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Molecular Characterisation Of Methicillin-Resistant Staphylococcus Aureus Isolated From Clinical Specimens In Tertiary Hospital Of North-East India.

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ABSTRACT:

Methicillin-resistant Staphylococcal aureus (MRSA) is one of the most important strains responsible for both Hospital and Community acquired infections (HA-MRSA and CA-MRSA). Spread by contact directly or indirectly.

Objective: To differentiate the Isolated MRSA into HA-MRSA and CA-MRSA and Molecular characterisation of Methicillin-resistant Staphylococcus aureus isolates from the clinical specimens .

Methods: A total of 348 MRSA isolates obtained from various clinical specimens and submitted to the Department of Microbiology of Regional Institute of Medical Sciences (RIMS), Imphal, during the period from October 2019 to November 2022, were studied. All isolates were confirmed as MRSA by using standard techniques [16]. PCR for detection of mec A and pvl genes was done and Antimicrobial Susceptibility testing of MRSA isolates was done by Kirby-Bauer disc diffusion method [24].

Results: All the 348 MRSA isolates, 124(35.63%) met the definition of HA-MRSA and 224 (64.36%) CA-MRSA. SCCmec typing was done in both community-acquired infection and healthcare-associated infection and mec types 4a, 66 (19.0%), III+V, 65 (18.7%), and 4a+V 63 (18.1%) were more frequently isolated. AST pattern showed that 84.57 percent (181/214) and 79.10 percent (106/134) were multidrug resistant (MDR)-CA-MRSA and MDR-HA-MRSA, respectively.

Conclusion: The emerging MDR resistance pattern of CA-MRSA (84.57%) has to be controlled with proper antibiotic stewardship. This emphasizes that the diagnosis of CA-MRSA should be done appropriately by standard diagnostic tools such as molecular characterization by PCR and antibiotic-susceptibility profile to avoid treatment failures and also proper infection prevention control measure especially contact precaution measure should be follow to prevent spread of MRSA in hospital as well as in community.

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Introduction:

Staphylococcus aureus is one of the first pathogens to be discovered because of its ability to cause a wide range of diseases and adapt to various environmental settings, it continue to be one of the main cause of infections in humans till date both in hospital as well as community-acquired infections. Methicillin-resistant Staphylococcal aureus (MRSA) is one of the most important strains responsible for both Hospital and Community acquired infections (HA-MRSA and CA-MRSA). The mecA gene, which is located on a mobile genetic element present on the staphylococcal cassette chromosome mec (SCCmec), encodes methicillin resistance, which results from altered penicillin-binding protein (PBP-2a)[1]. There are currently 14 SCCmec sequence types known[2,3]. The two important genotypic markers that differentiate hospital-acquired (HA)-MRSA strains from community-acquired (CA-MRSA) strains are the architecture of the staphylococcal cassette chromosome mec (SCCmec) type and the presence of the Panton-Valentine Leukocidin toxin[4]. HA-MRSA typically carried large SCCmec elements, types I, II, and III (34-67 kb)[5], however, CA-MRSA contained newly described smaller SCCmec elements, type IV (24 kb)[6]or, less frequently, V or a variant VT[7]. The PVL toxin is not linked to SCCmec types I, II, or III, but it is frequently linked to the presence of SCCmec types IV and occasionally to SCCmec types V or VT[4]. Methicillin-sensitive S. aureus (MSSA) strains acquiring the lukS-PV and lukF-PV genes for PVL production and the resulting PVL-positive MSSA gaining methicillin resistance through integration of the smaller, more mobile SCCmec types IV or V1 are thought to be evolutionary processes leading to the high association of PVL toxin in CA-MRSA strains[8]. A major concern is the spread of CA-MRSA to hospital environments across borders worldwide[9-12]. Infections with CA-MRSA vary from 2.5% to 39% in Asian nations[13]. In hospital settings, multidrug-resistant PVL-positive CA-MRSA has been found to occur commonly in India[14,15]. The present study was undertaken to compare and characterize community-and hospital-acquired MRSA in a tertiary care hospital in RIMS, Imphal Manipur.

Materials and Methods:

Settings and bacterial isolates: A total of 348 MRSA isolates obtained from various clinical specimens of blood (40), wound swabs (29), aspirate (12), pus (196), sputum (16), and urine (55) submitted to the Department of Microbiology of Regional Institute of Medical Sciences (RIMS), Imphal, during the period from October 2019 to November 2022, were studied. The study was approved by the ethics committee of Regional Institute of Medical Sciences, Imphal vide order No. A/206/REB/Prop(sp)72/48/2019. There is no conflict of interest in publication of this research paper and no financial grant is funded by any known organization.

Confirmation and storage of MRSA isolates: All isolates were confirmed as MRSA by using standard techniques[16]. The isolates were inoculated into the semi-solid nutrient agar and stored at -20°C until further study.

Case Definition: Health care-associated MRSA cases were defined as patients with

- An MRSAinfection identified after 48 hours of admission to a hospital.
- A history of hospitalization, surgery, dialysis, or residence in a long-term care facility within 1 year of the MRSA culture date.
- A permanent indwelling catheter or percutaneous medical device (eg, tracheostomy tube, gastrostomy tube, or Foley catheter) present at the time of culture or
- A known positive culture for MRSA prior to the study period.
- Cases that had none of the above features were classified as community-associated.[17,18].

Exclusion criteria: Any organism other than Staphylococcus aureus is proven by phenotypical tests. MSSA proved phenotypically and in mixed cultures.

