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Detection of mecA Gene in Methicillin-Resistant Staphylococcus aureus (MRSA) Isolated from Diabetic Ulcer Swab Samples

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ABSTRACT Diabetic ulcers are a major complication of diabetes mellitus and are highly susceptible to bacterial infection due to impaired immunity, poor vascularization, and prolonged hyperglycemia. Staphylococcus aureus is among the most frequently isolated pathogens in diabetic ulcers, and the emergence of methicillin-resistant Staphylococcus aureus (MRSA) poses a significant therapeutic challenge. Methicillin resistance is commonly associated with the presence of the mecA gene, which encodes penicillin-binding protein 2a (PBP2a) and confers resistance to β-lactam antibiotics. However, discrepancies between phenotypic resistance and genotypic confirmation have been increasingly reported, raising concerns regarding the accuracy of routine diagnostic methods. This study aimed to detect the presence of the mecA gene in phenotypically identified MRSA isolates from diabetic ulcer swab samples and to evaluate the concordance between phenotypic and genotypic detection methods. A descriptive quantitative study was conducted using 30 diabetic ulcer swab samples collected from patients attending a diabetes wound care clinic in Surabaya, Indonesia. Bacterial identification was performed using standard culture and biochemical tests. Phenotypic MRSA screening was carried out using the cefoxitin disk diffusion method in accordance with CLSI guidelines. Genotypic detection of the mecA gene was performed using conventional polymerase chain reaction (PCR) followed by agarose gel electrophoresis. Of the 30 samples, 19 (63%) were confirmed as S. aureus. Six isolates (31.6%) demonstrated phenotypic resistance to cefoxitin and were classified as presumptive MRSA. However, PCR analysis revealed that none of the phenotypically resistant isolates carried the mecA gene, while the positive control showed the expected 304 bp amplification product. In conclusion, phenotypic cefoxitin resistance in S. aureus isolates from diabetic ulcers does not necessarily correlate with the presence of the mecA gene, suggesting the involvement of alternative resistance mechanisms. These findings highlight the importance of integrating molecular diagnostics with conventional methods to ensure accurate MRSA detection and support rational antibiotic use in diabetic wound management.

INDEX TERMS Diabetic Ulcer, *Staphylococcus aureus*, MRSA, mecA Gene, Polymerase Chain Reaction

I. INTRODUCTION

Diabetic foot ulcers (DFUs) are among the most severe complications of diabetes mellitus and represent a significant global health burden due to their association with chronic infection, prolonged hospitalization, and increased risk of lower-extremity amputation [1], [2]. Pathophysiological conditions such as peripheral neuropathy, ischemia, and impaired immune function create a favorable environment for microbial colonization and persistent infection [3]. Among the pathogens isolated from diabetic ulcers, *Staphylococcus aureus* is consistently reported as the most prevalent bacterium, largely due to its virulence factors, biofilm-forming capacity, and ability to adapt to hostile wound environments [4]. The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has further complicated the management of diabetic wound infections, as MRSA infections are associated with limited therapeutic options and poorer clinical outcomes [5].

Accurate and timely detection of MRSA is therefore essential for guiding appropriate antimicrobial therapy and preventing the misuse of broad-spectrum antibiotics. Phenotypic methods, particularly the cefoxitin disk diffusion test, are widely used in routine clinical microbiology laboratories because they are inexpensive and simple to perform [6]. Cefoxitin is recommended as a surrogate marker for methicillin resistance because it effectively induces expression of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a) [7]. Nevertheless, several recent studies have demonstrated that phenotypic resistance does not always correspond with the presence of the *mecA* gene, leading to potential misclassification of MRSA when relying solely on disk diffusion testing [8].

State-of-the-art diagnostic strategies increasingly emphasize molecular approaches, particularly polymerase chain reaction (PCR)-based detection of the *mecA* gene, which is regarded as the reference standard for confirming

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MRSA [9]. Recent technological advances, including isothermal amplification methods, CRISPR-based diagnostics, and MALDI-TOF mass spectrometry–assisted resistance profiling, have further improved the sensitivity and specificity of MRSA detection [10]–[12]. However, the implementation of these advanced molecular techniques remains limited in many low- and middle-income countries due to financial constraints, infrastructure limitations, and insufficient technical expertise [13].

