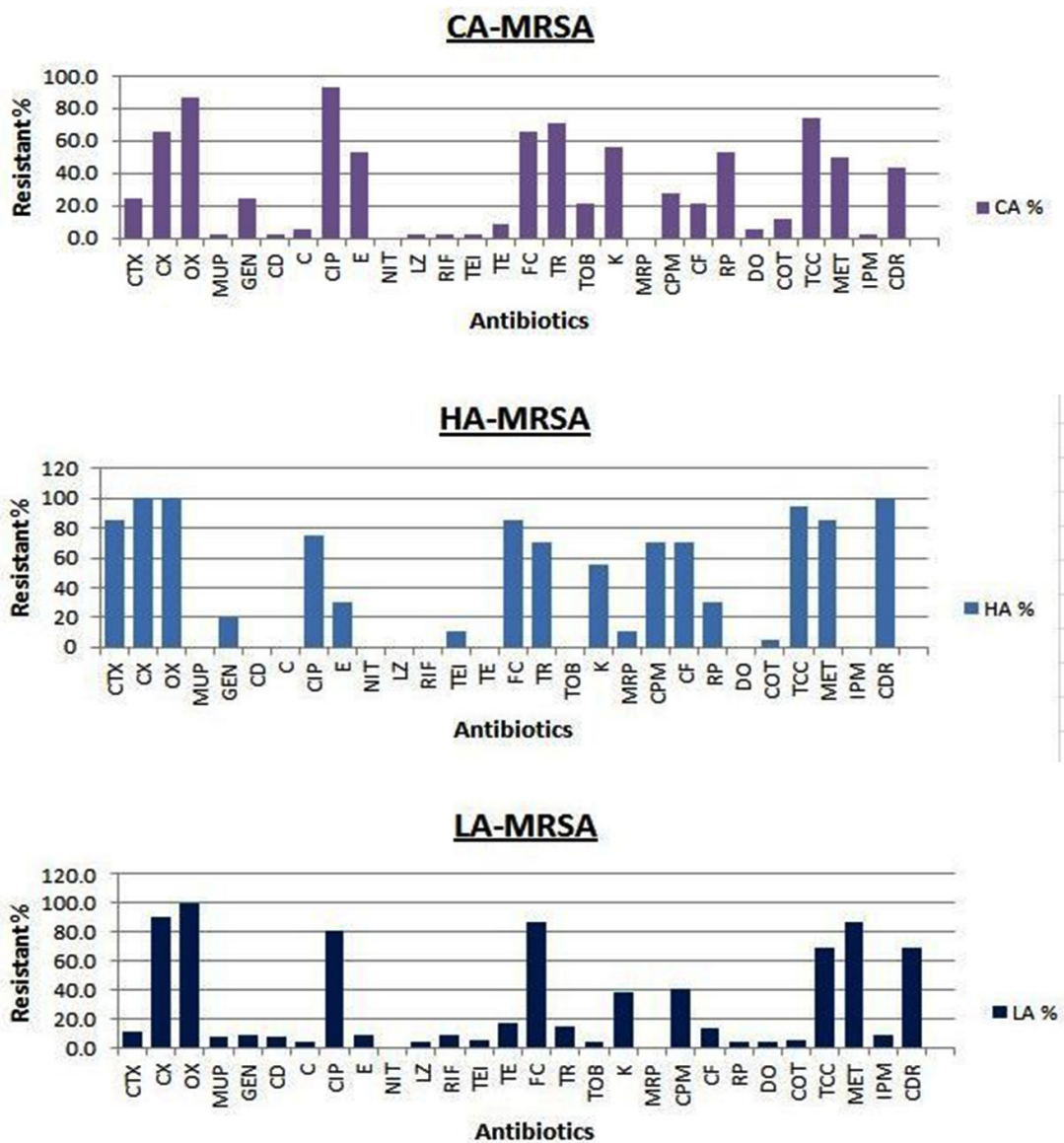


Figure 7. Resistance percentage of CA-MRSA, HA-MRSA and LA-MRSA isolates to different antimicrobial agents

MIC study revealed that except for Rifampicin, other 11 antibiotics mostly fell within the MIC range of 0.25-8 µg/ml. Vancomycin MIC was in the range of 0.25-8 µg/ml (Table 3).

It was found that all the LA-MRSA isolates were MDR and 5.77% among them were XDR with resistance shown against 10 to 14 antimicrobial classes.

MIC study revealed that all antibiotics mostly fell within the MIC range of 0.25-8 µg/ml (Table 4).

No Pan Drug Resistance (PDR) was detected among the isolates in this study.