DNA isolation: The DNA was extracted using the Qiagen Blood and Tissue kit, Hilden, Germany as per the manufacturer's instructions.

PCR for detection of mec A and pvl genes: The primer pairs for mec-A and pvl genes were taken from the published sequence by Olivera et al[19]and McClure et al[20], respectively. Primers were commercially obtained from Eurofins Genomics India Pvt. Ltd. Bangalore. PCR was performed by using a PCR kit (Qiagen, Hilden, Germany) with a slight modification of the final reaction volume of 25 µl (12.5µl master mix, 2.5µl primer mixed, 3µl of DNA template and 7µl of RNAnase-free water). Reference strains ATCC 43300 and 25923 were used as a positive and negative control for the mec-A gene, respectively and ATCC 43300 was used as a negative control for the pvl gene.

Multiplex PCR: Primers were selected from the published sequence of the SCCmec types I-III (Olivera et al)[19] IVa-Ivb (Okuma et al)[21].IVc-IVd (Hisata et al)[22].V (Zang et al)[23] and were commercially obtained from Eurofins Genomics India Pvt. Ltd. Bangalore.

Preparation of primer mix – The preparation of the primer mix was done as per the manufacturer's instructions for the multiplex PCR kit (Qiagen). For multiplex PCR, the primers were divided into two sets: set A was designed to amplify SCCmec type I, II, III, V, and mec-A. Whereas set B was designed to amplify SCCmec IVa, IVb, IVc, IVd, and mec-A. The mec-A gene was included in the protocol as an internal positive control.

SCCmec typing – To ensure the individual primer pairs were adequate for the amplification of all loci (gene fragments), the single-target PCR protocol[**23**] with each primer pair was conducted before the multiplex PCR optimization. Then PCR was performed by using a Qiagen Multiplex PCR kit with slight modifications. The reaction is run in two sets with two different sets of primers mixed. Multiplex PCR with set A primers consisting of 12.5µl mastermix, 2.5µl primer mix (set A), 3µl of DNA template, and 7µl of RNase-free water with a total reaction volume of 25µl.Multiplex primer set B included the same as in set A except for the (set B).Thermocycling conditions and visualization of products were done as per the manufacturer's instructions.

Antimicrobial Susceptibility testing of MRSA isolates: Kirby-Bauer disc diffusion method[**24**] was performed with the antibiotics discs (Hi-Media, Mumbai); Ciprofloxacin(5µg), erythromycin(15µg), clindamycin(2 µg), linezolid(30 µg), teicoplanin(30 µg), Vancomycin(30 µg), tetracycline(30 µg), tigecycline(15 µg), nitrofurantoin(30 µg), trimethoprim/sulfamethoxazole(25 µg) and Mupirocin High level. Vitek 2 systems were also used for anti-biotyping whenever required. The testing conditions and interpretation of the test were done as per Clinical and Laboratory Standards Institute (CLSI) criteria[**25**]. The MRSA isolates resistant to ≥ three non-beta lactam antibiotics were classified as multidrug-resistant MRSA (MDR-MRSA)[**26**].

Statistical Analysis:Categorical variables were analyzed using Chi-square and Fisher's exact tests using IBM SPSS statistics 20(IBM Corporation, USA).

Results:

All the 348 MRSA isolates, 124(35.63%) met the definition of HA-MRSA and 224 (64.36%) CA-MRSA. To test the presence of pvl toxin between the HA-MRSA and CA-MRSA, table 1 is introduced by using χ^2 -test as a statistical tool.

PVL toxin gene: It is found that pvl positive is visibly higher than that of negative pvl genes. This is found true in both the infection types. Nevertheless, the insignificant test value (P=0.196) indicates that there is no significant variation in the pattern of pvl genes between the CA-MRSA and HA-MRSA cases.

Table 1:Infection type-wise MRSA cases according to panton-valentine leukocidin (PVL)

Parameters		Panton valentine leukocydin (PVL)			χ^2 -value	df	P -value
		Positive	Negative	Total			
Infection type	Community-Acquired Infection	192(89.7%)	22(10.3%)	214(100.0%)	1.675	1	.196
	Health Care Associated Infection	114(85.1%)	20(14.9%)	134(100.0%)			
Total		306(87.9%)	42(12.1%)	348(100.0%)			

χ^2 -value; df: degree of freedom; P-value: probability due to chance factor

SCCmec typing: Comparison analysis of SCCmec typing between community-acquired infection and healthcare-associated infection is made by using χ^2-the test and its findings are outlined in Table 2. In this study, the mec types 4a 66 (19.0%), III+V 65 (18.7%), and 4a+V 63 (18.1%) were more frequently isolated. CA-MRSA are more likely to be of type 4a 51 (23.8%), 4a+V 39 (18.2%),