Furthermore, emerging evidence highlights the role of alternative resistance mechanisms that may produce phenotypic methicillin resistance in the absence of the *mecA* gene. These mechanisms include the presence of the *mecC* gene, hyperproduction of β-lactamase enzymes, and borderline oxacillin-resistant *Staphylococcus aureus* (BORSA), all of which can result in cefoxitin-resistant phenotypes without classical *mecA*-mediated resistance [14]–[16]. Failure to recognize these alternative mechanisms may lead to unnecessary use of last-line antibiotics, increased treatment costs, and acceleration of antimicrobial resistance [17].

Despite the growing global literature on MRSA, data on the molecular characterization of MRSA in diabetic ulcers remain limited in Indonesia. Most existing studies rely predominantly on phenotypic identification methods, with minimal integration of molecular confirmation, thereby restricting the understanding of true resistance mechanisms and local epidemiological patterns [18], [19]. This lack of comprehensive surveillance represents a critical research gap, particularly in the context of antimicrobial stewardship and evidence-based wound management.

Therefore, the aim of this study was to detect the presence of the *mecA* gene in phenotypically cefoxitin-resistant *Staphylococcus aureus* isolates obtained from diabetic ulcer swab samples and to evaluate the concordance between phenotypic and genotypic MRSA detection methods. The contributions of this study are threefold: (1) providing updated local data on *S. aureus* and MRSA prevalence in diabetic ulcers using combined phenotypic and molecular approaches; (2) identifying diagnostic discrepancies between cefoxitin resistance and *mecA* gene detection; and (3) offering evidence-based recommendations to improve MRSA diagnostic accuracy and antimicrobial stewardship in resource-limited clinical settings [20].

The remainder of this article is organized as follows. Section II describes the study design, sampling procedures, microbiological identification, and molecular analysis. Section III presents the results of phenotypic and genotypic testing. Section IV discusses the findings in relation to recent literature and study limitations, and Section V concludes the paper with implications for clinical practice and future research.

II. METHODS

A. Study Design and Setting

This study employed a descriptive quantitative laboratory-based design aimed at evaluating the concordance between phenotypic and genotypic detection of methicillin-resistant Staphylococcus aureus (MRSA) isolated from diabetic ulcer swab samples. The study was conducted prospectively between April and May 2025 at the Bacteriology and Molecular Biology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Surabaya, Indonesia. No randomization was applied, as the study focused on laboratory confirmation rather than interventional outcomes.

B. Study Population and Sample Collection

The study population consisted of patients diagnosed with diabetes mellitus presenting with diabetic foot ulcers who attended the Diabetes Wound Care Clinic in Surabaya. A total of 30 wound swab samples were collected using a consecutive sampling technique. Inclusion criteria were patients with clinically diagnosed diabetic foot ulcers classified as Wagner grade ≥1 and who had not received systemic antibiotic therapy within two weeks prior to sampling. Patients with non-diabetic ulcers or incomplete clinical data were excluded. The sample size was determined based on comparable molecular studies assessing MRSA prevalence in diabetic wounds, aiming to provide an initial estimation of resistance patterns [21].

Wound swab samples were obtained aseptically after cleaning the ulcer surface with sterile normal saline to remove superficial contaminants. Sterile cotton-tipped swabs were rotated firmly across the base of the ulcer to ensure adequate collection of viable bacteria. Samples were immediately placed into Amies transport medium and transported to the laboratory under cold-chain conditions for processing within 24 hours [22].

C. Bacterial Isolation and Identification

Upon arrival at the laboratory, swabs were inoculated onto Mannitol Salt Agar (MSA) and Blood Agar Plates (BAP) and incubated aerobically at 37 °C for 24 hours. Colonies exhibiting golden-yellow pigmentation on MSA and β-hemolysis on BAP were considered presumptive Staphylococcus aureus. These colonies were further identified using standard microbiological procedures, including Gram staining, catalase testing, tube coagulase testing, and mannitol fermentation assays. Only isolates confirmed as S. aureus through these tests were included in subsequent analyses [23].