Out of 104 *mecA* positive *Staphylococcus aureus* isolates, *tst-1* was detected in 8 (7.7%) isolates which comprises of 2 male, 5 female and 1 animal source. Detection of *tst-1* in human samples were 6.73% (7/104) and all are from hospital-associated samples. All 5 female isolates were from the pre and post-caesarian ward of the Gynaecology department. No *tst-1* was detected in CA-MRSA isolates.

A total of 34 (32.69%) isolates found harboring *lukPV* gene comprises of both human and animal isolates. Detection of *lukPV* gene in *mecA* positive samples were higher in Livestock (18/52) followed by hospital associated (9/20) and Community associated (7/32) samples. All *mecA* positive HA-MRSA and LA-MRSA carrying *lukPV* and/or *tst-1* found mostly resistant against a diverse classes of antibiotics viz. Penicillinase stable Penicillin, Cephalosporins, Steroidal, Fluoroquinolones, Cephamycin and β lactam + β lactamase Inhibitor combination. CA-MRSA carrying *lukPV* were mostly found resistant against Fluoroquinolones and Folate pathway inhibitor classes whereas CA-MRSA carrying *tst-1* were mostly resistant against Macrolides, Streptogramins and β lactam + β lactamase Inhibitor combination classes of antibiotics.

Details of MAR indexing results are provided in Table 5.

DISCUSSION

Asian Network for Surveillance of Resistant Pathogens (ANSORP) studies found that in Asian region, average MRSA prevalence was >50% with 25.5% and 67.4% being CA-MRSA and HA-MRSA, respectively. In India, they found

22.6% HA-MRSA and 4.3% CA-MRSA during the study period.²⁶ Most of the samples received for ICMR-AMR surveillance network study were from ICU, OPD and ward admitted patients with superficial or blood stream infections. The study reported an increase of MRSA infections from 33.78% in 2015 to 42.6% in 2021.^{7,27-28} LA-MRSA occurrence varies worldwide (0.028%-9.3%) due to different geographical locations, samplings and methodologies.²⁹ In India, it was reported to be higher (29.41%) in milk so as in Assam too. In some earlier studies in Assam, MRSA in animal meat was found to be 28.6%-48.57%.³⁰⁻³¹ Our study showed that *S. aureus* as well as MRSA prevalence was very high in this region than the reported nationwide prevalence. This may be attributed to the high humidity in this region with substantial heat during summer.

It was also seen that *S. aureus* isolation was the lowest from hospitals but HA-MRSA was the highest from hospitals. ICMR-AMR surveillance network study have found deep wound infections by MRSA to be very low.²⁷ In this study, however, we could not detect MRSA from wound infections, but a higher percentage (95.24%) of MRSA were detected from anterior nares samples. This was perceptibly due to the use of effective regime of antibacterial for treatment of the wounds in the hospitalized patients. Isolation of 95.24% of MRSA from anterior nares of the hospitalized patients signified a high prevalence of MRSA in hospitals. However, sample volume may have influenced this result.

Worldwide, MRSA isolates are mostly resistant to Cefoxitin, Oxacillin, Penicillin, Methicillin, Tetracyclines, most of the 1st generation Cephalosporins, Macrolides, Fusidic Acid and Mupirocin.³² In Asian countries including India, it was observed that MRSA isolates were mostly resistant against both 1st and 2nd generation Cephalosporins along with both Penicillinase-labile and Penicillinase-stable β – lactam antibiotics, Cephamycin, Macrolides and Tetracyclines. In our study, the HA-MRSA isolates that were found to be resistant against 3rd and 4th generation Cephalosporins showed more than 60% resistance against β–lactam + β–lactam inhibitor combination also. Resistance was also recorded against Ciprofloxacin, which is otherwise mostly found effective outside India. It might be due to

the indiscriminate and injudicious use of the Cephalosporins, β – lactam antibiotics and the common fluoroquinolone in this region. Resistance to the 2nd generation Cephalosporins was found to be very less in CA-MRSA, almost equivalent to 3rd and 4th generation Cephalosporins. Among LA-MRSA, resistance to the 2nd generation Cephalosporins was found to be very low compared to the 3rd and the 4th generation Cephalosporins. It was a very encouraging finding as currently, the 2nd generation Cephalosporins are rarely used in the treatment regime.