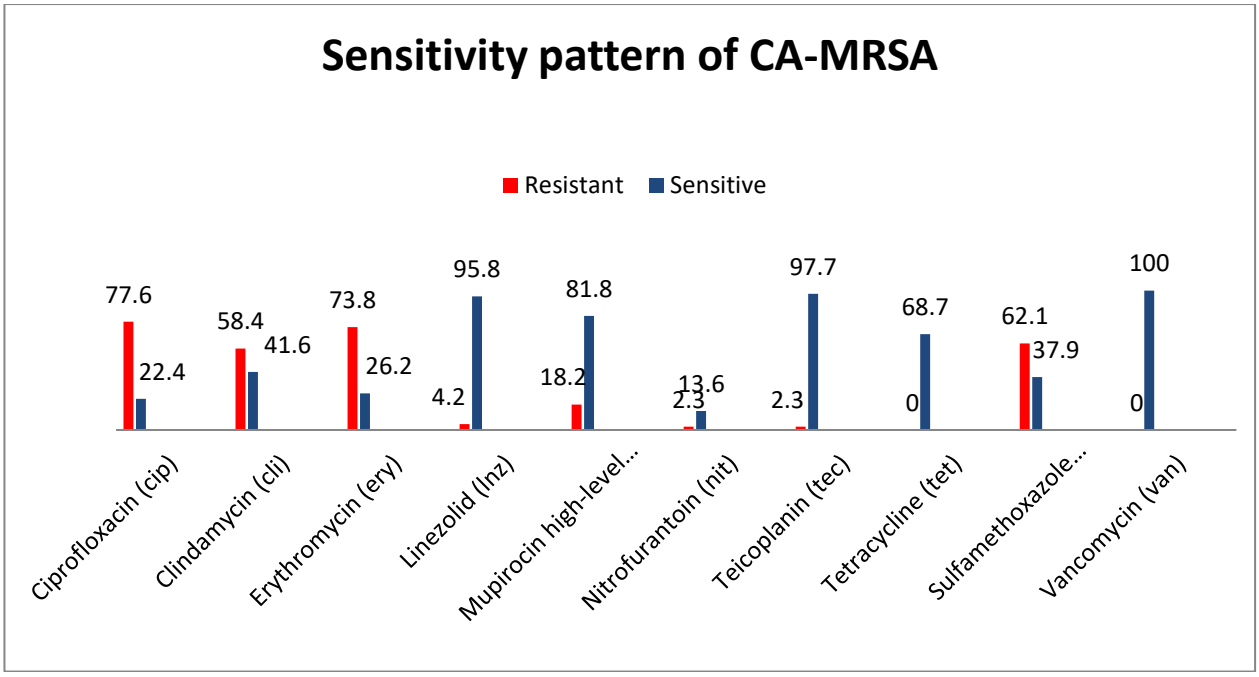
and V 37 (17.3%), whereas HA-MRSA are of type III+V 29 (21.6%), III 27 (20.1%), and 4a+V 24 (17.9%). When their variations were tested further, it was discovered that statistically, they were extremely highly significant. As a result, it is possible to conclude that SCCmec typing significantly distinguishes between infections acquired in the community and infections associated with healthcare. The significance of P<.001 supports this assertion.

Table:2 Infection type-wise MRSA cases according to mec typing

Mec typing	Infection type		Total	χ^2 **-value**	**df**	**P -value**
	Community-Acquired Infection	Health Care Associated Infection				
4a	51(23.8%)	15(11.2%)	66(19.0%)			
4a+III	3(1.4%)	2(1.5%)	5(1.4%)			
4a+III+V	7(3.3%)	7(5.2%)	14(4.0%)			
4a+V	39(18.2%)	24(17.9%)	63(18.1%)			
I+II	-	4(3.0%)	4(1.1%)			
II+III	1(0.5%)	2(1.5%)	3(0.9%)	32.699	10	<.001
II+III+V	-	2(1.5%)	2(0.6%)			
III	18(8.4%)	27(20.1%)	45(12.9%)			
III+V	36(16.8%)	29(21.6%)	65(18.7%)			
V	37(17.3%)	15(11.2%)	52(14.9%)			
Not amplified	22(10.3%)	7(5.2%)	29()8.3%			
Total	214(100.0%)	134(100.0%)	348(100.0%)			

χ^2 -value; df: degree of freedom; P-value: probability due to chance factor

Antimicrobial susceptibility pattern of MRSA isolates: In this study, 12 antibiotics were used to understand the antibiotic resistance profiles of MRSA isolates. CA-MRSA and HA-MRSA showed insignificant ($p > 0.05$) in their susceptibility, except for the mupirocin high level ($p=0.005$) showing CA-MRSA with a higher percentage of sensitivity (81.8%) compared to HA-MRSA (68.7%).In our study, CA-MRSA isolates resistant to three or more classes of antibiotics were found. These isolates were resistant to ciprofloxacin (77.6%), clindamycin (58.4%), erythromycin (73.8%), levofloxacin (75.70%) and sulfamethoxazole-trimethoprim (SXT) (62.1%). All (100%) MRSA isolates showed no resistance to vancomycin. In our study, the occurrence of MDR-MRSA is high (82.47%).AST pattern showed that 84.57 percent (181/214) and 79.10 percent (106/134) were multidrug resistant (MDR)-CA-MRSA and MDR-HA-MRSA, respectively.



