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Molecular Detection of mecA and mecC Genes in Urinary *Staphylococcus aureus* Isolates From Pregnant Women: Implications for Methicillin Resistance Screening

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Abstract

Urinary tract infections are common during pregnancy and require careful antimicrobial selection to ensure foetal safety. The emergence of methicillin-resistant *Staphylococcus aureus* poses additional challenges, particularly in settings where routine phenotypic screening for methicillin resistance is limited. This study aimed to provide molecular data on the mecA and mecC genes in urinary *Staphylococcus aureus* isolates from pregnant women. A cross-sectional study was conducted over a two-month period among 67 pregnant women. Midstream urine samples were cultured using standard microbiological methods. Bacterial isolates were identified, and antimicrobial susceptibility testing was performed using a routine multidisc panel that did not contain oxacillin or cefoxitin. *Staphylococcus aureus* isolates were further subjected to polymerase chain reaction to detect the mecA and mecC genes. Of the 67 urine samples analysed, 54 (80.6%) yielded significant bacterial growth. *Staphylococcus saprophyticus* was the most common isolate, accounting for 63.0%, followed by *Staphylococcus aureus* (20.4%). Polymerase chain reaction analysis of *S. aureus* isolates revealed the mecA gene in 7 isolates (70.0%). The mecC gene was not detected in any isolate, while the remaining three isolates (30.0%) were negative for both genes. The study provides baseline molecular evidence of MecA-mediated methicillin resistance among urinary *Staphylococcus aureus* isolates from pregnant women. The findings highlight the clinical importance of incorporating oxacillin or cefoxitin into routine antimicrobial susceptibility testing panels for *Staphylococcus aureus*, as reliance on antibiotics that do not reliably detect methicillin resistance may lead to missed diagnoses with implications for patient management.

Keywords: *Staphylococcus aureus*, mecA, mecC, pregnancy, Urinary tract infections

Introduction

Urinary tract infections (UTIs) are among the most frequently encountered bacterial infections during pregnancy and remain a significant cause of maternal morbidity worldwide (Zeng el.,, 2022; Sujith et al., 2024). Physiological and anatomical changes in pregnancy increase susceptibility to infection, while inappropriate or delayed treatment may result in adverse outcomes such as pyelonephritis, preterm labour, and low birth weight (Pradhan et al., 2023;WHO, 2023). Antimicrobial management of UTIs in pregnancy is particularly challenging because therapeutic options are restricted by foetal safety considerations, making accurate pathogen identification and resistance detection essential (WHO, 2023; Angelescu et al., 2021).

While Gram-negative bacteria, particularly Escherichia coli, have long been viewed as the primary uropathogens, Gram-positive organisms are also acknowledged contributors to urinary infections, especially among pregnant women (Pradhan et al., 2023). Notably, *Staphylococcus saprophyticus* and *Staphylococcus aureus* play significant roles, with *S. aureus* drawing particular clinical attention due to its capacity to acquire and disseminate antimicrobial resistance determinants (Ndamason et al., .2019; Kot et al., 2022; Ehlers, 2023). Infections caused by resistant strains of *S. aureus* may lead to treatment failures and prolonged illness if resistance is not promptly identified. (Urriago-Osorio et al. 2025; Touaitia et al., 2025).

Methicillin resistance in *Staphylococcus aureus* is primarily mediated by the mecA gene, which encodes an altered penicillin-binding protein (PBP2a) with reduced affinity for β-lactam antibiotics (Abebe & Birhanu, 2023; Peacock & Paterson, 2015). Additionally, a homologous gene, mecC, has been identified, conferring a similar form of resistance, although its prevalence is believed to be lower globally (Lee et al., 2022). The presence of these genes grants resistance to nearly all β-lactam agents, irrespective of *in vitro* susceptibility to other antibiotics. This highlights the critical necessity for reliable methicillin resistance screening in routine laboratory practices (Roch et al., 2019).

Current international guidelines recommend using oxacillin or cefoxitin as indicator agents for phenotypic detection of methicillin resistance during antimicrobial susceptibility testing, as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2024). However, in most routine diagnostic settings, antimicrobial susceptibility panels may include multiple antibiotics, such as fluoroquinolones, aminoglycosides, and cephalosporins, while omitting oxacillin or cefoxitin. Relying on antibiotics that do not reliably indicate methicillin resistance may fail to identify methicillin-resistant S. aureus, potentially leading to inappropriate therapy and adverse clinical outcomes.

Molecular detection of methicillin resistance genes provides direct evidence of resistance mechanisms, regardless of phenotypic expression, and is widely utilised for confirmatory testing and epidemiological surveillance (Lee et al., 2022). In pregnancy-specific contexts, molecular data are especially valuable for identifying diagnostic gaps in routine susceptibility testing, considering the limited antimicrobial options available and the potential consequences of undetected resistance.