D. Phenotypic Detection of MRSA

Phenotypic screening for methicillin resistance was performed using the cefoxitin disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (2022). Bacterial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard and evenly inoculated onto Mueller–Hinton agar plates. A 30 µg cefoxitin disk was placed on the agar surface, and plates were incubated at 35 °C for 18–24 hours. Inhibition zone diameters were measured in millimeters, and isolates with zone diameters ≤21 mm were interpreted as cefoxitin-resistant and classified as presumptive MRSA [24].

E. Genomic DNA Extraction

Genomic DNA was extracted from all cefoxitin-resistant isolates using the boiling method, a cost-effective and widely used technique for clinical bacterial DNA preparation. Briefly, a single bacterial colony was suspended in 200 µL of TE buffer and heated at 100 °C for 10 minutes. The

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suspension was then centrifuged at 10,000 rpm for 10 minutes, and the supernatant containing crude DNA was collected and stored at −20 °C until PCR analysis. DNA purity was assessed spectrophotometrically, and samples with acceptable A260/A280 ratios were used for amplification [25].

F. PCR Amplification of the mecA Gene

Detection of the mecA gene was performed using conventional polymerase chain reaction (PCR). Amplification was carried out using specific primers targeting a 304 bp fragment of the mecA gene. Each 25 µL reaction mixture consisted of 12.5 µL of 2× PCR master mix, 1 µL of forward primer, 1 µL of reverse primer, 2 µL of DNA template, and 8.5 µL of nuclease-free water. PCR cycling conditions included an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds, with a final extension at 72 °C for 5 minutes. A known mecA-positive strain was used as a positive control, while nuclease-free water served as a negative control [26], [27].

G. Agarose Gel Electrophoresis

PCR products were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Electrophoresis was conducted at 100 V for 45 minutes in 1× TBE buffer alongside a 100 bp DNA ladder. Amplified DNA bands were visualized under ultraviolet illumination, and the presence of a 304 bp band was interpreted as positive for the mecA gene [28].

H. Ethical Considerations and Data Analysis

Ethical approval for this study was obtained from the institutional ethics committee of Poltekkes Kemenkes Surabaya. All patient data were anonymized prior to analysis. Data were analyzed descriptively and presented as frequencies and percentages to describe the distribution of S. aureus, phenotypic MRSA, and mecA gene detection results. No inferential statistical analysis was performed, as the study aimed to describe diagnostic concordance rather than establish causal associations [29].

III. RESULTS

Out of 30 diabetic ulcer swabs, 19 (63%) yielded S. aureus isolates. Six (31.6%) of these were cefoxitin-resistant, indicating presumptive MRSA. However, none of the resistant isolates were positive for the mecA gene, with only the control sample showing the expected 304 bp PCR band. After performing standard culture and identification methods, 19 of the 30 samples (63%) yielded *Staphylococcus aureus* isolates. This result reinforces findings from previous studies that have consistently reported *S. aureus* as the most predominant pathogen in diabetic ulcers due to its ability to produce biofilms, secrete various toxins, and resist phagocytosis[4][18]. These virulence factors contribute to delayed wound healing, chronic inflammation, and increased risk of complications such as osteomyelitis and limb amputation [3]

The isolates were subjected to cefoxitin disk diffusion testing following CLSI guidelines to identify phenotypic methicillin resistance. Six of the 19 *S. aureus* isolates (31.6%) exhibited resistance to cefoxitin, thereby meeting the criteria for presumptive MRSA. Cefoxitin is considered a surrogate marker for the detection of the *mecA* gene product, PBP2a, due to its stability and stronger induction of *mecA* expression compared to oxacillin [8]. The remaining 13 isolates (68.4%) were susceptible to cefoxitin, suggesting sensitivity to β-lactam antibiotics. The detailed cefoxitin resistance results are shown in TABLE 1.

