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Mec-A Positive Methicillin-Susceptible *Staphylococcus Aureus* as a Public Health Concern: A Case Series

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Abstract

Background: *Staphylococcus aureus*, an opportunistic microorganism, is the leading cause of severe bloodstream infections, including sepsis and endocarditis, which can be life-threatening. Therefore, *S. aureus* infection poses a significant public health challenge, particularly in developing nations. mec-a is a genetic element commonly found in Methicillin-resistant *Staphylococcus aureus* (MRSA) strains that characterises the S. aureus resistance phenotype.

Methods: Clinical infection samples obtained from blood were collected and categorised as MRSA or Methicillin-sensitive Staphylococcus aureus (MSSA) using the VITEK-2 compact device. Subsequently, specific samples were gathered as case series owing to their unique characteristics. Resistance genes were detected using conventional polymerase chain reaction (PCR), followed by visualisation through electrophoresis.

Results: Our findings were based on the identification of five instances of MSSA among samples obtained from a tertiary hospital's microbiology laboratory. Using the VITEK-2 antimicrobial susceptibility profile, these cases were determined to be MSSA. Subsequently, we conducted PCR, which revealed the presence of a mec-a-positive strain. Upon re-examination using Mueller–Hinton agar, the five strains were confirmed to be MSSA. Further analysis demonstrated that all strains were positive for Panton-Valentine leucocidin *(pvl)* and exfoliative toxin A *(eta) gens.*

Conclusions: The positive mec-A MSSA results should serve as a warning to clinicians that a resistant strain is forthcoming. mec-A continues to be the benchmark for confirming the resistance phenotype. Additional research is essential to explore this strain.

Keywords: MSSA, mec-a, *Staphylococcus aureus*.

Introduction

Staphylococci are Gram-positive cocci characterized by their unique growth pattern, which resembles that of a cluster of grapes. This appearance forms the basis of their

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definition. Although some specimens may appear as single cells, pairs or short chains, this characteristic pattern remains the most distinctive feature of Staphylococci (1). Among them, *Staphylococcus aureus* (*S. aureus*) is well-known due to its significant clinical implications. It is a major human bacterial pathogen and is considered a commensal organism in humans but can cause infections that lead to bacteraemia (2). The precise incidence of *S. aureus* bacteraemia is difficult to ascertain, but surveillance data from several developed countries have reported incidence rates ranging from 20 to 50 per 100,000 (3).

Staphylococcus aureus bacteraemia was once a leading cause of high mortality rates, with pre-antibiotic era figures reaching as high as 75% to 83%. Nonetheless, the introduction of antibiotics in the 1940s and the 1950s significantly improved patient outcomes. However, the emergence of antibiotic resistance may offset these gains (4). Methicillin-resistant *Staphylococcus aureus* (MRSA), commonly referred to as MRSA, has emerged as one of the most significant modern pathogens. Initially identified in the 1960s, the prevalence of MRSA increased significantly in the 1990s. Its rapid spread undermined the achievements of the antibiotic era, further exacerbated by the decline in the development of novel antibiotics (5).

The mecA gene is a component of the 21- to 60-kilobase staphylococcal chromosome cassette mec (SCCmec), a mobile genetic element that may additionally comprise other genetic structures, including Tn554, pUB110, and pT181, which confer resistance to non-beta-lactam antibiotics (6). Methicillin-resistant *Staphylococcus aureus* (MRSA) expresses penicillin-binding protein 2a (PBP2a), a process that is regulated by the mecA gene (1). Protein-bound polysaccharides (PBPs), a group of enzymes found in the cell membrane of *S. aureus*, are responsible for catalysing the trans-peptidation process that leads to the formation of peptidoglycan chains, which serve as cross-linkers in the cell wall. PBP2a has an extremely low affinity for beta-lactam antibiotics, which allows MRSA to survive even when exposed to high concentrations of antimicrobial agents (7).

The expression of mecA is a critical determinant of the MRSA phenotype; however, it is widely acknowledged that numerous factors can impact the MRSA phenotype (8,9). Typically, these factors are more widely recognised in mecA-negative MRSA cases. Although mecA-positive MSSA has been identified, a comprehensive report of such cases in humans is lacking thus far (10). These strains have also been documented in animals. The emergence of mecA-positive MSSA obscures the antibiotic resistance profile. Treating these cases with broad-spectrum antibiotics or empiric treatment like in ordinary MSSA cases could accelerate the pathogen's resistance phenotype; however, overly aggressive treatment could also lead to potential hazards (11).

