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Prevalence Assessment of the *mecC* Gene Among MRSA Clinical Isolates in a Malaysian Teaching Hospital

(Penilaian Kelaziman Gen *mecC* dalam Kalangan Pencilan Klinikal MRSA di Sebuah Hospital Pengajar di Malaysia)

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) resistance is predominantly mediated by the *mecA* gene; a novel homologue, *mecC*, has emerged among livestock-associated MRSA, and to date no data exist on the prevalence of human *mecC*-MRSA in Malaysia. This retrospective cross-sectional study aimed to determine the prevalence of *mecC* gene carriage and characterise the antimicrobial susceptibility profiles of 89 archived clinical MRSA isolates from 2023, using conventional PCR for gene detection and the VITEK 2 system for antimicrobial susceptibility testing. All isolates were *mecA*-positive, and none carried *mecC*. High resistance rates were observed against ciprofloxacin (89.9%, MIC_{90} ≥8 µg/mL), levofloxacin (88.8%, MIC_{90} ≥8 µg/mL), moxifloxacin (88.8%, MIC_{90} 4 µg/mL), erythromycin (66.3%, MIC_{90} ≥8 µg/mL) and clindamycin (65.2%, MIC_{90} ≤0.25 µg/mL). All isolates remained susceptible to vancomycin (MIC_{90} 1 µg/mL), linezolid (MIC_{90} 2 µg/mL), quinupristin/dalfopristin (MIC_{90} ≤0.25 µg/mL), tigecycline (MIC_{90} 0.25 µg/mL), nitrofurantoin (MIC_{90} 32 µg/mL) and rifampicin (MIC_{90} ≤0.5 µg/mL). Susceptibility to trimethoprim/sulphamethoxazole and tetracycline was 95.5% (MIC_{90} ≤10 µg/mL and ≤1 µg/mL, respectively). One *mecA*-positive isolate was susceptible to oxacillin (MIC 0.5 µg/mL) and negative on cefoxitin screening, consistent with the oxacillin-susceptible MRSA (OS-MRSA) phenotype. This study confirms *mecA* as the sole methicillin-resistance determinant in this hospital; the absence of *mecC* likely reflects limited livestock exposure in this urban population, although surveillance in agricultural regions remains warranted. The detection of OS-MRSA highlights the limitations of relying solely on phenotypic screening and supports incorporating molecular assays to prevent misclassification.

Keywords: *mecA*; *mecC*; microbial sensitivity tests; MRSA; prevalence

ABSTRAK

Rintangan *Staphylococcus aureus* rintang metisilin (MRSA) lazimnya dimediasi oleh gen *mecA*; gen baharu, *mecC*, turut dikenal pasti memberikan rintangan metisilin, khususnya dalam MRSA berkait ternakan dan setakat ini tiada data kelaziman *mecC*-MRSA pada manusia di Malaysia. Kajian keratan rentas retrospektif ini bertujuan untuk menentukan kelaziman pembawaan gen *mecC* serta mencirikan profil kerentanan antibiotik dalam kalangan 89 pencilan MRSA klinikal terarkib pada tahun 2023, menggunakan amplifikasi PCR konvensional untuk pengesanan gen dan sistem VITEK 2 untuk ujian kerentanan antibiotik. Kesemua pencilan didapati positif *mecA* dan tiada yang membawa *mecC*. Kadar kerintangan tinggi diperhatikan terhadap siprofloksasin (89.9%, MIC_{90} ≥8 µg/mL), levofloksasin (88.8%, MIC_{90} ≥8 µg/mL), moksifloksasin (88.8%, MIC_{90} 4 µg/mL), eritromisin (66.3%, MIC_{90} ≥8 µg/mL) dan klindamisin (65.2%, MIC_{90} ≤0.25 µg/mL). Kesemua pencilan kekal rentan terhadap vankomisin (MIC_{90} 1 µg/mL), linezolid (MIC_{90} 2 µg/mL), kinupristin/dalfopristin (MIC_{90} ≤0.25 µg/mL), tigesiklin (MIC_{90} 0.25 µg/mL), nitrofurantoin (MIC_{90} 32 µg/mL) dan rifampicin (MIC_{90} ≤0.5 µg/mL). Kerentanan terhadap trimetoprim/sulfametoksazol dan tetrasiklin adalah 95.5% (MIC_{90} ≤10 µg/mL dan ≤1 µg/mL). Satu pencilan positif *mecA* rentan terhadap oksasilin (MIC 0.5 µg/mL) dan negatif dalam saringan sefoksitin, selaras dengan fenotip MRSA rentan oksasilin (OS-MRSA). Kajian ini mengesahkan *mecA* sebagai penentu tunggal kerintangan metisilin dalam kalangan MRSA klinikal di hospital ini; ketiadaan *mecC* mungkin mencerminkan pendedahan ternakan yang terhad dalam populasi bandar, walaupun pemantauan di kawasan pertanian tetap diperlukan. Pengesanan OS-MRSA menekankan keterbatasan

penggunaan kaedah saringan fenotip semata-mata serta menyokong penggabungan ujian molekul bagi mengelakkan salah pengelasan.