TABLE 1
Phenotypic Results of Cefoxitin Resistance in *S. aureus* Isolates

Isolate Code	Cefoxitin Resistance	Presumptive MRSA
002	Resistant	**yes**
004	Resistant	**yes**
010	Resistant	**yes**
018	Resistant	**yes**
019	Resistant	**yes**
022	Resitant	**yes**

To verify whether methicillin resistance in these isolates was due to the presence of the *mecA* gene, conventional PCR was performed on all six cefoxitin-resistant isolates using specific primer pairs targeting the *mecA* region. Surprisingly, none of the tested clinical isolates produced amplification bands at the expected 304 base pair (bp) size. Only the positive control yielded the correct band, confirming that the PCR protocol, reagents, and primers were functioning properly.

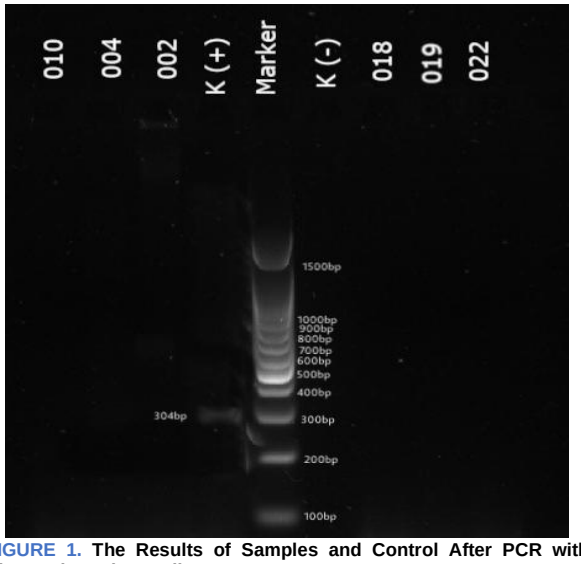

FIGURE 1. **The Results of Samples and Control After PCR with Electrophoresis Reading**

This discordance between phenotypic and genotypic results is a notable finding. While cefoxitin disk diffusion remains a valuable initial screening method for MRSA, it is not definitive. Several studies have documented cases where *S. aureus* isolates exhibit phenotypic resistance to cefoxitin but lack the *mecA* gene [2,7,9]. Possible explanations include the presence of other resistance determinants such as the *mecC* gene, a homolog of *mecA* that also encodes a penicillin-binding protein with low affinity for β-lactams but is not detected by conventional *mecA* primers [12]. In addition, overproduction of β-lactamase enzymes or

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mutations in regulatory genes such as *blaZ* may contribute to resistance phenotypes without *mecA* involvement[5].

The absence of *mecA* gene detection in phenotypically resistant isolates may also reflect borderline oxacillin-resistant *S. aureus* (BORSA), a strain category that exhibits intermediate resistance to β-lactams due to cell wall thickening or altered PBP expression but does not possess *mecA* or *mecC* genes [18]. BORSA strains often produce ambiguous or false-positive results in disk diffusion testing, underscoring the importance of PCR confirmation, particularly in clinical settings where treatment decisions hinge on accurate susceptibility data.

Electrophoresis of PCR products using 1.5% agarose gel with ethidium bromide confirmed the absence of amplification in all tested isolates. A 100 bp DNA ladder was used for size estimation, and a clear 304 bp band was only visible in the positive control lane, thereby validating the accuracy and sensitivity of the gel electrophoresis system[19].

Furthermore, recent studies have demonstrated that other genetic mechanisms such as modifications in the fem gene complex and altered expression of native penicillin-binding proteins (PBPs) can lead to resistance patterns similar to MRSA even in the absence of the mecA gene. For instance, strains showing borderline oxacillin resistance (BORSA) have been reported with thickened cell walls or altered PBPs that reduce the efficacy of β-lactams without carrying mecA or mecC [20], [21].

Additionally, the clinical management of MRSA infections, particularly in diabetic wounds, remains challenging due to biofilm formation which reduces antibiotic penetration and promotes chronic infection. A 2023 systematic review emphasized that biofilm-associated MRSA strains are up to 1000 times more resistant to antibiotics compared to planktonic cells, suggesting the necessity for adjunctive therapies such as enzymatic debridement or quorum sensing inhibitors [22].