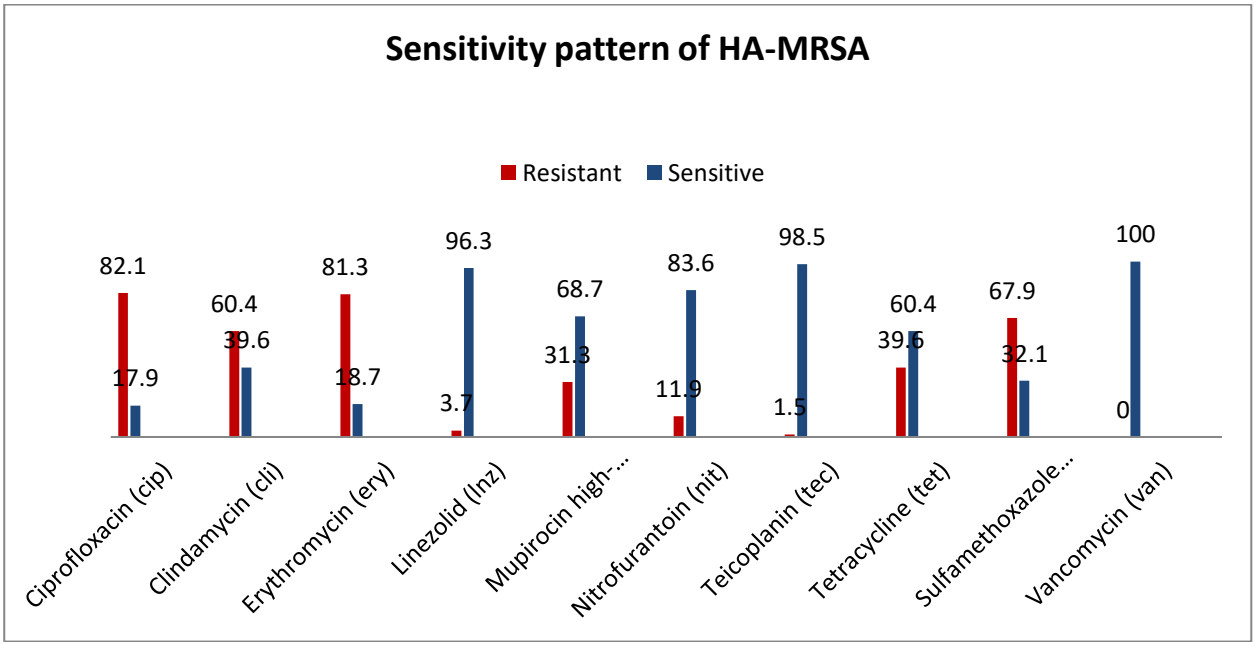
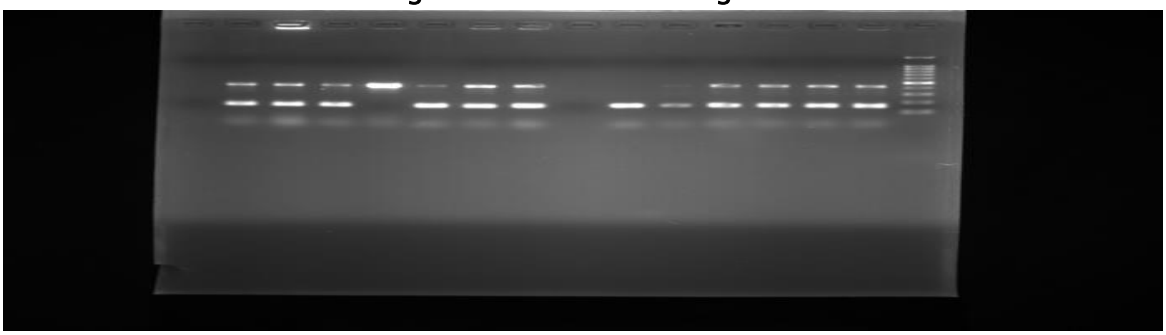
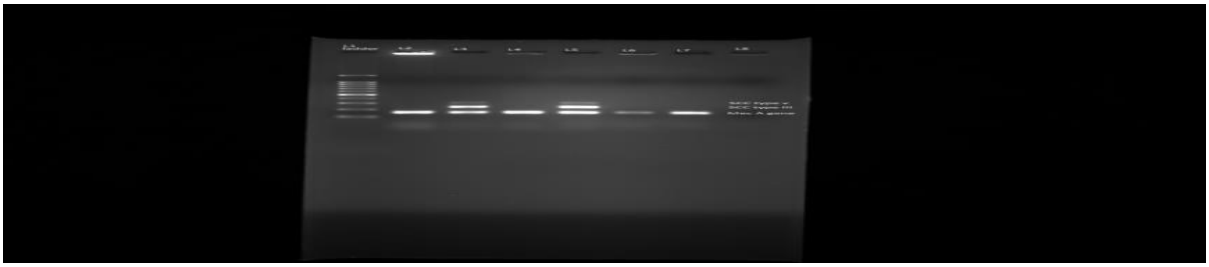


Fig 1: PCR mecA and PVL gene



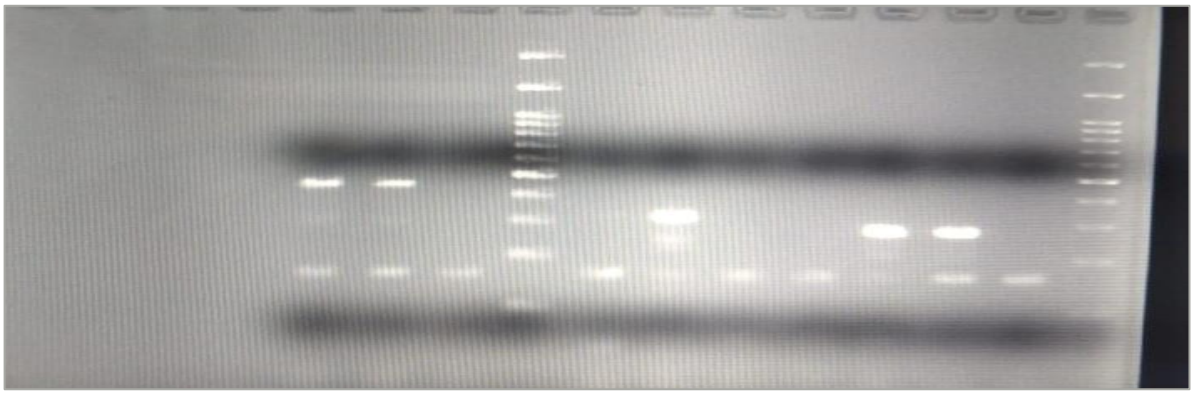
Lane 1: 1500 bp, lane 2 positive control MRSA ATCC 700699, lane3,4,5,6,9,10,11,13,14,15,16 clinical samples positive for mec-A (162bp) and PVL (433bp).Lane 8 Negative control mec-A and PVL and lane 12 positive control PVL.