Kata kunci: Kelaziman; *mecA*; *mecC*; MRSA; ujian kepekaan mikrob

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a significant global health threat and is broadly classified into three epidemiological forms: community-associated (CA-MRSA), hospital-associated (HA-MRSA) and livestock-associated MRSA (LA-MRSA) (Paterson, Harrison & Holmes 2014). Resistance is primarily mediated by the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with low affinity for *β*-lactam antibiotics (Peacock & Paterson 2015). While phenotypic detection via cefoxitin disc diffusion or cefoxitin/oxacillin broth microdilution is most frequently employed in clinical laboratories, molecular detection of the *mecA* gene by polymerase chain reaction (PCR) remains the diagnostic gold standard (CDC 2024).

However, the emergence of the *mecA* homologue, *mecC*, has introduced additional complexity to the traditional MRSA diagnostic algorithms. The gene was first identified through whole-genome sequencing of a bovine-associated *S. aureus* strain (LGA251) in 2007. The *mecC*-carrying isolate was phenotypically methicillin-resistant but tested negative for *mecA* by PCR and for PBP2a by latex agglutination (García-Álvarez et al. 2011). This discordance highlighted a significant diagnostic gap and raised concerns regarding the potential misclassification of *mecC*-MRSA, as current protocols are primarily designed for *mecA* detection (Kriegeskorte et al. 2018). Given the recognised potential for zoonotic transmission of *mecC*-positive MRSA (Albert et al. 2023), and the lack of human *mecC* prevalence data in Malaysia, this study aims to assess the prevalence of *mecC* and characterise the antibiotic susceptibility profiles of clinical MRSA isolates in Hospital Canselor Tuanku Muhriz (HCTM) UKM, a major 1000-bed tertiary hospital in Kuala Lumpur serving the diverse population of the Klang Valley. Contemporary global surveillance confirms that *mecC*-MRSA remains an epidemiologically minor but persistent genotype relative to *mecA*-MRSA (Suleiman, Bhattacharya & Islam 2025), and genomic evidence continues to point to unresolved cross-species transmission pathways between wildlife/livestock reservoirs and humans (Miller et al. 2024), underscoring the continued relevance of regional prevalence surveillance such as the present study.

MATERIALS AND METHODS

STUDY DESIGN AND ISOLATE COLLECTION

This retrospective cross-sectional study analysed archived clinical MRSA isolates collected between 1 January and 31 December 2023 at the Microbiology Laboratory/ Infection Control Unit, Hospital Canselor Tuanku Muhriz UKM (HCTM UKM). The archive comprised MRSA isolates recovered from two categories of clinical specimens: (i) diagnostic specimens, i.e., specimens submitted for the investigation of suspected active infection, comprising wound swabs/pus aspirate, blood, respiratory specimens, urine and tissue/bone; and (ii) screening specimens, comprising nasal swabs collected for MRSA carriage surveillance rather than active infection. All diagnostic isolates had previously been identified as MRSA by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), followed by cefoxitin disc diffusion or oxacillin minimum inhibitory concentration (MIC) testing. Screening isolates were identified based on positive DNase and coagulase tests, followed by cefoxitin disc diffusion. All the isolates were preserved on cryopreservation beads at –80 °C until retrieval for this study.

SAMPLE SIZE

The sample size was calculated using the following finite population correction formula (Naing, Winn & Rusli 2006). Using a total archived population (N) of 310, a 95% confidence level (Z=1.96), 5% precision (d=0.05), and an expected prevalence of 16% based on recent livestock-associated *mecC* prevalence in Malaysia (Aklilu & Ying 2020), the minimum required sample size was calculated to be 87 isolates.

SAMPLING METHOD

From the archived collection of 310 MRSA isolates, we excluded 165 duplicates (isolates from the same patient) and isolates with incomplete phenotypic data, leaving 145 eligible isolates. From this pool, 90 isolates were randomly selected for *mecA* and *mecC* PCR detection using an online random number generator (https://www.calculator.net/random-number-generator.html). One isolate was subsequently excluded after it was confirmed to be a methicillin-susceptible *S. aureus* (MSSA) strain (due to the absence of both *mec* genes and the typical MRSA resistance phenotype), suggesting a misclassification at the time of archiving. This resulted in a final study cohort of 89 isolates, comprising 79 diagnostic and 10 screening specimens. All screening specimens were collected via nasal swabs. Among the diagnostic specimens, 27 (34.2%) were from bone or tissue specimens, 22 (27.8%) from respiratory sources, 16 (20.3%) from pus or swab samples, 13 (16.5%) from blood, and one (1.3%) isolate was from urine.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing (AST) was performed using phenotypic methods. To ensure a standardised dataset across all isolates, susceptibility profiles were determined by measuring the minimum inhibitory concentration (MIC) using the VITEK 2 automated system (bioMérieux, France) with AST-GP67 cards. Since the VITEK 2 system was only introduced to the hospital in the last quarter of 2023, prior archived diagnostic isolates tested via the Kirby-Bauer disc diffusion method (n=71)(hence lacked MIC data), as well as screening isolates that were only tested for oxacillin resistance (n=10), were retested using the VITEK 2 system.

Bacterial suspensions were prepared according to the manufacturer's instructions. Fresh colonies from overnight cultures on Columbia blood agar were suspended in 0.45% saline solution and adjusted to a turbidity of 0.50–0.63 McFarland units. The prepared suspensions were loaded into the instrument together with the AST-GP67 test cards. The AST-GP67 card panel includes a qualitative cefoxitin screen and MIC testing for the following antibiotics: oxacillin, penicillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, vancomycin, tetracycline, tigecycline, nitrofurantoin, rifampicin, and trimethoprim/sulfamethoxazole. The panel also includes testing for inducible clindamycin resistance (ICR), which, if positive, will automatically report clindamycin as 'resistant', regardless of the initial MIC. AST was performed automatically, with an incubation period up to 18 h for *Staphylococcus* spp. Upon completion, MIC values were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints. MIC_{50} and MIC_{90} values were also calculated for each antibiotic.