Moreover, given that mec-A-positive MSSA is relatively rare, a comprehensive analysis of this pathogen is essential. One such investigation involves identifying the associated virulence genes. The presence of numerous virulence factors is directly linked to the ability of a pathogen to cause infection. Numerous strains of *S. aureus*, particularly MRSA, discharge one or more distinct staphylococcal exotoxins, including staphylococcal enterotoxins, which are essential pathogenic components of the superantigen family (12).

Panton–Valentine leukocidin (PVL) is a bacterial toxin comprising two separate components: LukS-PV and LukF-PV. Numerous studies have demonstrated a connection between the presence of PVL genes and severe infections, leading researchers to classify PVL as an epidemiological marker of severe diseases. Consequently, in certain nations, public health measures have been implemented for individuals infected with PVL-producing strains (13).

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Exfoliative toxins (ETs) are pathogenic factors secreted by staphylococci and are critical components of staphylococcal skin infections. They are virulence agents that target desmoglein 1 (Dsg1) in mammals and play key roles in exfoliation. Studies have shown that exfoliation caused by ETs is observed in a variety of host organisms, yet the susceptibility to this effect varies, suggesting a degree of host specificity (14).

This study aimed to analyze the clinical, microbial and molecular characteristics of mecA-positive MSSA strains.

Materials and Methods

This was a case series observational study employing a cross-sectional design, which aimed to examine the occurrence of mecA-positive MSSA within our urban tertiary referral centre, specifically accepting patients from North Sumatra, Sumatra Island. Informed consent was obtained from the patients.

Due to the scarcity of these cases, purposive sampling with rigorous inclusion criteria was used. Our description encompassed cases of mecA-positive MSSA regardless of demographic characteristics. The isolates were obtained from Adam Malik General Hospital. Subsequently, the samples were cultured on nutrient agar medium, and colonies suspected to be *S. aureus* were subjected to Gram staining and catalase, oxidase, and coagulase tests. These colonies were further cultured in mannitol salt agar (MSA) medium, and antibiotic susceptibility was determined using the VITEK-2 Compact system. After a 24-hour incubation period, the suspension was transferred to an Eppendorf tube and stored at -80 °C. The corresponding clinical data of the patients were collected either through direct examination or by consulting medical records. In cases of interest, cefoxitin disk diffusion (DD) tests were performed using Muller–Hinton agar, with the CLSI criteria applied to define resistance, which was determined by the diameter of non-growth below 21 mm (15).

DNA extraction

DNA was extracted from bacterial cells using the Presto Mini gDNA Bacteria Kit (Geneaid, Germany). One million bacterial cells were added to a sterile 1.5 mL tube and spun at 9500 G for 1 min to separate the cells from the liquid. The supernatant was removed and replaced with 200 µL of buffer, including 0.8 mL of lysozyme, which was mixed by vortexing. The solution was incubated at 20 °C for 5 min, and then 20 µL of proteinase K was added and mixed again. The mixture was heated at 70 °C for 10 min, followed by the addition of 200 µL of absolute ethanol. The solution was mixed once more and centrifuged to separate the liquid from the DNA. DNA was then extracted using a GD column, and 400 µL of wash buffer was added to the collection tube, which was centrifuged. The liquid was then removed and replaced with 600 µL of wash buffer, followed by the addition of 100 µL elution buffer. The tube was then stored at -20 °C.

mecA gene identification

For the polymerase chain reaction (PCR) of the mecA gene, specific primers were employed following a search of the NCBI search engine. The primer specifications are detailed in Table 1.

Table 1. Primer specifications.

Gene		Accession Number	Sequence (5'-3')	Amplitude Size (bp)	Reference
mecA	**F**	OR255918.1	AAAATCGATGGTAAAGGTTGGC	533	Shopsin *et al.*, 1999 (16).
	R		AGTTCTGCAGTACCGGATTTGC		
pvl	***F***	OP997649.1	ACAAGCAAAAGAATACAGCG	575	Hesari *et al.*, 2018 (17).
	R		GTTTTTGGCTGCTTCTCTT		
eta	***F***	CP127551.1	TTTGCTTTCTTGATTTGGATT	464	Mohseni *et al.*, 2018 (18).
	R		GATGTGTTCGGTTTGATTGAC		

***F: Forward, R: Revers.**

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Next, a PCR master mix was created by first liquefying GoTaq Green Master Mix 2x, primers (forward and reverse), nuclease-free water, and the DNA template. These were then vortexed and spun for 10 s. The PCR mix was created with samples to obtain one sample. The mixture was vortexed to ensure suspension homogeneity. Finally, the DNA template was then added, and thermal cycling was performed.