Fig 2: SCCmec typing of MRSA



Lane 1:1500bp, lane 2,3,4,5,6,7 clinical samples were positive for mec-A (162bp), and lane 3,5 clinical samples were positive for SCCmec type III (243bp) and V (325bp).

Fig 3: SCCmec typing of MRSA



Lane 1,9: 1500bp lane2,3,4,5,6,7,8,10,11,12 clinical samples positive for mec-A (162bp),lane 3,4 clinical isolates positive for SCCmec type II (284)bp, lane 7 clinical samples positive for SCCmec type V (325)bp, lane 11,12clinical samples positive for SCCmec type IVa (450)bp.

Discussion:

In the present study, we evaluated the presence of PVL toxin as a marker of virulence factor of both CA-MRSA and HA-MRSA isolates. Several studies have reported the PVL as a reliable marker of CA-MRSA strains[**27,28**]. Our findings are in agreement with the reports from Ireland[**29**], and Finland[**30**]. Our finding of PVL genes in (85%) of HA-SCCmec isolates is not surprising since SCCmec IV/V strains have been associated largely with SSTIs and PVL production[**31**]. Our study documents the emergence of MRSA isolates typical of CA genotypes in patients with HA-MRSA. Remarkably, at our institute a large proportion (38·5%) of isolates classified epidemiologically as HA-MRSA had a CA genotype. These data confirm the reported spread of CA-MRSA SCCmec IV/V strains in hospital settings in Europe, the USA[**32,33**]and India[**34,35**]. Given the vulnerable population within the hospital setting, it is unclear how infections with isolates that contain SCCmec IV/V may differ in symptoms and severity from those caused by the traditional HA-SCCmec I/II/III isolates. The introduction of SCCmec IV/V strains into our hospital population did not result in a change in the spectrum or severity of illness in this group. Compared to patients with CA-SCCmec IV/V SSTIs, it was observed that HA-SCCmec IV/V SSTIs were significantly associated with malignancy and trauma-related infection. Patients with these conditions are predisposed to use healthcare facilities, are generally exposed to antibiotics, tend to have interventions performed, and hence present opportunities to contract MRSA in the healthcare

facility. Exactly why the SCCmec IV/V strains are successful in hospital settings such as ours remains unknown but mathematical models predict the replacement of traditional HA-MRSA strains by CA-MRSA strains, due to their higher growth rate and greater genetic fitness[36,37].

In this study, SCC mec typing reveals that MRSA isolates with single DNA fragment band IVa 66(19.0%) were more predominant in CA-MRSA (51(23.8%) as compared to HA-MRSA 15(11.2%). The SCCmec type IVa has been reported as the most common type Berglund et al., 2009[38]. Type IV SCC*mec* is prevalent in CA-MRSA strains which may be due to short (21 to 25 kb) and lack of any antibiotic resistance genes other than mecA[39].A similar study in Mumbai, India also reported the presence of SCCmec type IV in 34% of isolates collected during three years. The SCCmec IV MRSA (EMRSA-15) strain is a global pandemic HA-MRSA clone and interestingly it is recovered from both inpatients and outpatients in our hospital. This suggests that outpatients may represent an important reservoir for MRSA dissemination within the hospital when admitted as inpatients[40]. The combination of SCCmec III plus V (18.7%) is on the rise in our study followed by SCCmec type IVa plus V ((18.1%). Similarly, Zang et al[23] reported the presence of double bands in 1:1 percent of clinical isolates of MRSA of these combinations; SCCmec type I plus II (1.1%), II plus III(0.9%), II plus III plus V (0.6%), which corroborated the study results of Lawung et al[41]. from Thailand and Bhutia et al[8]. in Sikkim. SCCmec type III has been reported to be the predominant MRSA in the Asian continent except in Korea and Japan[42,43]. The present study showed a low occurrence of type III MRSA isolates in Manipur (12.9%) compared to that reported from other parts of India[42-44].D'Souza et al[34] from Mumbai India reported that SCCmec type V (41.01%) was higher than type III (24.55%). Our study suggests that SCCmec type IVa and V seem to be emerging MRSA in this part of India. The percentage of non-typeable MRSA isolates in our study is (8.3%), which was higher than the earlier reports from Korea (1.35%)[45], Canada (1.77%)[23] India (4%)[42]. In contrast to our findings, a study from Taiwan reported that 81 % of MRSA isolates were non-typable[46]. The non-type-ability observed in the current study could be due to the presence of other rare SCCmec elements among the test MRSA isolates, which were not looked for.