Despite the clinical importance of methicillin-resistant S. aureus, data on the molecular presence of mecA and mecC genes in urinary isolates from pregnant women remain limited in many settings. Basic evidence is crucial for guiding laboratory practices and ensuring adherence to recommended screening protocols for methicillin resistance.

Therefore, this study aimed to detect both mecA and mecC genes among urinary Staphylococcus aureus isolates from pregnant women and to emphasise the importance of including oxacillin and cefoxitin in routine antimicrobial susceptibility testing for S. aureus, as methicillin resistance might be missed if these agents are not tested.

Methodology

Study Design

This cross-sectional, laboratory-based study aimed to investigate urinary bacterial isolates from pregnant women. It involved microbiological culture, bacterial isolate identification, antimicrobial susceptibility testing, and molecular detection of methicillin resistance genes in *Staphylococcus aureus* isolates.

Study Area

The research was conducted at the Niger Delta University Teaching Hospital (NDUTH) in Okolobiri, Bayelsa State, South-South Nigeria. This tertiary healthcare facility serves as a referral center and provides antenatal care services to women from Yenagoa and surrounding communities.

Ethical Approval

Ethical approval was obtained from the Research and Ethics Committee of the Niger Delta University Teaching Hospital, Okolobiri, before commencement of the study. Informed consent was obtained from all participants before sample collection.

Sample Size

A total of 67 midstream urine samples were collected from pregnant women attending antenatal clinics at NDUTH during the study period. The sample size represented all eligible samples obtained within the study timeframe.

Sample Collection

Midstream clean-catch urine samples were collected using sterile, wide-neck, leak-proof containers, and participants were instructed on proper collection techniques. Samples were labelled with unique identification codes and transported to the microbiology laboratory within two hours of collection for analysis.

Culture and Identification of Isolates

Urine samples were mixed gently and inoculated onto Cysteine Lactose Electrolyte Deficient (CLED) agar using a calibrated wire loop. Inoculation was also performed on Nutrient Agar for general bacterial growth. Culture plates were incubated aerobically at 37 °C for 24 hours.

Bacterial growth was examined, and distinct colonies were selected based on morphological features. Pure isolates were obtained by sub-culturing onto fresh Nutrient Agar. Preliminary identification was performed using Gram staining, followed by relevant biochemical tests, including catalase, coagulase, indole, and citrate tests, depending on the Gram reaction and morphology of the isolates.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Bacterial suspensions were prepared from overnight cultures and standardised to the

0.5 McFarland turbidity standard before inoculation. Plates were incubated at 37 °C for 18–24 hours, and inhibition zones were measured and interpreted according to CLSI breakpoints. Susceptibility testing involved a routine multidisc panel including β-lactams, fluoroquinolones, aminoglycosides, and other commonly prescribed agents; oxacillin and cefoxitin were not included in the testing panel.

Molecular Detection of mecA and mecC Genes

Polymerase chain reaction was used to detect the mecA and mecC genes in confirmed *Staphylococcus aureus* isolates. All molecular biology reagents and consumables were obtained from Inqaba Biotec (South Africa) unless otherwise stated. PCR amplification was performed using an ABI 9700 Applied Biosystems thermal cycler.

PCR Reaction Mixture

PCR reactions for both mecA and mecC genes were conducted in a 30 µL volume. Each mixture included 2× DreamTaq Master Mix (Inqaba Biotec, South Africa), which contains Taq DNA polymerase, dNTPs, and $MgCl_2$, along with 0.4 µM of each primer and 50 ng of extracted genomic DNA as the template.

PCR Amplification Conditions

Amplification of mecA gene

The mecA gene was amplified using the forward primer mecA-F (5′-TGGCTATCGTGTCACAATCG-3′) and reverse primer mecA-R (5′-CTGGAACTTGTTGAGCAGAG-3′). Thermal cycling conditions comprised an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 40 seconds, and extension at 72 °C for 50 seconds, with a final extension at 72 °C for 5 minutes. The expected amplicon size was approximately 300 bp.

Amplification of mecC gene

Amplification of the mecC gene was performed using the forward primer mecC F (5′-TGAACGAAGCAACAGTACACC-3′) and reverse primer mecC R (5′-AGATCTTTTCCGTTTTCAGCCT-3′). PCR cycling conditions included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 50 °C for 45 seconds, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 2 minutes.

Agarose Gel Electrophoresis

PCR products were resolved on a 1% agarose gel at 200 V for 15 minutes and visualised using a blue-light imaging system. Amplification was confirmed by the presence of bands corresponding to the expected product sizes.

Statistical Analysis

Data were analysed descriptively using frequencies and percentages. Results were presented in tables and figures as appropriate.