DNA EXTRACTION FOR PCR AMPLIFICATION

Cryopreserved isolates were thawed at room temperature and inoculated onto Columbia agar with 5% sheep blood (Isolab Sdn. Bhd., Malaysia) and grown overnight at 37 °C in 5% CO_2. All isolates demonstrated viable bacterial growth. DNA was extracted from fresh colonies using the GF-1 Bacterial DNA Extraction Kit (Vivantis Technologies, Malaysia) according to the manufacturer's instructions. DNA concentration and purity were assessed using the Eppendorf BioSpectrometer® fluorescence system (Eppendorf AG, Germany). The extracted DNA was stored at –40 °C until further processing.

PCR REACTION MIXTURE

Each PCR reaction was performed in a total volume of 25 µL, containing: 15 µL of 2× DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA); 1 µL each of the gene target's forward and reverse primers at a final concentration of 0.4 µM each; 1 µL each of the internal control's forward and reverse primers (Integrated DNA Technologies, Singapore) at a final concentration of 0.4 µM each; 4 µL of nuclease-free water; and 2 µL of extracted DNA template. Amplification of *mecA* and *mecC* targets was performed in separate reactions. Specific primers targeting *mecA*, *mecC*, and the *Staphylococcus* genus-specific 16S rRNA gene (internal control) used for amplification are shown in Table 1. A previously characterised MRSA strain (M106_2017), sequenced by the UKM Medical Molecular Biology Institute (UMBI), Malaysia, was used as the *mecA*-positive control (Ang et al. 2022). *Staphylococcus aureus* ATCC BAA-2313 (Strain M10/0148) was used as the *mecC*-positive control (Goncalves et al. 2023), while *Staphylococcus aureus* ATCC 29213 (methicillin-sensitive *Staphylococcus aureus*) was included as the negative control.

PCR CYCLING CONDITIONS

PCR was performed using a T100 Thermal Cycler (Bio-Rad, USA) under the following conditions:

i) *mecA* (Pournajaf et al. 2014)

Initial denaturation at 95 °C for 3 min, followed by 33 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 30 s, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 6 min.

ii) *mecC* (Stegger et al. 2012)

Initial denaturation at 94 °C for 15 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 1 min, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min.

GEL ELECTROPHORESIS

PCR products were analysed by electrophoresis on a 2% agarose gel prepared in 1× Tris-borate-EDTA (TBE) buffer (Vivantis Technologies, Malaysia) and stained with 10 µL of FloroSafe DNA Stain (1st BASE, Singapore). Electrophoresis was conducted at 100 volts for 40 min. A 100 bp DNA ladder (Biohelix, Taiwan) was loaded in the first lane as a molecular size marker. Gel images were captured using the AlphaImager HP gel documentation system (ProteinSimple, USA).

ETHICAL APPROVAL

This study was approved by the Research Ethics Committee, Universiti Kebangsaan Malaysia (RECUKM) (Date: 9 July 2024, Number: JEP-2024-511).

TABLE 1. Primer sequences used in this study

Target gene	Nucleotide sequence (5′ - 3 ′)	Amplicon size (bp)	Reference
mecA			
Forward	AAAATCGATGGTAAAGGTTGGC	533	(Murakami et al. 1991)
Reverse	AGTTCTGGAGTACCGGATTTGC		
mecC			
Forward	GAAAAAAAGGCTTAGAACGCCTC	138	(Stegger et al. 2012)
Reverse	GAAGATCTTTTCCGTTTTCAGC		
16S rRNA			
Staph756F	AACTCTGTTATTAGGGAAGAACA	756	(Mcclure et al. 2006)
Staph750R	CCACCTTCCTCCGGTTTGTCACC		

RESULTS

PREVALENCE OF *mecC*

Conventional PCR was utilised to detect the *mecA* and *mecC* genes. Positive results were indicated by the presence of single amplicon bands at 533 bp for *mecA* and 138 bp for *mecC*. All 89 isolates were positive for the *mecA* gene (Figure 1), whereas no band corresponding to *mecC* was detected (Figure 2). These results confirm that none of the clinical isolates in this study harboured the *mecC* gene.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antimicrobial susceptibility testing (AST) parameters are detailed in Table 2 and Figure 3. All 89 isolates were resistant to penicillin ($MIC_{50/90} \geq 0.5$ µg/mL). High resistance rates were also observed among the fluoroquinolones, specifically ciprofloxacin (89.9%, n = 80; $MIC_{50/90} \geq 8$ µg/mL), levofloxacin (88.8%, n = 79; $MIC_{50/90} \geq 8$ µg/mL), moxifloxacin (88.8%, n = 80; $MIC_{50/90}$ 2/4 µg/mL), as well as erythromycin (66.3%, n = 59; $MIC_{50/90} \geq 8$ µg/mL). Although the MIC_{50} and MIC_{90} of clindamycin were low (both ≤ 0.25 µg/mL), inducible clindamycin resistance was common. Fifty isolates (56.2%) exhibited inducible clindamycin resistance while 8 (9.0%) exhibited constitutive resistance. Accounting for both mechanisms, the overall clindamycin non-susceptibility rate reached 65.2% (n = 58), leaving only 34.8% (n = 31) of the cohort susceptible and ICR-negative. In contrast, all 89 isolates remained fully susceptible to vancomycin (MIC_{90} = 1 µg/mL), linezolid (MIC_{90} = 2 µg/mL), quinupristin/dalfopristin ($MIC_{90} \leq 0.25$ µg/mL), tigecycline (MIC_{90} = 0.25 µg/mL), nitrofurantoin (MIC_{90} = 32 µg/mL), and rifampicin ($MIC_{90} \leq 0.5$ µg/mL). High susceptibility was also recorded for trimethoprim/sulfamethoxazole and tetracycline (both 95.5%, n = 85; $MIC_{90} \leq 10$ µg/mL and ≤ 1 µg/mL, respectively), as well as gentamicin (92.1%, n = 82; $MIC_{90} \leq 0.5$ µg/mL).