Apart from genotypic markers, MRSA is also categorized based on the susceptibility pattern to various antibiotics[8,47,48]. Several studies have shown that CA-MRSA is more susceptible to non-β-lactam antibiotics compared to HA-MRSA[48].In this study, 11 antibiotics were used to understand the antibiotic resistance profiles of MRSA isolates. CA-MRSA and HA-MRSA showed insignificant ($p > 0.05$) in their susceptibility. Except for the mupirocin high level ($p=0.005$) showing CA-MRSA with a higher percentage of sensitivity (81.8%) compared to HA-MRSA (68.7%). Susceptibility to non-β-lactam antibiotics has been previously reported by several investigators[34,48,49].In this study, MRSA from patients with hospital-associated risk factors and harboring the SCCmec type IV and SCCmec type V genes shows higher antibiotic resistance. In our study, CA-MRSA isolates resistant to three or more classes of antibiotics were found. These isolates were resistant to ciprofloxacin (77.6%), clindamycin (58.4%), erythromycin (73.8%), levofloxacin (75.70%) and sulfamethoxazole-trimethoprim (SXT) (62.1%). All (100%) MRSA isolates showed no resistance to vancomycin. A study from India reported CA-MRSA resistant to gentamicin (69%), erythromycin (62%), cotrimoxazole (58.6%), and ciprofloxacin (79.3%)[50]. Similarly, the Malaysian National Surveillance of Antibiotic Resistance program for the year 2007 reported that MRSA strains from 12 major hospitals were resistant to erythromycin, gentamicin, and co-trimoxazole at rates of 95, 93.5, and 89.3 %, respectively. MDR CA-MRSA has been reported from India and worldwide[50].In our study, the occurrence of MDR-MRSA is high (82.47%).AST pattern showed that 84.57 percent (181/214) and 79.10 percent (106/134) were multidrug-resistant (MDR)-CA-MRSA and MDR-HA-MRSA, respectively.

Conclusion:

The emerging MDR resistance pattern of CA-MRSA (84.57%) has to be controlled with proper antibiotic stewardship. CA-MRSA isolated from patients with hospital-associated risk factors with MDR similar to HA-MRSA can lead to the spread of multidrug-resistant virulent strains of CA-MRSA in the hospital and in the community. The molecular characterization results show an increasing trend in the prevalence of MRSA in the general population and the presence of CA-MRSA in the hospital environment as well as in patients with hospital-associated risk factors. This emphasizes that the diagnosis of CA-MRSA should not be strictly based on the risk factors but on standard diagnostic tools such as molecular characterization by PCR and antibiotic-susceptibility profile to avoid treatment failures.

References

1. De Lencastre, H.; Oliveira, D.; Tomasz, A. Antibiotic resistant Staphylococcus aureus: A paradigm of adaptive power. *Curr. Opin. Microbiol.* 2007, *10*, 428–435.
2. Andrade, M.M.; Luiz, W.B.; Souza, R.D.S.O.; Amorim, J.H. The History of Methicillin-Resistant Staphylococcus aureus in Brazil.*Can. J. Infect. Dis. Med. Microbiol.* 2020, *2020*, 1–18.
3. Lakhundi S, Zhang K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev. 2018 Sep 12;31(4):e00020-18. doi: 10.1128/CMR.00020-18. PMID: 30209034; PMCID: PMC6148192.
4. Boyle-Vavra S, Daum RS. Community-acquired methicillin- resistant *Staphylococcus aureus*: the role of Panton-Valentine Leukocidin. *Lab Invest* 2007; *87* : 3-9.
5. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, *et al*. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001; *45* : 1323-36.
6. Ma XX, Ito T, Tiensasitorn C, Jamklang M, Chongtrakool P, Boyle-Vavra S, *et al*. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2002; *46* : 1147-52.
7. Boyle-Vavra S, Ereshefsky B, Wang CC, Daum RS. Successful multiresistant community-associated methicillin-resistant *Staphylococcus aureus* lineage from Taipei, Taiwan, that carries either the novel Staphylococcal chromosome cassette *mec* (SCC*mec*) type V_T or SCC*mec* Type IV. *J Clin Microbiol* 2005; *43* : 4719-30.
8. Bhutia KO, Singh T, Adhikari L, Biswas S. Molecular characterization of community- & hospital-acquired methicillin-resistant & methicillin-sensitive Staphylococcus aureus isolates in Sikkim. Indian J Med Res. 2015 Sep;142(3):330-5. doi: 10.4103/0971-5916.166600. PMID: 26458350; PMCID: PMC4669869.
9. Kobayashi, T.; Nakaminami, H.; Ohtani, H.; Yamada, K.; Nasu, Y.; Takadama, S.; Noguchi, N.; Fujii, T.; Matsumoto, T. An outbreak of severe infectious diseases caused by methicillin-resistant Staphylococcus aureus USA300 clone among hospitalized patients and nursing staff in a tertiary care university hospital. *J. Infect. Chemother.* 2020, *26*, 76–81.
10. Abimannan, N.; Sumathi, G.; Krishnarajasekhar, O.; Sinha, B.; Krishnan, P. Clonal Clusters and Virulence Factors of Methicillin- Resistant Staphylococcus Aureus: Evidence for Community-Acquired Methicillin-Resistant Staphylococcus Aureus Infiltration into Hospital Settings in Chennai, South India. *Indian J. Med. Microbiol.* 2019, *37*, 326–336.