Results

Table 1 summarises the demographic characteristics of the pregnant women enrolled in the study. The majority of women were aged 26-35 years (46.3%), followed closely by those aged 18-25 years (44.8%), while participants aged 35 years or older accounted for 9.0% of the study population.

Table 1: Demographic Characteristics of Pregnant Women Enrolled in the study (n = 67)

Variable	Frequency (n)	Percentage (%)
Age group (years)		
18–25	30	44.8
26–35	31	46.3
>35	6	9.0
Total	67	100

The distribution of uropathogens is shown in Table 2. 54 samples yielded significant bacterial growth, with *Staphylococcus saprophyticus* the most common bacterium, accounting for 63.0% of the isolates. This was followed by Staphylococcus aureus (20.4%) and Enterococcus spp. (9.3%). Gram-negative bacteria were less frequently isolated, with *Klebsiella pneumoniae* and *Escherichia coli* accounting for 3.7% and 1.9% of isolates, respectively.

Table 2: Distribution of Uropathogens Isolated from Urine Samples of Pregnant Women (n = 54)

Bacterial isolate	Frequency (n)	Percentage (%)
Staphylococcus saprophyticus	34	63.0
Staphylococcus aureus	11	20.4
Enterococcus spp.	5	9.3
Klebsiella pneumoniae	2	3.7
Escherichia coli	1	1.9
Total	**54**	**100**

The detection of mecA and mecC genes among the *Staphylococcus aureus* isolates is shown in Table 3. PCR results showed that the mecA gene was detected in 7 isolates (70.0%), whereas the mecC gene was not detected in any isolate.

Table 3: Molecular Detection of *mecA* and *mecC* Genes among *Staphylococcus aureus* Isolates (n = 10)

Resistance gene profile	Frequency (n)	Percentage (%)
mecA detected	7	70.0
mecC detected	0	0.0
Neither *mecA* nor *mecC* detected	3	30.0
Total analysed by PCR	**10**	**100**

PCR amplification of the mecA and mecC was performed on Staphylococcus aureus to assess the presence of methicillin resistance determinants. As shown in Figure 1, amplification bands corresponding to the expected mecA amplicon size (approx 300bp) were observed in several isolates. In contrast, Figure 2 shows no amplification bands expected for the mecC amplicon size, indicating the absence of the mecC gene in the isolates analysed.

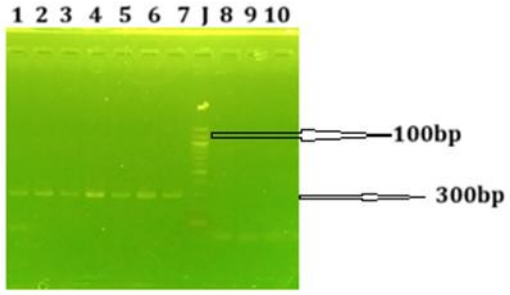

Figure 1: Agarose Gel Electrophoresis of *mecA* Gene Amplification in *Staphylococcus aureus* Isolates

Agarose gel electrophoresis showing PCR amplification of the *mecA* gene in *Staphylococcus aureus* isolates. Lanes 1–7 show positive amplification bands at approximately 300 bp, corresponding to the expected size of the *mecA* gene. Lane J represents the 100 bp molecular weight ladder.

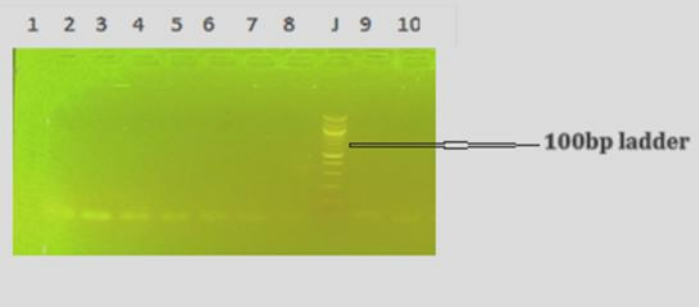

Figure 2: Agarose Gel Electrophoresis of *mecC* Gene Amplification in Selected Isolates

Agarose gel electrophoresis displaying PCR analysis of the mecC gene in Staphylococcus aureus isolates. No amplification bands were observed at the expected size (~500 bp), which indicates the mecC gene is absent in the tested isolates. Lane J represents the 100 bp molecular weight ladder.

Discussion

This study presents baseline molecular evidence of methicillin resistance in *Staphylococcus aureus* isolates from pregnant women attending a teaching hospital. The presence of the mecA gene in 70.0% of these isolates indicates that clinically significant resistance determinants are present within this population.