INCIDENTAL FINDING OF OXACILLIN-SUSCEPTIBLE MRSA (OS-MRSA)

Of the 89 *mecA*-positive MRSA isolates, 88 exhibited the expected resistance to both oxacillin (MIC ≥ 4 µg/mL) and cefoxitin. However, one isolate was consistently susceptible to oxacillin (MIC = 0.5 µg/mL) and tested negative on the VITEK 2 cefoxitin screen. The presence of the *mecA* gene in this isolate was confirmed by PCR in triplicate (Figure 4). This finding is consistent with an oxacillin-susceptible MRSA (OS-MRSA) phenotype (Table 3). To verify the discrepancy with the previously reported cefoxitin-resistant disc diffusion result from 2023, cefoxitin (30 µg) disc diffusion testing was repeated. It yielded a similarly susceptible result (zone of inhibition = 30 mm).

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene is reportedly less common than those harbouring its homologue, *mecA*, with global prevalence rates of approximately 2.4% and 14.6%, respectively (Suleiman, Bhattacharya & Islam 2025). Although *mecC*-MRSA infections in humans are still rare, they pose significant diagnostic challenges, as many routinely used phenotypic and genotypic assays fail to detect this gene with complete accuracy (Kriegeskorte et al. 2018; Paterson et al. 2014). Moreover, severe and fatal infections attributable to *mecC*-MRSA have been reported (García-Garrote et al. 2014).

At the time of writing, no published data are available on the prevalence of *mecC*-MRSA among human clinical isolates in Malaysia. This study therefore provides empirical baseline data on *mecC* carriage among clinical MRSA isolates in a Malaysian tertiary hospital setting, addressing a previously unexamined gap in national antimicrobial resistance surveillance. Although *mecC* was not detected in this cohort, this finding is still worth noting and is consistent with reports from neighbouring Singapore (Chew, Lin & Teo 2021) and Turkey (Ceylan, Sumbul & Doymaz 2022; Cikman et al. 2019; Erdoğan,

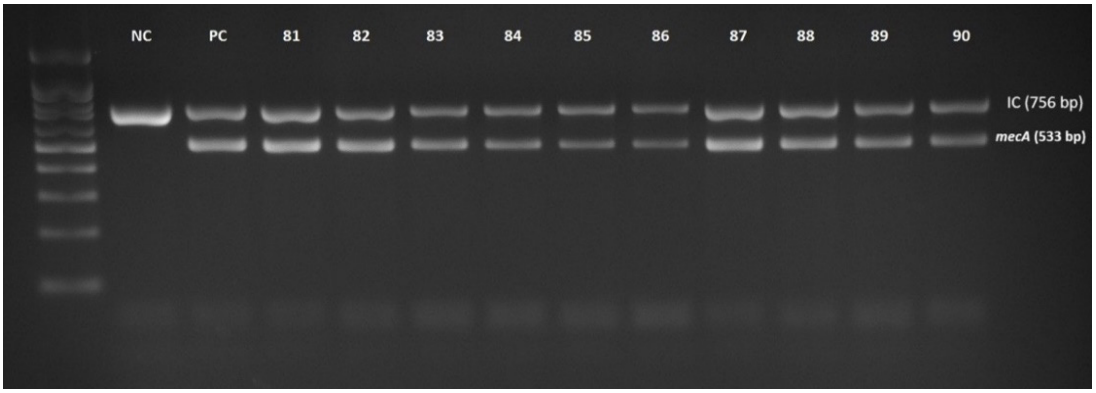


FIGURE 1. Representative gel image showing *mecA* (533 bp) and 16S rRNA (IC/internal control, 756 bp) amplification by conventional PCR. Lanes (left to right): 100 bp DNA ladder, negative control (NC), positive control (PC), and test samples. *mecA* detected in samples 81-90