The discordance between phenotypic resistance and mecA gene detection highlights the limitations of relying solely on disk diffusion testing. Several possibilities, such as mecC-mediated resistance or borderline oxacillin-resistant S. aureus (BORSA), could explain cefoxitin resistance in the absence of mecA. Biofilm formation and other resistance mechanisms may also play a role.

In addition to the cefoxitin resistance testing, all 19 *Staphylococcus aureus* isolates underwent further phenotypic verification, including coagulase and catalase testing, confirming their classification as *S. aureus*. Among the six cefoxitin-resistant isolates, the inhibition zones consistently measured ≤21 mm, aligning with the Clinical and Laboratory Standards Institute (CLSI) 2022 criteria for presumptive MRSA identification. These isolates were considered phenotypically resistant and selected for genotypic analysis.

The PCR analysis targeting the *mecA* gene revealed that none of the six phenotypically resistant isolates amplified the expected 304 bp DNA fragment. Only the positive control yielded a visible band, confirming the functionality of the PCR reagents and the reliability of the thermal cycling conditions. This indicates a lack of *mecA*-mediated resistance in the clinical isolates, despite their cefoxitin-resistant phenotype.

To rule out false negatives due to low DNA concentration or poor template quality, the extracted DNA samples were quantified using a spectrophotometer, and all values were within acceptable purity (A260/A280 ~1.8–2.0). Furthermore, each PCR run included both positive and negative controls, and the assays were repeated to confirm consistency. No amplification was observed in any of the test samples across two independent PCR rounds.

This discrepancy between phenotypic resistance and genotypic negativity highlights the complexity of MRSA identification and suggests that alternative mechanisms, such as *mecC*, hyperproduction of β-lactamase, or other PBP modifications, may be contributing to the observed resistance. These findings underscore the need for additional molecular assays and broader genomic surveillance in future studies to fully characterize the resistance profiles of clinical *S. aureus* isolates.

These findings echo international reports and emphasize the importance of integrating molecular diagnostics into routine MRSA screening, especially in resource-limited settings. By doing so, clinicians can make more informed decisions, potentially avoiding unnecessary use of advanced antibiotics. Small sample size and limited gene targets (e.g., no mecC screening) may restrict the generalizability of findings.

These results highlight the limitations of relying solely on phenotypic methods for MRSA detection, particularly in resource-limited laboratories where genotypic methods are not routinely employed. False positives could lead to the unnecessary use of second-line antibiotics such as vancomycin or linezolid, which are more expensive and associated with higher toxicity risks. Therefore, the integration of molecular diagnostics into routine microbial surveillance programs is highly recommended to ensure appropriate therapeutic strategies and prevent the overuse of broad-spectrum antibiotics.

In summary, this study demonstrates a significant discrepancy between phenotypic cefoxitin resistance and genotypic confirmation via *mecA* PCR in *S. aureus* isolates from diabetic ulcer samples. These findings are consistent with previous literature and emphasize the importance of using a combination of phenotypic and molecular methods in MRSA surveillance and antimicrobial stewardship efforts.

IV. DISCUSSION

A. INTERPRETATION OF THE FINDINGS

This study demonstrated that Staphylococcus aureus was the predominant bacterial species isolated from diabetic ulcer swab samples, accounting for 63% of all specimens analyzed. This finding is consistent with the well-established role of S. aureus as a major pathogen in chronic diabetic wounds, attributable to its extensive virulence repertoire, biofilm-forming capacity, and ability to evade host immune responses [30]. Biofilm formation in particular is known to promote bacterial persistence and reduce antibiotic penetration, thereby contributing to chronic infection and delayed wound healing.

Among the confirmed S. aureus isolates, 31.6% exhibited phenotypic resistance to cefoxitin and were therefore

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classified as presumptive MRSA according to CLSI criteria. Cefoxitin resistance is widely accepted as a reliable phenotypic indicator of methicillin resistance due to its strong induction of penicillin-binding protein 2a (PBP2a) expression [31]. However, a key finding of this study was the absence of the mecA gene in all phenotypically resistant isolates, despite the successful amplification of the positive control. This discordance between phenotypic and genotypic results suggests that methicillin resistance in these isolates may not be mediated by the classical mecA-dependent mechanism.