Electrophoresis of PCR products

The PCR products were run on a 2% agarose gel (Merck, Germany) using a mixture of 1 ul DNA loading dye and 5 ul PCR products. The gel was electrophoresed at 80 V.

Statistical analysis

The data were analysed using Microsoft Excel. Patient demographics and clinical, microbial, and molecular characteristics were reported as percentage frequencies, and descriptive comparisons were performed.

Results

Five cases of *mecA*-positive MSSA were detected between January 2022 and December 2022. These cases tested positive for mecA expression but were identified as MSSA using the VITEK-2 Compact system. The cefoxitin DD test performed on Mueller–Hinton agar confirmed that all pathogens were susceptible to methicillin. Four of the five patients were geriatric, and the majority were male. The majority of patients were diagnosed with SAB, with four of five patients having pneumonia and the fifth having cellulitis. Three patients recovered, and two died during treatment. The demographic characteristics of the patients are presented in Table 2.

Table 2. Patient demographic characteristics

Patient	Age	Gender	Infection Foci	Outcome
1	26	L	Pneumonia	Recovery
2	71	L	Pneumonia	Recovery
3	69	P	Pneumonia	Death
4	54	L	Cellulitis	Recovery
5	66	L	Pneumonia	Death

Clinically, most patients exhibited leukocytosis, while one patient had a relatively typical leukocyte count. One patient had a TB comorbidity, requiring standard TB treatment in addition to broad-spectrum antibiotics. Patients who received meropenem were of interest, particularly those who died and were administered vancomycin. This suggests that the clinician had doubts about the culture results and their association with broad-spectrum antibiotics. The duration of hospital stay for the two fatal cases was relatively long, lasting 40 days and 17 days. The clinical characteristics are shown in Table 3.

Table 3. Patient clinical characteristics.

Patient ID	Leucocyte (/uL)	Procalcitonin (ng/mL)	Antibiotics at First Encounter	Antibiotics at Treatment Ending	Length of Hospital Stay (day)
1	12,870	40,42	Anti-tuberculosis treatment; Ceftriaxone; Meropenem	Anti-tuberculosis treatment; Ceftriaxone; Meropenem	15
2	14,610	-	Ceftriaxone	Cefixime	8
3	19,820	3,44	Levofloxacin; Ceftriaxone	Azithromycin; Cefixime	40
4	19,110	-	Ampicillin; Chloramphenicol; Clindamycin	Ampicillin; Chloramphenicol; Clindamycin	8
5	5020	26,4	Meropenem	Ceftazidime; Vancomycin	17

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Following the collection of patient samples, PCR was performed to assess the expression of mecA. In each sample, mecA was detected at a length of 533 bp, corresponding to the expected size, as indicated by the specific primers employed (Fig. 1).