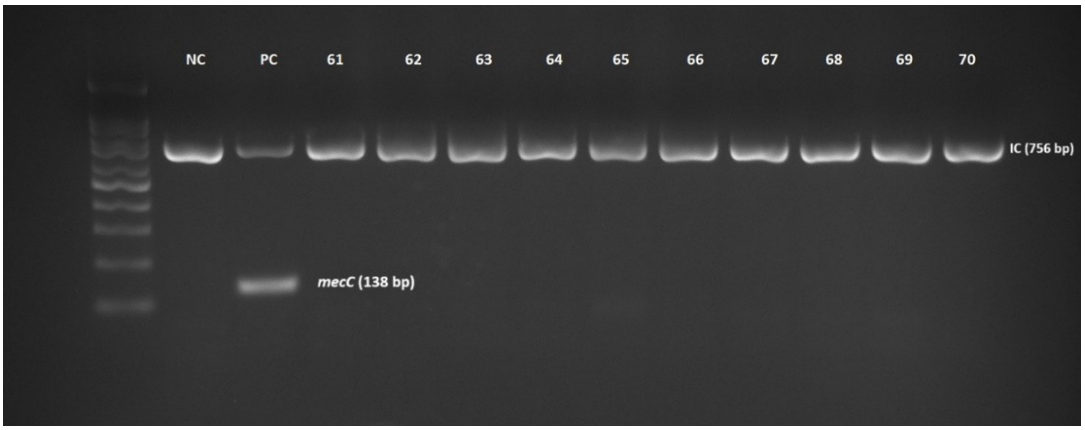


FIGURE 2. Representative gel image of conventional PCR for the detection of *mecC* (138 bp) and 16S rRNA (IC/internal control, 756 bp). Lanes (left to right): 100 bp DNA ladder, negative control (NC), positive control (PC), and test samples. Samples 61-70 show no visible *mecC* bands

Acer & İnal 2024), where *mecC*-MRSA has similarly been undetected in human isolates. This regional distribution likely reflects the limited direct livestock exposure in predominantly urban populations, where agricultural and farming activities are minimal (Chew, Lin & Teo 2021). In contrast, *mecC*-MRSA has been reported primarily in patients from several European countries, including England, Denmark, Slovenia, and Spain (Dermota et al. 2015; García-Garrote et al. 2014; Harrison et al. 2017), as well as in Egypt (Shebl et al. 2020) and Pakistan (Idrees et al. 2023; Khan et al. 2020). Notably, many *mecC*-positive isolates were found to harbour both *mecA* and *mecC* resistance genes simultaneously (Idrees et al. 2023).

Beyond human hosts, *mecC*-MRSA has been widely detected across diverse animal populations. In Europe, it is particularly prevalent among hedgehogs (*Erinaceus europaeus*), with carriage rates approaching 60% (Bengtsson et al. 2017; Rasmussen et al. 2019), suggesting that hedgehogs may act as a natural reservoir (Dube et al. 2021). Larsen et al. (2022) further proposed that livestock-associated *mecC*-MRSA may have emerged even before the introduction of antibiotics, likely driven by natural selection from the colonisation of a *β*-lactam-producing dermatophyte, *Trichophyton erinacei* in hedgehogs (Larsen et al. 2022). Additional reservoirs include dairy cattle; the species in which *mecC* was first discovered (Bietrix et al. 2019; García-Álvarez et al. 2011), as well as sheep (Giacinti et al. 2017), cats (Worthing et al. 2016) and horses (Albert et al. 2023). In addition to colonising multiple host species, *mecC*-harbouring isolates may persist in farm environments for many years, further complicating eradication efforts (Bietrix et al. 2019).

In Malaysia, despite no human *mecC*-MRSA being detected in our study, *mecC* has been identified in 15.8%

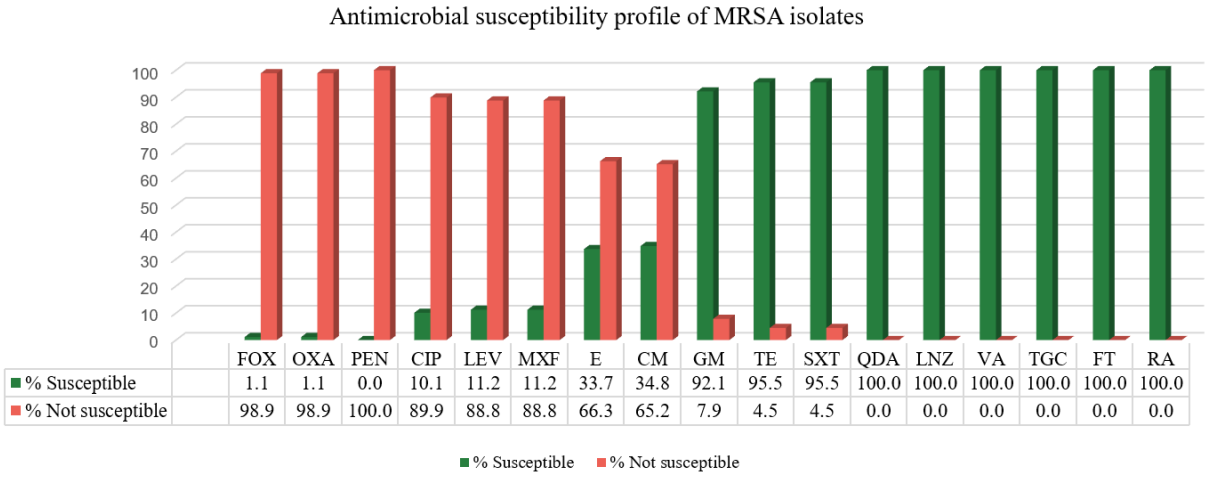
TABLE 2. MIC_{50} and MIC_{90} of clinical MRSA isolates (n = 89) by antibiotic, VITEK 2 AST-GP67