The lack of mecA detection indicates the possible involvement of alternative resistance pathways, such as hyperproduction of β-lactamase enzymes, alterations in native penicillin-binding proteins, or borderline oxacillin-resistant Staphylococcus aureus (BORSA). These mechanisms can confer reduced susceptibility to β-lactam antibiotics while escaping detection by standard mecA-targeted PCR assays [32]. This finding highlights the complexity of MRSA diagnostics and underscores the limitations of relying exclusively on phenotypic screening methods in clinical laboratories.

B. COMPARISON WITH PREVIOUS STUDIES

The prevalence of phenotypic MRSA observed in this study aligns with several recent reports investigating diabetic foot infections, which describe MRSA rates ranging from 20% to 40% depending on geographic region and healthcare setting [33], [34]. Similar to the present findings, multiple studies have documented discrepancies between cefoxitin resistance and mecA gene detection. For instance, Rostami et al. reported phenotypic resistance in S. aureus isolates that lacked detectable mecA, attributing this phenomenon to non-mecA-mediated resistance mechanisms [35].

In contrast, other investigations conducted in tertiary care hospitals have reported a high concordance between phenotypic MRSA identification and mecA gene presence, with detection rates approaching 100% [36]. Such differences may reflect variations in local antimicrobial usage patterns, infection control practices, and the molecular epidemiology of circulating S. aureus strains. Settings with intensive antibiotic exposure may exert selective pressure favoring classical mecA-harboring MRSA, whereas community-based or outpatient settings may harbor a greater proportion of BORSA or atypical resistant strains.

Recent studies have also highlighted the emergence of mecC-positive MRSA strains, which are not detected by conventional mecA PCR primers and may contribute to false-negative genotypic results [37]. Although mecC screening was not performed in the present study, its potential involvement cannot be excluded and may partially explain the observed phenotype–genotype discordance. Furthermore, advances in molecular diagnostics, including whole-genome sequencing and multiplex PCR assays, have revealed increasing genetic diversity among MRSA isolates, reinforcing the need for expanded molecular surveillance [38].

Overall, the findings of this study are consistent with growing evidence that phenotypic resistance alone is insufficient to accurately characterize MRSA and that regional variations in resistance mechanisms must be considered when interpreting laboratory results.

C. LIMITATIONS, WEAKNESSES, AND IMPLICATIONS

Several limitations of this study should be acknowledged. First, the relatively small sample size limits the generalizability of the findings and may not fully represent the broader population of diabetic ulcer patients. Second, molecular analysis was restricted to detection of the mecA gene; other resistance determinants such as mecC, blaZ, or mutations affecting penicillin-binding proteins were not investigated. As a result, the precise genetic basis of methicillin resistance in the phenotypically resistant isolates could not be definitively determined.

Additionally, this study employed a descriptive design without longitudinal follow-up, precluding assessment of clinical outcomes or treatment responses associated with different resistance profiles. The absence of advanced molecular techniques, such as whole-genome sequencing, also limited the depth of genetic characterization achievable within the available laboratory resources.

Despite these limitations, the findings have important clinical and public health implications. The demonstrated discordance between phenotypic MRSA screening and mecA gene detection underscores the risk of misclassification when phenotypic methods are used in isolation. False-positive MRSA identification may lead to unnecessary use of second-line antibiotics such as vancomycin or linezolid, increasing treatment costs and the risk of adverse drug reactions [39].

From an antimicrobial stewardship perspective, integrating molecular confirmation into routine diagnostics particularly in high-risk populations such as diabetic ulcer patients could enhance diagnostic accuracy and promote rational antibiotic use. In resource-limited settings, targeted molecular testing for isolates exhibiting phenotypic resistance may represent a feasible compromise between cost and diagnostic precision.