11. Choo, E.J. Community-Associated Methicillin-Resistant Staphylococcus aureus in Nosocomial Infections. *Infect. Chemother.* 2017,*49*, 158-159.
12. Kateete, D.P.; Bwanga, F.; Seni, J.; Mayanja, R.; Kigozi, E.; Mujuni, B.; Ashaba, F.K.; Baluku, H.; Najjuka, C.F.; Källander, K.; et al. CA-MRSA and HA-MRSA coexist in community and hospital settings in Uganda. *Antimicrob. Resist. Infect. Control.* 2019, *8*, 1-9.
13. Chuang, Y.Y.; Huang, Y.C. Molecular epidemiology of community associated meticillin-resistant Staphylococcus aureus in Asia.*Lancet Infect. Dis.* 2013, *13*, 698-708.
14. Dhawan, B.; Rao, C.; Udo, E.E.; Gadepalli, R.; Vishnubhatla, S.; Kapil, A. Dissemination of methicillin-resistant Staphylococcus aureus SCCmec type IV and SCCmec type V epidemic clones in a tertiary hospital: Challenge to infection control. *Epidemiol. Infect.* 2015, *143*, 343-353.
15. Yamuna, D.; Francis, Y.; Doss, G.P.; Balaji, V. Molecular characterization of Panton-Valentine leukocidin (PVL) toxin-encoding phages from South India. *New Microbes New Infect.* 2017, *20*, 34-38.
16. Baird D. Staphylococcus: cluster-forming Gram-positivecocci. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie & McCartney practical medical microbiology,* 14th ed. New York: Churchill Livingstone; 1996. p. 245-61.
17. Timothy S. Naimi, Kathleen H, Kathryn Como-Sabetti, Stephanie M. Borchardt, David J. Boxrud, Jerome Etienne et al. Comparison of Communityand Health Care-Associated Methicillin-Resistant *Staphylococcus aureus* Infection JAMA, December 10, 2003—Vol 290, No. 22.
18. McClure J, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, *et al*. Novel multiplex PCR assay for detection of the *staphylococcal* virulence marker panton-valentine leukocidin genes and simultaneous discrimination of methicillin- susceptible from -resistant *staphylococci*. *J Clin Microbiol* 2006; *44* : 1141-4.
19. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid-identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002; *46* : 2155-61.
20. McClure J, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, *et al*. Novel multiplex PCR assay for detection of the *staphylococcal* virulence marker panton-valentine leukocidin genes and simultaneous discrimination of methicillin- susceptible from -resistant *staphylococci*. *J Clin Microbiol* 2006; *44* : 1141-4.
21. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, *et al*. Dissemination of new methicillin-resistant *staphylooccus aureus* clones in the community. *J Clin Microbiol* 2002; *40* : 4289-94.
22. Hisata K, Kuwahara-Arai K, Yamanoto M, Ito T, Nakatomi Y, Cui L, *et al*. Dissemination of methicillin-resistant *Staphylococci* among healthy Japanese children. *J Clin Microbiol* 2005; *43* : 3364-72.
23. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005; *43* : 5026-33.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; *45* : 493-6.
25. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*; 18th informational supplement. Approved standard M100-S18; vol, 28. No1. Wayne, PA: CLSI; 2008.

26. Charlebois ED, Perdreau-Remington F, Kreiswirth B, Bangsberg DR, Ciccarone D, Diep BA, *et al*. Origins of community strains of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2004; *39* : 47-54.
27. McDonald RR, Antonishyn NA, Hansen T, Snook LA, Nagle E, Mulvey MR, Levett PN, Horsman GB. Development of a triplex real-time PCR assay for detection of Panton-Valentine leukocidin toxin genes in clinical isolates of methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2005 Dec;43(12):6147-9. doi: 10.1128/JCM.43.12.6147-6149.2005. PMID: 16333116; PMCID: PMC1317231.
28. Rubin IM, Hansen TA, Klingenberg AM, Petersen AM, Worning P, Westh H, Bartels MD. A Sporadic Four-Year Hospital Outbreak of a ST97-IVa MRSA With Half of the Patients First Identified in the Community. Front Microbiol. 2018 Jul 10;9:1494. doi: 10.3389/fmicb.2018.01494. PMID: 30042745; PMCID: PMC6048196.
29. Roosney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine Leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community- acquired MRSA strains in Ireland. *J Clin Microbiol* 2007; *45* : 2554-63.
30. Kardén-Lilja M, Ibrahem S, Vuopio-Varkila J, Salmenlinna S, Lyytikäinen O, Siira L, *et al*. Panton-Valentine leukocidin genes and staphylococcal chromosomal cassette *mec* types amongst Finnish community-acquired methicillin-resistant *Staphylococcus aureus* strains, 1997-1999. *Eur J Clin Microbiol Infect Dis* 2007; *26* : 729-33.
31. Valsesia G, Rossi M, Bertschy S, Pfyffer GE. Emergence of SCCmec type IV and SCCmec type V methicillin-resistant Staphylococcus aureus containing the Panton-Valentine leukocidin genes in a large academic teaching hospital in central Switzerland: external invaders or persisting circulators? J Clin Microbiol. 2010 Mar;48(3):720-7. doi: 10.1128/JCM.01890-09. Epub 2009 Dec 30. PMID: 20042625; PMCID: PMC2832439.
32. Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R; National Nosocomial Infections Surveillance System. Changes in the epidemiology of methicillin-resistant Staphylococcus aureus in intensive care units in US hospitals, 1992-2003. Clin Infect Dis. 2006 Feb 1;42(3):389-91. doi: 10.1086/499367. Epub 2005 Dec 19. PMID: 16392087.
33. Otter JA, French GL. Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Europe. Lancet Infect Dis. 2010 Apr;10(4):227-39. doi: 10.1016/S1473-3099(10)70053-0. PMID: 20334846.
34. D'Souza N, Rodrigues C, Mehta A. Molecular characterization of methicillin-resistant Staphylococcus aureus with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India. J Clin Microbiol. 2010 May;48(5):1806-11. doi: 10.1128/JCM.01867-09. Epub 2010 Mar 29. PMID: 20351212; PMCID: PMC2863868.
35. Shambat S, Nadig S, Prabhakara S, Bes M, Etienne J, Arakere G. Clonal complexes and virulence factors of Staphylococcus aureus from several cities in India. BMC Microbiol. 2012 May 1;12:64. doi: 10.1186/1471-2180-12-64. PMID: 22548694; PMCID: PMC3438138.
36. D'Agata EM, Webb GF, Horn MA, Moellering RC Jr, Ruan S. Modeling the invasion of community-acquired methicillin-resistant Staphylococcus aureus into hospitals. Clin Infect Dis. 2009 Feb 1;48(3):274-84. doi: 10.1086/595844. PMID: 19137654; PMCID: PMC2666259.
37. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant Staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA strains? Clin Infect Dis. 2008 Mar 15;46(6):787-94. doi: 10.1086/528716. PMID: 18266611.