Antibiotic	MIC range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Penicillin	≥ 0.5	≥ 0.5	≥ 0.5
Oxacillin	0.5 – ≥ 4	≥ 4	≥ 4
Gentamicin	≤ 0.5 – ≥ 16	≤ 0.5	≤ 0.5
Ciprofloxacin	≤ 0.5 – ≥ 8	≥ 8	≥ 8
Levofloxacin	≤ 0.12 – ≥ 8	≥ 8	≥ 8
Moxifloxacin	≤ 0.25 – 4	2	4
Erythromycin	≤ 0.25 – ≥ 8	≥ 8	≥ 8
Clindamycin	≤ 0.25 – ≥ 8	≤ 0.25	≤ 0.25
Quinupristin/dalfopristin[a]	≤ 0.25 – 1	≤ 0.25	≤ 0.25
Linezolid	1 – 4	2	2
Vancomycin	≤ 0.5 – 2	≤ 0.5	1
Tetracycline	≤ 1 – ≥ 16	≤ 1	≤ 1
Tigecycline	≤ 0.12 – 0.5	≤ 0.12	0.25
Nitrofurantoin	≤ 16 – 32	32	32
Rifampicin	≤ 0.5	≤ 0.5	≤ 0.5
Trimethoprim/sulfamethoxazole[b]	≤ 10 – ≥ 320	≤ 10	≤ 10

MIC_{50}/MIC_{90}: the MIC at or below which ≥50%/≥90% of isolates were inhibited, respectively. ≤ and ≥ denote values at the lowest/highest concentration tested on the VITEK 2 AST-GP67 card; the true MIC may lie beyond this range. Cefoxitin (FOX) is a qualitative screen only (88/89, 98.9% positive; 1/89, 1.1% negative) and is not included above

[a]MIC is reported as the concentration of the first antimicrobial (quinupristin)

[b]MIC is reported as the sum concentrations of trimethoprim and sulfamethoxazole



FOX: cefoxitin, OXA: oxacillin, PEN: penicillin, CIP: ciprofloxacin, LEV: levofloxacin, MXF: moxifloxacin, E: erythromycin, CM: clindamycin, GM: gentamicin, TE: tetracycline, SXT: trimethoprim/sulfamethoxazole, QDA: quinupristin/dalfopristin, LNZ: linezolid, VA: vancomycin, TGC: tigecycline, FT: Nitrofurantoin, RA: rifampicin

FIGURE 3. Antimicrobial susceptibility and resistance profile of MRSA isolates, including the OS-MRSA strain. The cefoxitin screen test provides a qualitative result; a negative result indicates susceptibility. 'Not susceptible' includes both intermediate and resistant categories

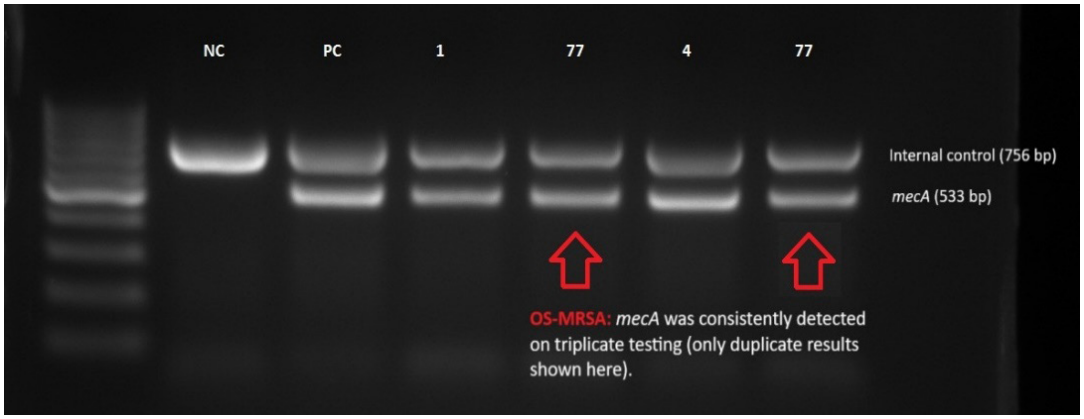


FIGURE 4. Repeat PCR detection of *mecA* (533 bp) with internal control (756 bp). Lanes (left to right): 100 bp DNA ladder, negative control (NC), positive control (PC) and test samples (1, 4, 77). *mecA* was consistently amplified in OS-MRSA isolate 77 (duplicate results shown)

TABLE 3. Antibiotic susceptibility profile of the oxacillin-susceptible MRSA (OS-MRSA) isolate (n=1)

Antibiotic	MIC (μg/mL)	Interpretation
Cefoxitin[a]	-	Negative
Oxacillin	0.5	Susceptible
Penicillin	≥ 0.5	Resistant
Gentamicin	≤ 0.5	Susceptible
Ciprofloxacin	≥ 8	Resistant
Levofloxacin	≥ 8	Resistant
Moxifloxacin	2	Resistant
Erythromycin	≤ 0.25	Susceptible
Clindamycin	≤ 0.25	Susceptible
Quinupristin/dalfopristin	≤ 0.25	Susceptible
Linezolid	2	Susceptible
Vancomycin	1	Susceptible
Tetracycline	≥ 16	Resistant
Tigecycline	≤ 0.12	Susceptible
Nitrofurantoin	≤ 16	Susceptible
Rifampicin	≤ 0.5	Susceptible
Trimethoprim/sulfamethoxazole	≤ 10	Susceptible

[a]The cefoxitin screen test provides a qualitative result. A positive cefoxitin screen result corresponds to resistance by cefoxitin disc diffusion, whereas a negative result corresponds to susceptibility. MIC: minimum inhibitory concentration

of MRSA isolates recovered from dairy cattle (Aklilu & Ying 2020). Its absence among human isolates, despite its presence in livestock, may be attributed to differences in study settings and population exposure. Our study was conducted in an urban tertiary hospital in the capital city, where possible contact with farm animals and agricultural environments is minimal. In contrast, the livestock-associated study was performed in a northern state with active dairy farming, where environmental and occupational exposure to livestock is considerably higher. Hence, additional surveillance studies in agriculturally active regions are warranted to determine the potential for *mecC*-MRSA carriage among individuals with direct animal contact.