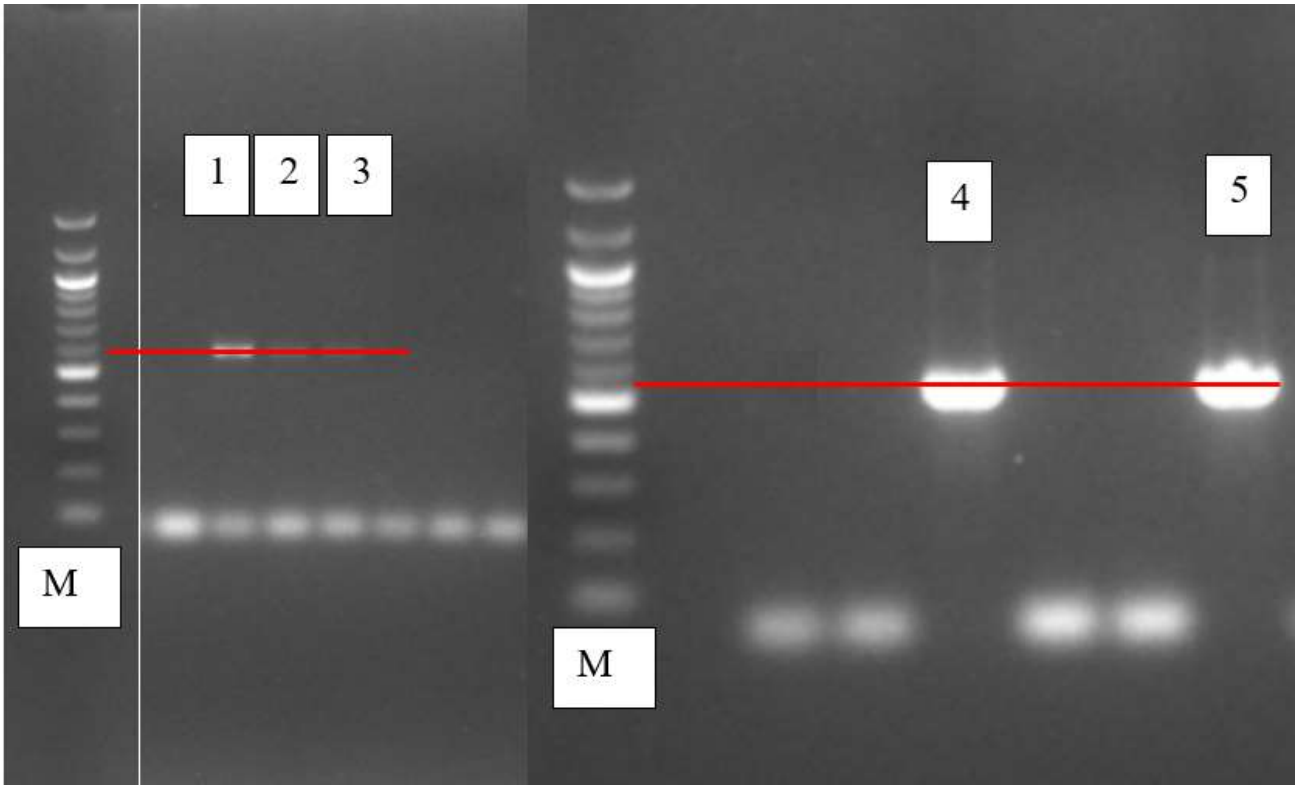

Fig. 1. Electrophoresis result of mecA PCR amplification showing *mecA* (+) MSS; (M: Marker; red line: mecA detection in 533 bp); (1): sample 1; (2): sample 2; (3): sample 3; (4): sample 4; (5) sample 5. The figure shows electrophoresis visualization which revealed expression of mecA in 533 bp for all five samples in all MSSA sample that we tested.

A second examination was conducted using the cefoxitin DD test on Muller–Hinton agar to confirm the diagnosis and prevent misdiagnosis resulting from human error. The results from all cases revealed a diameter consistent with the interpretation of sensitivity. The microbiological and molecular data of these cases are presented in Table 4.

The visualisations of the cefoxitin DD test are presented in Fig. 2.

Table 4. Patient molecular characteristics.

Patient	VITEK-2 Compact Results	mecA Expression	Cefoxitin DD Test (cm)	Other Virulence Genes
1	MSSA	(+)	31.2	pvl (+), eta (+)
2	MSSA	(+)	32.1	pvl (+), eta (+)
3	MSSA	(+)	29.6	pvl (+), eta (+)
4	MSSA	(+)	29.7	pvl (+), eta (+)
5	MSSA	(+)	27	pvl (+), eta (+)

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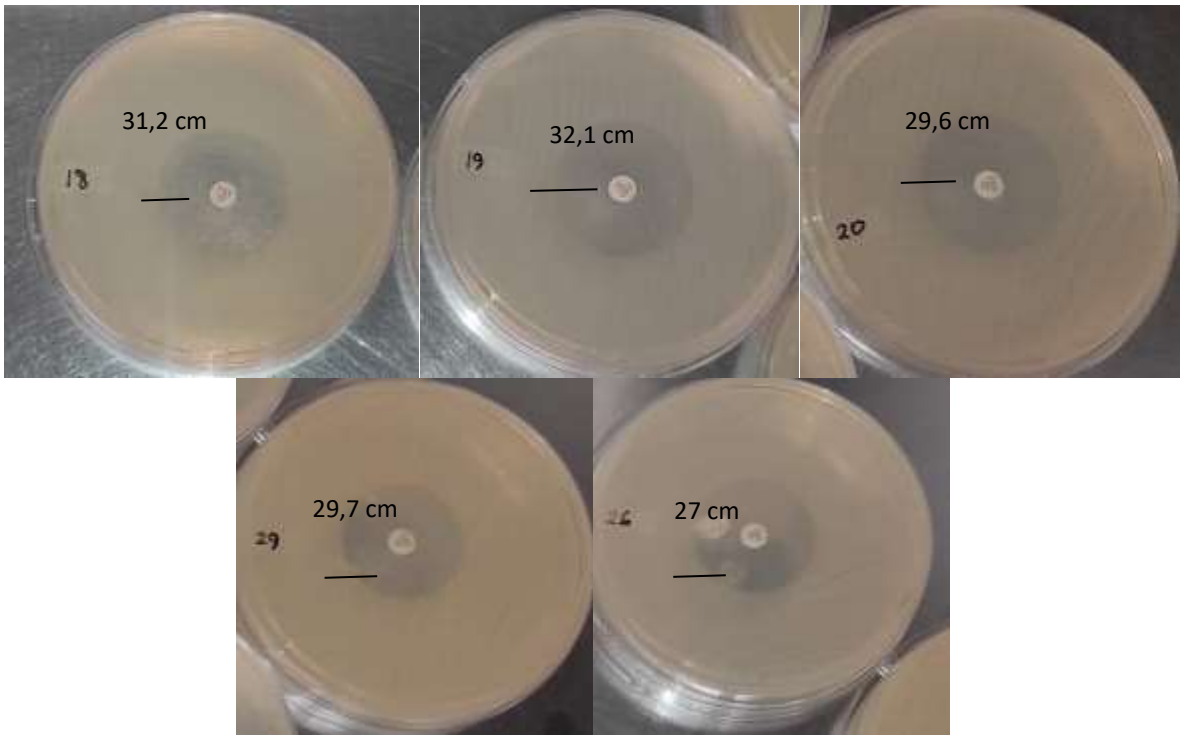