38. Berglund C, Ito T, Ma XX, Ikeda M, Watanabe S, Söderquist B, Hiramatsu K. Genetic diversity of methicillin-resistant Staphylococcus aureus carrying type IV SCCmec in Orebro County and the western region of Sweden. J Antimicrob Chemother. 2009 Jan;63(1):32-41. doi: 10.1093/jac/dkn435. Epub 2008 Nov 11. PMID: 19001453.
39. Hiramatsu K, Katayama Y, Yuzawa H, Ito T. Molecular genetics of methicillin-resistant Staphylococcus aureus. Int J Med Microbiol. 2002 Jul;292(2):67-74. doi: 10.1078/1438-4221-00192. PMID: 12195737.
40. Alcoceba E, Mena A, Cruz Pérez M, Ruiz de Gopegui E, Padilla E, Gil J, Ramírez A, Gallegos C, Serra A, Pérez JL, Oliver A. Molecular epidemiology of methicillin-resistant Staphylococcus aureus in Majorcan hospitals: high prevalence of the epidemic clone EMRSA-15. Clin Microbiol Infect. 2007 Jun;13(6):599-605. doi: 10.1111/j.1469-0691.2007.01703.x. Epub 2007 Mar 22. PMID: 17378930.
41. Lawung R, Chuong LV, Cherdtrakulkiat R, Srisarin A, Prachayasittikul V. Revelation of staphylococcal cassette chromosome mec types in methicillin-resistant Staphylococcus aureus isolates from Thailand and Vietnam. J Microbiol Methods. 2014 Dec;107:8-12. doi: 10.1016/j.mimet.2014.08.024. Epub 2014 Sep 6. PMID: 25205542.
42. Arakere G, Nadig S, Ito T, Ma XX, Hiramatsu K. A novel type-III staphylococcal cassette chromosome mec (SCCmec) variant among Indian isolates of methicillin-resistant Staphylococcus aureus. FEMS Microbiol Lett. 2009 Mar;292(1):141-8. doi: 10.1111/j.1574-6968.2008.01482.x. Epub 2009 Jan 29. PMID: 19187210.
43. Shabir S, Hardy KJ, Abbasi WS, McMurray CL, Malik SA, Wattal C, Hawkey PM. Epidemiological typing of meticillin-resistant Staphylococcus aureus isolates from Pakistan and India. J Med Microbiol. 2010 Mar;59(Pt 3):330-337. doi: 10.1099/jmm.0.014910-0. Epub 2009 Nov 19. PMID: 19926728.
44. Nadig S, Namburi P , Raghunath D, Arakere G. Genotyping27.of methicillin-resistant Staphylococcus aureus isolates from Indian hospitals. Curr Sci 2006; 91 :1364-9.
45. Ko KS, Lee JY, Suh JY, Oh WS, Peck KR, Lee NY, Song JH. Distribution of major genotypes among methicillin-resistant Staphylococcus aureus clones in Asian countries. J Clin Microbiol. 2005 Jan;43(1):421-6. doi: 10.1128/JCM.43.1.421-426.2005. PMID: 15635004; PMCID: PMC540159.
46. Wang CC, Lo WT, Chu ML, Siu LK. Epidemiological typing of community-acquired methicillin-resistant Staphylococcus aureus isolates from children in Taiwan. Clin Infect Dis. 2004 Aug 15;39(4):481-7. doi: 10.1086/422642. Epub 2004 Jul 28. PMID: 15356810.
47. Fey PD, Saïd-Salim B, Rupp ME, Hinrichs SH, Boxrud DJ, Davis CC, Kreiswirth BN, Schlievert PM. Comparative molecular analysis of community- or hospital-acquired methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2003 Jan;47(1):196-203. doi: 10.1128/AAC.47.1.196-203.2003. PMID: 12499191; PMCID: PMC149027.
48. P R V, M J. A comparative analysis of community acquired and hospital acquired methicillin resistant Staphylococcus aureus. J Clin Diagn Res. 2013 Jul;7(7):1339-42. doi: 10.7860/JCDR/2013/5302.3139. Epub 2013 Jul 1. PMID: 23998061; PMCID: PMC3749631.
49. Shenoy MS, Bhat GK, Kishore A, Hassan MK. Significance of MRSA strains in community associated skin and soft tissue infections. Indian J Med Microbiol. 2010 Apr-Jun;28(2):152-4. doi: 10.4103/0255-0857.62494. PMID: 20404463.
50. Preeja PP, Kumar SH, Shetty V. Prevalence and Characterization of Methicillin-Resistant *Staphylococcus aureus* from Community- and Hospital-Associated Infections: A

Tertiary Care Center Study. Antibiotics (Basel). 2021 Feb 18;10(2):197. doi: 10.3390/antibiotics10020197. PMID: 33670648; PMCID: PMC7922968.