Furthermore, these findings highlight the need for continued local surveillance of antimicrobial resistance mechanisms and the development of context-specific diagnostic algorithms. Future studies should incorporate larger sample sizes, expanded gene targets, and advanced molecular tools to provide a more comprehensive understanding of MRSA epidemiology in diabetic wound infections [40].

V. CONCLUSION

This study was conducted to detect the presence of the *mecA* gene in phenotypically cefoxitin-resistant *Staphylococcus aureus* isolates obtained from diabetic ulcer swab samples and to evaluate the concordance between phenotypic and genotypic methods for methicillin-resistant *S. aureus* (MRSA) identification. Of the 30 diabetic ulcer specimens analyzed, 19 samples (63%) were confirmed as *S. aureus* through standard microbiological identification. Phenotypic screening using the cefoxitin disk diffusion method revealed that 6 of these isolates (31.6%) exhibited resistance and were classified as presumptive MRSA according to CLSI criteria. However, molecular analysis using conventional PCR demonstrated that none of the phenotypically resistant

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isolates carried the *mecA* gene, while the positive control consistently produced the expected 304 bp amplification product. This clear discordance indicates that phenotypic cefoxitin resistance in *S. aureus* isolated from diabetic ulcers does not necessarily correlate with *mecA*-mediated methicillin resistance. The findings suggest that alternative resistance mechanisms such as β-lactamase hyperproduction, borderline oxacillin-resistant *S. aureus* (BORSA), or the presence of other resistance determinants not assessed in this study may contribute to the observed resistance profiles. Clinically, these results underscore the limitations of relying solely on phenotypic methods for MRSA detection, as misclassification may lead to unnecessary use of last-line antibiotics, increased treatment costs, and potential adverse drug effects. From a diagnostic and antimicrobial stewardship perspective, the integration of molecular confirmation alongside routine phenotypic testing is essential to improve accuracy and optimize therapeutic decision-making, particularly in high-risk populations such as patients with diabetic ulcers. Future research should involve larger sample sizes, include broader molecular targets such as *mecC* and β-lactamase-related genes, and apply advanced genomic approaches to comprehensively characterize resistance mechanisms. Additionally, studies correlating molecular resistance profiles with clinical outcomes would provide valuable insights for improving infection management and guiding evidence-based treatment strategies in diabetic wound care.

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DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTION

All authors contributed substantially to the conception and design of the study. Aliyya Noor Izzati performed sample collection, laboratory experiments, data acquisition, and initial manuscript drafting. Suliati and Lully Hanni Endarini contributed to methodology development, laboratory supervision, and data interpretation. Syamsul Arifin provided critical revisions, supervised the overall research process, and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

DECLARATIONS

ETHICAL APPROVAL

This study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Institutional Ethics Committee of Poltekkes Kemenkes Surabaya. Written informed consent was obtained from all participants prior to sample collection.

CONSENT FOR PUBLICATION PARTICIPANTS.