The predominance of *Staphylococcus saprophyticus* observed in this study is consistent with previous reports that have identified it as a common gram-positive uropathogen among women of reproductive age (Kot et al., 2022). Although *S. aureus* was less frequently isolated than *S. saprophyticus*, its presence is clinically important, particularly given antimicrobial resistance and limited treatment options during pregnancy (Tandogdu & Wagenlehner, 2023).

Methicillin resistance in *S. aureus* is primarily mediated by the *mecA* gene, which encodes an altered penicillin-binding protein with reduced affinity for β-lactam antibiotics. This mechanism confers resistance to virtually all β-lactam agents in clinical practice, regardless of apparent *in vitro* susceptibility to other antibiotics (Lee et al., 2022; CLSI, 2024). In the present study, *mecA* was detected in a substantial proportion of isolates, while *mecC* was not identified. The absence of *mecC* is consistent with the existing literature, which indicates its relatively low prevalence compared with *mecA*, particularly outside specific geographic regions (Lee et al., 2022).

Analysis of antimicrobial susceptibility data revealed resistance to multiple β-lactam antibiotics among the *S. aureus* isolates tested. Among isolates tested, resistance to amoxicillin–clavulanate, ceftriaxone, and cefuroxime was frequently observed. Several isolates exhibited resistance to all tested β-lactam agents, including third-generation cephalosporins and β-lactam/β-lactamase inhibitor combinations.

Notably, resistance to ceftriaxone and cefuroxime was observed even in isolates that remained susceptible to non-β-lactam agents, such as fluoroquinolones and aminoglycosides. This finding indicates that apparent susceptibility to other antibiotic classes does not reliably predict β-lactam activity against *S. aureus*. Where available, imipenem susceptibility results did not negate resistance to other β-lactams and should not be interpreted as evidence of methicillin susceptibility.

When considered alongside the molecular findings, detection of the *mecA* gene in 7 of the 10 *S. aureus* isolates analysed by PCR provides a mechanistic explanation for the observed β-lactam resistance. The *mecA* gene encodes an altered penicillin-binding protein that confers resistance to virtually all β-lactam antibiotics, irrespective of *in vitro* susceptibility to non-indicator agents. Accordingly, the resistance patterns observed for ceftriaxone, cefuroxime, and amoxicillin–clavulanate are consistent with *mecA*-mediated methicillin resistance.

Importantly, these findings demonstrate that testing multiple β-lactam antibiotics alone is insufficient for the reliable detection of methicillin resistance (González-Vázquez et al., 2024 ; Sanchini, 2022). In the absence of oxacillin or cefoxitin, methicillin resistance cannot be confidently inferred, even when extensive antimicrobial susceptibility panels are employed. This limitation underscores the risk of missed or delayed recognition of methicillin-resistant *Staphylococcus aureus* in routine laboratory practice when recommended indicator agents are omitted.

A key implication of this study is that methicillin resistance determinants may be present even when routine antimicrobial susceptibility testing panels include multiple antibiotic classes but omit oxacillin or cefoxitin. Although antibiotics such as ceftriaxone, cefuroxime, fluoroquinolones, and aminoglycosides are commonly tested, they do not reliably indicate methicillin resistance in *S. aureus* (CLSI, 2024). Reliance on such agents alone may therefore fail to detect methicillin-resistant strains, potentially leading to inappropriate antimicrobial therapy and adverse clinical outcomes.

While molecular methods such as PCR are not routinely used for susceptibility testing in many diagnostic laboratories, their application in this study provides confirmatory evidence of underlying resistance mechanisms. It highlights the limitations of incomplete phenotypic testing. Importantly, these findings do not suggest that molecular testing should replace phenotypic methods. Rather, they reinforce existing guideline recommendations that oxacillin or cefoxitin should be incorporated into routine antimicrobial susceptibility testing for *S. aureus* as reliable and cost-effective screening agents for methicillin resistance (CLSI, 2024).

The pregnancy-specific context of this study further underscores the clinical relevance of these findings. Foetal safety considerations limit antimicrobial treatment options during pregnancy, and undetected methicillin resistance may compromise effective therapy and increase the risk of treatment failure (World Health Organisation [WHO], 2023; Angelescu et al., 2021). Strengthening routine laboratory screening practices is therefore essential to support safe and informed antimicrobial decision-making in antenatal care settings.

This study has some limitations. Although *S. aureus* was the primary organism of interest and molecular analysis was conducted on nearly all available isolates, phenotypic methicillin resistance testing using oxacillin or cefoxitin was not performed. This reflects common laboratory practice in which these indicator agents may be omitted from routine testing panels. In addition, the single-centre design and relatively short study duration may limit the generalisability of the findings. Nonetheless, the study provides important baseline molecular data and highlights a practical diagnostic gap with direct implications for routine laboratory practice.

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