Isolates showing an MIC breakpoint value falling between 4– 8 $\mu\text{g/ml}$ which was earlier 8-16 $\mu\text{g/ml}$ prior to 2006,³³ are considered as Vancomycin Intermediate *S.aureus* (VISA). After the first report of VISA,³⁴ it is being an emerging concern in the MRSA treatment regime. It is believed that presence of MIC values towards the higher end of 2 $\mu\text{g/ml}$ in Vancomycin susceptible isolates would make it slowly a less favourable antibiotic.³⁵ ICMR AMRSN study⁸ found only 2.3% VISA strains among human associated MRSA. In our study, we didn't find any VISA among HA-MRSA, but 18.75% and 26.92% of CA-MRSA and LA-MRSA, respectively, were found to be VISA isolates, clearly indicating the different patterns of MRSA prevalence in this region.

Oxacillin resistant *S. aureus* whose MIC value falls between 0.5– 4 $\mu\text{g/ml}$ are characterized as BORSA³⁶ and they are predominantly *mecA* negative. But, Yu-Tsung Huang³⁷ reported *mecA* positive, Oxacillin resistant BORSA strains which also had an MIC of 0.5– 4 $\mu\text{g/ml}$. We found 4 no of CA-MRSA isolates which were *mecA* positive but Cefoxitin and Oxacillin susceptible falling under the range of BORSA. Marilyn Chung³⁸ had mentioned such isolates as Oxacillin susceptible MRSA (OS-MRSA). Out of the four isolates, two were resistant to 6 groups of antibiotics (Aminoglycosides, Fluoroquinolones, Macrolides, Tetracyclin, Streptogramins and Folate pathway inhibitors) and other two were resistant to Fluoroquinolones, Folate pathway inhibitor, Macrolides, and Streptogramins, respectively. All the four isolates were susceptible to β -lactam + β -lactam inhibitor combination drug. Details study of these four isolates are further needed.

MDR strains of CA-MRSA were mostly resistant to 4-17 antibiotics classes

which were mainly Fluoroquinolones, Steroidal, Streptogramins, β -lactam antibiotics with β -lactam inhibitors, Folate pathway inhibitor (Trimethoprim), Macrolides, 3rd generation Cephalosporins (Cefdinir), Aminoglycosides (Kanamycin), Penicillinase resistant penicillin and Cephamycin. High prevalence of MDR strains of CA-MRSA was also observed in China.³⁹ Healthy individuals are a major asymptomatic reservoir of MRSA that may lead to its spread within the community.⁴⁰⁻⁴¹ Interestingly, susceptibility of 2nd generation Cephalosporin (Cefaclor) and low resistance of Methicillin indicated restricted or obsolete use of these two drugs in this region.

MDR strains of HA-MRSA were mostly resistant to 5 -11 antibiotics classes consisting of mainly Cephamycin, Penicillinase resistant penicillin, Aminoglycoside (Kanamycin), Fluoroquinolones, Steroidal, 2nd/3rd/4th generation Cephalosporins, Folate pathway inhibitor (Trimethoprim) and β -lactam + β -lactam inhibitor combination drugs. All three generation Cephalosporins resistance indicated the drug abuse in human treatment regime and left with only choice of Glycopeptides.

MDR strains of LA-MRSA were mostly resistant to 4 -14 antibiotic classes mainly consisting of Cephamycin, Penicillinase resistant penicillin, Fluoroquinolones, Steroidal, 3rd generation Cephalosporin and β -lactam antibiotic with β -lactam inhibitor. Interestingly, above all these, MRSA from dogs showed resistance to Pseudomonic acid, Lincosamide, Ansamycin, Imipenem, Cephalosporins (all generation) and Folate pathway inhibitor. Small animals like dogs are mostly treated at the Veterinary Clinical Complex rather than other animals and they are more often treated with antibiotics. That's why the higher level of antimicrobial resistance was observed.

Macrolide-Lincosamide-Streptogramin B (MLSB) regime are the most commonly used antibiotics against MRSA infections to spare the high-end antibiotics of Glycopeptides (Vancomycin) and Oxazolidinones (Linezolid) groups. Clindamycin resistance was found to be very high among HA-MRSA (100%) and LA-MRSA (69.23%), and moderate among CA-MRSA (43.75%) isolates. These findings are comparable to two earlier findings in other part of India⁴²

and abroad.⁴³ Though Clindamycin resistance was found to be higher in Hospital and Livestock samples, Erythromycin resistance was very less. This definitely indicates the possibility of using Clindamycin as an alternative therapy for *Staphylococcus* infection in India, especially in Assam, both in human and animals.