Several other published studies have also demonstrated a potential zoonotic risk. Strong evidence of cross-species

transmission of *mecC* isolates has been demonstrated through genomic sequencing (Albert et al. 2023; Harrison et al. 2013). In these studies, close animal contact emerged as a key risk factor, with affected patients either living near farms or working closely with animals (Albert et al. 2023; Harrison et al. 2013). Conversely, *mecC*-MRSA has not been detected in populations with minimal animal exposure, such as military personnel (Ganesan et al. 2013). Furthermore, isolates from both human and animal sources frequently belong to the same clonal complex, CC130, suggesting a shared evolutionary origin (Dierikx et al. 2023; Mališová et al. 2020). However, Dierikx et al. (2023) noted that despite their genetic relatedness, there was insufficient evidence to confirm direct hedgehog-to-human transmission when a threshold of 25 allelic differences in whole-genome multilocus sequence typing (wgMLST) was applied (Dierikx et al. 2023). Similarly, Miller et al. (2024) observed considerable variations in the *SCCmec* elements carrying *mecC* between human and hedgehog isolates, suggesting the possible existence of other unidentified reservoirs (Miller et al. 2024). In addition to living hosts, *mecC*-MRSA has been detected in raw cow's milk (Bietrix et al. 2019), dairy products (Taban, Hassankhani & Aytac 2021) and processed meat (De Melo et al. 2020). However, further research is required to determine whether *mecC*-MRSA present in contaminated food can be transmitted to humans and subsequently cause infection.

The detection of *mecC*-MRSA across multiple reservoirs, including wildlife, livestock and food products, highlights the complex ecological interconnections between environmental, agricultural, and human ecosystems that facilitate the dissemination of antimicrobial resistance. Therefore, the presence of *mecC*-positive MRSA in local livestock, even in the absence of human cases, underscores the need for adopting a coordinated One Health approach (Algammal et al. 2020). Such an integrative framework is essential for strengthening surveillance systems, interrupting potential transmission pathways and promoting judicious antimicrobial use across all sectors, thereby preventing the spillover of resistance genes into human populations.

The role of *mecC* as the genetic determinant of methicillin resistance has been confirmed by Ballhausen et al. (2014), who demonstrated reduced cefoxitin and oxacillin minimum inhibitory concentrations (MICs) to susceptible categories in a *mecC* knockout strain, followed by restoration of the resistance properties upon complementation with wild type *mecC*. The same study also showed that *mecC* transcription is inducible by oxacillin (Ballhausen et al. 2014). Accordingly, *mecC*-carrying strains are recognised as MRSA. However, the affinity of PBP2c (the protein encoded by *mecC*) for cefoxitin and oxacillin differs (Kim et al. 2012), often resulting in the peculiar 'cefoxitin-resistant but oxacillin-susceptible' phenotype (Kriegeskorte et al. 2018). As a result, cefoxitin-based susceptibility testing is superior to oxacillin for MRSA screening, as it reliably identifies all *mecC*-MRSA isolates (Kriegeskorte et al. 2018; Skov et al. 2014). Most established phenotypic and genotypic confirmatory tests for MRSA were initially designed to target *mecA* or its protein product, PBP2a. Consequently, these assays often fail to identify *mecC*-positive strains, leading to misclassification (Kriegeskorte et al. 2018; Shore et al. 2011).

Another incidental finding in this study was the identification of a *mecA*-positive isolate that remained susceptible to both oxacillin and cefoxitin, consistent with the oxacillin-susceptible MRSA (OS-MRSA) phenotype. This strain was recovered from the sputum of a 77-year-old man who was clinically diagnosed with community-acquired pneumonia. Historical laboratory records from 2023 showed that the isolate had previously been classified as MRSA based on cefoxitin resistance by disc diffusion. The patient was subsequently treated with parenteral vancomycin for five days and was discharged home without complications. The discrepancy between the previous and current AST results of this isolate may be attributed to several possibilities: (i) the earlier AST result was erroneous and the isolate was in fact an OS-MRSA; (ii) an isolated pre-analytical or technical error occurred during specimen or isolate handling, such as inadvertent mislabelling at the time of archiving; or (iii) loss of resistance phenotype during long-term storage. We emphasise that this represents a single discrepancy identified through retrospective re-testing of one archived isolate, rather than evidence of a systemic issue within the laboratory's current AST or specimen-handling workflow, both of which remain subject to routine internal quality control and external quality assessment (EQA) participation. Although -80 °C freeze storage has generally been shown to preserve bacterial viability and phenotypic resistance, a two-year surveillance study of 360 cryopreserved MRSA isolates found no significant decline in the methicillin-resistant population after prolonged storage or repeated freeze–thaw cycles (Veguilla et al. 2008). Nonetheless, storage-related reductions in antimicrobial resistance phenotype have been documented in other bacterial species under specific conditions (Kiet et al. 2022). Taken together, this evidence suggests that possibility (iii) is comparatively less likely to explain the discrepancy observed in our isolate, lending relatively greater weight to possibilities (i) and (ii); however, without whole-genome sequencing or re-culture from the original clinical specimen, these explanations could not be definitively distinguished.