Fig. 2. Cefoxitin DD test results of all cases with respective labels. The line described zone of inhibition diameter. All figures were the result of cefoxitin DD test acquired from sample after electrophoresis determination. We found a large zone of inhibition in all five samples.

Discussion

We documented five cases of mecA-positive MSSA in our urban tertiary referral centre. Given the significant number of patients we received at the Adam Malik General Hospital, this represents an exceedingly small fraction. This observation is consistent with Chen et al.'s research in Taiwan, which identified six mecA-positive MSSA cases through oxacillin testing. Following cefoxitin screening, three cases with mecA-positive MSSA remained. Upon conducting a VITEK-2 Compact examination, six additional cases of mecA-positive MSSA were detected (12). Our study's novelty is that we comprehensively analysed the clinical and molecular characteristics of our cases. On the other hand, Chen et al. focused on reporting typing results.

From Chen et al.'s epidemiology typing, it was discovered that the majority of the unique specimens were CC59:SCCmec V:pvl (+), identified in wound samples collected from various locations and years, suggesting that these findings were not indicative of an outbreak (19).

Liu et al. reported 17 cases of oxacillin-susceptible mecA-positive *S. aureus* in their centre. These cases were found to be quite diverse, with 10 belonging to ST59, three to ST965, 11 carrying SCCmec type IV and five carrying SCCmec type V. Only one of these cases was PVL-positive, and 16 had one- or two-point mutations within the mecA promoter. These point mutations may explain the phenotype, but upon further examination, only two cases met the CLSI criterion of 21 mm as a cutoff for mecA positivity (20, 21).

Proulx et al.'s research is more sophisticated in that it demonstrates the transformation of mecA-positive MSSA into MRSA. The authors posit two specific mechanisms for this transformation: (1) precise excision of the transposable element IS1181 from within mecA and (2) slip-strand replication errors within mecA. Hypothesis 2 proposes that all mecA-positive MSSA will eventually revert to MRSA because of the shared sequence properties of all mecA genes. This phenomenon is especially prevalent when antibiotic therapy is initiated (11).

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Finally, a novel finding in our study was pvl and eta expression in these unique pathogens. The presence of these genes may indicate an increased risk of worsening infection within the group. To the best of our knowledge, no other study has reported this result.

Laboratory techniques that combine genotypic and phenotypic testing can offer a more comprehensive view of infecting strains to clinicians, thus enabling a more effective therapeutic strategy. By distinguishing between MRSA, MSSA and mecA-positive MSSA, clinicians can employ a combination of beta-lactams and secondary agents to control any minor revertant populations that may arise during therapy. These methods provide a more complete picture of the infecting strains, which can lead to a more effective treatment plan (11). This is also consistent with Amara's studies, which suggest the D-test for assessing inducible clindamycin resistance in daily routine work combined with a laboratory framework (22). Torki et al. adapted this approach in infertile men by combining PCR and culture-based methods, thus identifying the infectious cause of infertility (23).

Ethics Committee Code

This study was approved by the Universitas Sumatera Utara Ethics Committee (letter number 540/KEPK/USU/2022).

Written Informed Consent

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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