Prevalence of Toxic shock syndrome toxin varies in Asian countries with detection of as high as 75%⁴⁴ in Japan to as low as 2.9%⁴⁵ in China. A recent study in Assam,¹⁶ *tst-1* was detected in 50.79% MRSA consisting samples from hospital, community and environment, out of which only 7.9% were detected in hospital samples. In this study we have detected 35% hospital isolates harboring the *tst-1* gene in MRSA strain which is corroborating with the earlier findings. Presence of *tst-1* was thought to be connected with female menstrual hygiene with the use of tampons and menstrual cups, but later it was also detected in non-menstrual females and men. Most of the studies are concerned with mTSS and subsequent detection of *tst-1*. In this study, we have detected 5 no of MRSA strain from pregnant women in pre and post caesarian ward of the Gynecology department, which was first from this region. Mere detection of *tst-1* doesn't necessarily lead to the manifestation of disease as the level of gene expression controls the disease outcome. But the detection of the same in pregnant women is crucial as it has been reported that maternal carriage of MRSA plays a vital role in the neonatal carriage. No significance difference of antibiotic resistance was noticed in MRSA strain from mother and their neonates⁴⁵ and the birth with highly resistant MRSA strain is a matter of concern. We found these five MRSA strains were highly resistant (Mean- 11.5 antibiotics, Mean MAR Index- 0.421). They were mostly resistant to Cephamycin, Penicillinase stable Penicillin, Fluoroquinolones, Nitrofurans, Steroidal, Cephalosporins, Folate pathway inhibitor (only Trimethoprim) and β lactam+ β lactamase inhibitor combination classes suggesting highly MDR strain.

Staphylococcus aureus isolates harboring *lukPV* in HA-MRSA and CA-MRSA were found to have higher antibiotic resistance with mean MAR Index 0.409 and 0.342 respectively than a lesser mean MAR Index of 0.289 for LA-MRSA.

It definitely implies the use of higher antibiotics in human to cure severe *Staphylococcus aureus* infection than animals. Though *lukPV* was mostly found associated with CA-MRSA, we detected highest no in LA-MRSA with maximum in raw milk from bovine (14/18) and lowest in dog (1/18). In earlier studies, 41.6%- 47.6% *Staphylococcus aureus* isolates from bovine found harboring *lukPV* gene in India.⁴⁶⁻⁴⁷ It is probably due the practice of milking with hand in this region.

Strength and Limitations

In this study, we could not isolate MRSA from wound samples in human. Anterior nares work as a reservoir of MRSA, and isolation of MRSA from patients in hospital settings indicates the risk of nosocomial infection. MLST and *SCCmec* typing of these strains are underway in the lab for an even better understanding of the MRSA distribution.

CONCLUSION

As higher number of MDR and a few XDR isolates were reported in this region, Vancomycin resistance may be lurking around the corner. Uses of Glycopeptides to treat staphylococcal infection has to be very carefully monitored to prevent the growing Vancomycin resistance. Mupirocin, Gentamicin, Co-trimoxazole, 2nd generation Cephalosporins, Linezolid and Rifampicin may act as good alternatives to Glycopeptides to treat MRSA infections. In fact, restricted use of 2nd generation Cephalosporins in treatment may act as a much needed relief in the MRSA treatment. Antibiotic resistance to different classes in HA-MRSA and LA-MRSA are higher than CA-MRSA in this region shows the increased and abundant use of antibiotics in treatment regime. Increase of higher generation Cephalosporins resistance is a matter of concern. The clinical set up needs to look seriously on the uses of higher classes of antibiotics indiscriminately during treatment regime without assessing the susceptibility patterns. A detailed study of *SCCmec* typing and MLST will shade more light into the distribution of clonal complexes in livestock and human in this region. Uses of menstrual cups and tampons are very limited in this region. As we have detected *tst-1* in pregnant woman with high MDR, it is

further necessary to go for their expression study at protein level along with neonatal carriage study.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at <https://doi.org/10.22207/JPAM.17.2.25>

Additional file: Additional Table S1-S4.

ACKNOWLEDGMENTS

The authors would like to thank Advanced Level State Biotech Hub, CVSc, AAU, Khanapara for providing with necessary help to carry out the research. Also thankful to Remya Parameswar Iyer, Kendriya Vidyalaya, IIT, Guwahati for her support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

NKD, PB, PJH conceptualized the study and performed methodology. PB, PJH performed formal analysis. NKD, MC, PK performed Investigation. NKD, PK, GD, RD, PB collected resources. NKD, PK wrote original draft. PJH, PB wrote, reviewed and edited the manuscript. PJH supervised the study. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

This study was approved by Institutional Ethics Committee, Gauhati Medical College & Hospital, Guwahati-781032 bearing approval no "MC/190/2007/Pt-11/MAR-2020/19" dated 04/06/2020 and Institutional Ethics Committee, Gauhati University, Guwahati- 781014 bearing approval no "GUIEC/2021/034" dated 29/09/2021.

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