Reports of OS-MRSA in Malaysia are scarce. In China, OS-MRSA accounts for approximately 9% of paediatric cases (Ma et al. 2021), whereas prevalence

among adults varies widely, ranging from 2% to 18% (Conceição et al. 2015; Liu et al. 2021). Among bloodstream infections, OS-MRSA has constituted up to 27% of isolates initially identified as MSSA and has been associated with a mortality rate of 27% (Duarte et al. 2024). Unlike *mecC*-harbouring MRSA, for which cefoxitin remains a reliable screening marker (Kriegeskorte et al. 2018), OS-MRSA poses a greater diagnostic challenge because it exhibits phenotypic susceptibility to both cefoxitin and oxacillin, as observed in this study (Liang et al. 2022; Ma et al. 2021). Inappropriate treatment with *β*-lactam antibiotics may induce reversion to a resistant phenotype, potentially leading to therapeutic failure (Liang et al. 2022). Genomic investigations of OS-MRSA have yielded heterogeneous findings. Liang et al. (2022) and Tao (2025) reported that point mutations in the *mecA* gene can disrupt PBP2a function, leading to phenotypic *β*-lactam susceptibility. In contrast, Boonsiri et al. (2020) found no mutations in the *mec* or *bla* gene regions among 43 clinical OS-MRSA isolates, suggesting that additional or alternative mechanisms may have contributed to this phenotype.

In our study, all MRSA isolates were penicillin-resistant, and a high proportion (89%) were resistant to all quinolones, as well as erythromycin (66.3%) and clindamycin (65.2%). This pattern is consistent with findings from the same hospital in 2018-2019 (Mohd Rohaizat et al. 2022). Although the raw clindamycin MIC_{50} and MIC_{90} values were low (both ≤ 0.25 μg/mL), the high overall non-susceptibility was driven predominantly by the high prevalence of inducible clindamycin resistance (56.2%). This discordance highlights the importance of reflex testing for inducible clindamycin resistance in *Staphylococcus* spp., rather than relying solely on raw MIC values, to prevent therapeutic failure.

For trimethoprim/sulfamethoxazole, although high resistance rates have been reported elsewhere (Gebremeskel, Alemayehu & Ali 2022; Mzee et al. 2021), the present findings showed the opposite, demonstrating a 96% susceptibility rate, consistent with local data from 2019 (Mohd Rohaizat et al. 2022). This high susceptibility rate may reflect relatively low community exposure, as trimethoprim/sulfamethoxazole is not routinely used as a first-line agent against MRSA in local practice. All MRSA isolates in this study were susceptible to vancomycin, linezolid, quinupristin/dalfopristin, tigecycline, nitrofurantoin, and rifampicin, indicating that vancomycin remains an appropriate empirical therapy against suspected MRSA infections. The MIC_{50}/MIC_{90} values further support this conclusion: vancomycin MIC_{50} and MIC_{90} values of ≤ 0.5 μg/mL and 1 μg/mL, respectively, remain well below the ≥ 2 μg/mL threshold associated with reduced vancomycin susceptibility ('MIC creep') and treatment failure (Sakoulas et al. 2004), reassuring for its continued first-line empirical use in this setting. Similarly narrow, low MIC_{50}/MIC_{90} margins were observed for linezolid (2 μg/mL) and tigecycline (≤ 0.12 –0.25 μg/mL), the two reserve agents in this panel. Conversely, ciprofloxacin, levofloxacin, and erythromycin all showed MIC_{50} and MIC_{90} values at or beyond the highest tested/breakpoint concentration (≥ 8 μg/mL), corroborating the high phenotypic resistance rates already reported and reinforcing that these agents offer little clinical utility for empirical MRSA therapy in this population.

Several limitations should be considered while interpreting these findings. This investigation was conducted at a single urban centre, which may limit the generalisability of the absence of the *mecC* gene to the broader national context. The relatively small sample size and unequal distribution of diagnostic and screening specimens constrained the depth and robustness of the analyses. The retrospective design precluded further investigation of the isolate with discrepant antimicrobial susceptibility results. Financial constraints prevented the use of whole-genome or additional molecular analyses to further characterise the potential OS-MRSA strain. Additionally, phenotypically MSSA isolates were not included, although they may potentially harbour the *mecC* gene (Kriegeskorte et al. 2018), thereby representing an additional reservoir for future study.

CONCLUSION

The findings of this study demonstrate the absence of the *mecC* gene among clinical MRSA isolates and confirm that *mecA* remains the predominant determinant of methicillin resistance in this setting. Although *mecC* was not detected, these results provide a valuable baseline for ongoing surveillance. Further research, particularly in regions with significant agricultural activity, is necessary to better characterise the prevalence and epidemiology of *mecC*-positive MRSA in Malaysia. The identification of a potential OS-MRSA isolate underscores the limitations of relying solely on phenotypic methods for MRSA screening. Incorporating molecular assays for resistance gene detection into routine diagnostics would improve the accuracy of MRSA variant identification and reduce the risk of misclassification. The antimicrobial susceptibility patterns observed were consistent with previous local reports, with high resistance rates to quinolones, erythromycin and clindamycin. At the same time, vancomycin remains susceptible and can be used as a first-line agent against MRSA infections in our setting.

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