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

[1] B. A. Lipsky et al., "IWGDF guideline on the diagnosis and treatment of foot infection in persons with diabetes," Diabetes Metab. Res. Rev., vol. 36, no. S1, 2020.
[2] J. Aragón-Sánchez, "Diabetic foot infections," World J. Diabetes, vol. 11, no. 6, 2020.
[3] Y. Zhang et al., "Biofilm-associated antibiotic resistance in Staphylococcus aureus," Front. Microbiol., vol. 14, 2023.
[4] WHO, Global priority list of antibiotic-resistant bacteria, Geneva, 2020.
[5] CLSI, Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed., 2022.
[6] S. Lakhundi and K. Zhang, "Methicillin-resistant Staphylococcus aureus," Clin. Microbiol. Rev., vol. 31, no. 4, 2018.
[7] S. Rostami et al., "Comparison of mecA PCR and cefoxitin disk diffusion," Afr. J. Microbiol. Res., vol. 7, 2021.
[8] K. Becker et al., "Rapid molecular detection of MRSA," J. Clin. Microbiol., vol. 58, 2020.
[9] H. Zhu et al., "PCR: past, present, and future," Biotechniques, vol. 69, 2020.
[10] W. Chen et al., "CRISPR-based detection of MRSA," Front. Microbiol., vol. 15, 2024.
[11] P. Santos et al., "MALDI-TOF MS biomarkers for MRSA," Clin. Microbiol. Infect., vol. 31, 2025.
[12] A. C. Fluit et al., "Antimicrobial resistance in developing countries," Lancet Infect. Dis., vol. 21, 2021.
[13] C. Lozano et al., "Clinical relevance of mecC-positive MRSA," Microorganisms, vol. 8, 2020.
[14] M. M. Hryniewicz and K. Garbacz, "BORSA: an underestimated problem," J. Med. Microbiol., vol. 69, 2020.
[15] V. Panchal et al., "High-level β-lactam resistance mechanisms," PLoS Pathog., vol. 16, 2020.
[16] F. M. Lowy, "Antimicrobial resistance in Staphylococcus aureus," J. Clin. Invest., vol. 131, 2021.
[17] D. Erikawati et al., "MRSA prevalence in Indonesia," Acta Med. Indones., vol. 52, 2020.
[18] E. Warganegara et al., "Phenotypic MRSA detection limitations," J. Kedokt. Brawijaya, vol. 34, 2022.
[19] M. Ayu et al., "Detection of mecA gene using PCR," J. Biol. Tropis, vol. 24, 2024.
[20] S. Otto, "MRSA virulence and spread," Cell. Microbiol., vol. 24, 2022.
[21] L. A. Lavery et al., "Risk factors for MRSA infection in diabetic foot ulcers," Int. Wound J., vol. 17, no. 6, 2020.
[22] J. Aragón-Sánchez, "Management of diabetic foot infections," World J. Diabetes, vol. 11, no. 6, 2020.
[23] K. Becker et al., "Laboratory diagnosis of Staphylococcus aureus," Clin. Microbiol. Rev., vol. 33, no. 3, 2020.
[24] CLSI, Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed., 2022.
[25] H. Zhu et al., "PCR: past, present, and future," Biotechniques, vol. 69, no. 4, 2020.
[26] M. Ayu et al., "Detection of the mecA gene in MRSA using PCR," J. Biol. Tropis, vol. 24, no. 1, 2024.
[27] S. Rostami et al., "Comparison of phenotypic and genotypic MRSA detection," Afr. J. Microbiol. Res., vol. 15, 2021.
[28] W. Chen et al., "Molecular approaches for MRSA detection," Front. Microbiol., vol. 15, 2024.

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Vol. 6 No. 1, pp. 39-45, February 2026

[29] E. Erikawati et al., "MRSA surveillance in Indonesia," Acta Med. Indones., vol. 52, no. 4, 2020.
[30] Y. Zhang et al., "Biofilm-associated antibiotic resistance in Staphylococcus aureus," Front. Microbiol., vol. 14, 2023.
[31] CLSI, Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed., 2022.
[32] M. M. Hryniewicz and K. Garbacz, "Borderline oxacillin-resistant Staphylococcus aureus (BORSA)," J. Med. Microbiol., vol. 69, 2020.
[33] L. A. Lavery et al., "Microbiology of diabetic foot infections," Int. Wound J., vol. 17, no. 6, 2020.
[34] J. Aragón-Sánchez, "Clinical significance of MRSA in diabetic foot infections," World J. Diabetes, vol. 11, 2020.
[35] S. Rostami et al., "Comparison of phenotypic and genotypic MRSA detection," Afr. J. Microbiol. Res., vol. 15, 2021.
[36] K. Becker et al., "Emerging MRSA detection strategies," J. Clin. Microbiol., vol. 58, 2020.
[37] C. Lozano et al., "Clinical relevance of mecC-positive MRSA," Microorganisms, vol. 8, 2020.
[38] W. Chen et al., "Molecular approaches for MRSA characterization," Front. Microbiol., vol. 15, 2024.
[39] F. M. Lowy, "Antimicrobial resistance in Staphylococcus aureus," J. Clin. Invest., vol. 131, 2021.
[40] WHO, Global Antimicrobial Resistance Surveillance System (GLASS) Report, 2023.