Occurrence of Antimicrobial Resistance Uropathogenic *Staphylococcus aureus* Isolates from Pregnant Women Attending Antenatal Clinics within Ilorin


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**A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.**

**Abstract**

**Background:** *Staphylococcus aureus* associated with urinary tract infection (UTI) has become a serious health problem especially with the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) due to the acquisition of *mecA* gene leading to increasing maternal and perinatal burden. This study aimed to evaluate the prevalence of antimicrobial resistance, β-lactamase production and methicillin resistance among uropathogenic *S. aureus* among pregnant women attending selected antenatal clinics in Ilorin.

**Methods:** Forty-five (45) out of 79 presumptive uropathogenic *S. aureus* isolated over a period of 12 months from urine samples of pregnant women were identified using standard bacteriological methods. Antibiogram studies was performed using gentamicin (CN-10µg), ciprofloxacin (CIP-5µg), ofloxacin (OFX-5µg), tetracycline (TE-30µg), sulphamethaxozole-trimethoprim (SXT-25µg), ampicillin (AMP-10 µg), penicillin G (P-10 units), nitrofurantoin (F-30 µg) and cefoxitin (FOX-30 µg) for the detection of MRSA by disc diffusion method. Furthermore, detection of β-lactamase producing *S. aureus* (BL-PSA) was carried out using Iodometric paper strip method.

**Results:** Of the 45 *S. aureus* isolates, 80% were BL-PSA, MRSA (87%), exhibiting high resistance to penicillin G (97.8%), ampicillin (95.5%), tetracycline (77.8%) and sulphamethoxazole trimethoprim (64.4%). In addition, 56% were multidrug-resistant (MDR) exhibiting 20 different phenotypes with CN-P-SXT-TE-AMP-FOX (15.6%) being the majority. Notwithstanding, *S. aureus* isolates showed high sensitivity to nitrofurantoin (93.3%) and ofloxacin (91.1%).

**Conclusion:** This study established an increasing resistance of *S. aureus* to different classes of antibiotics which emphasize the need for constant surveillance to monitor antimicrobial resistance trends. Routine screening for BL-PSA and MRSA among uropathogenic *S. aureus* is also advocated in order to reduce the development and spread of MDR isolates.

**Keywords:** Antimicrobial resistance; *Staphylococcus aureus*; UTI; Pregnant women
INTRODUCTION

In pregnancy, urinary tract infection (UTI) associated with bacterial infection has been the most commonly reported (Delzell and Lefevre, 2000). This is escalated by predisposing factors such as the physiological, hormonal, mechanical influences during pregnancy characterized by general reduction in systemic immunity thus creating suitable environment for the growth of both commensal and non-commensal bacteria (Kumar, 2019). Incriminated bacteria in UTI are Gram positive and Gram negative bacteria (Klines and Lewis, 2018). However, recent studies have reported the increasing predominance of Staphylococcus species including S. aureus (Assouma et al., 2023).

S. aureus are opportunistic bacteria and its isolation in urine is frequently due to Staphylococci bacteremia (Muder et al., 2006). More than 80% of S. aureus generally exhibits resistance to penicillin and ampicillin as well as to several other classes of antibiotics leading to the emergence of multidrug-resistant strains that harbors multidrug-resistant genes commonly acquired and spread via horizontal genes transfers via plasmid, transposons and staphylococcal cassette (Turner et al., 2019). The commonly coded and described resistant determinants to penicillins among the S. aureus are the blaZ-mediated production of β-lactamase (also called penicillinase), the mec genes (commonly known for mecA coding in methicillin resistant Staphylococcus aureus, MRSA) and rarely mecB and mecC that produces an additional altered penicillin-binding protein, PBP2a (Foster, 2017).

METHODOLOGY

Isolates collection and Identification of uropathogenic S. aureus

Of the 79 presumptive uropathogenic S. aureus previously isolated from urine samples of pregnant women attending selected antenatal clinics within Ilorin metropolis (Bello et al. 2020), 45 non-duplicated S. aureus exhibiting varying antibiotic resistance were selected for this study.

Presumptive S. aureus isolates were identified using standard bacteriological methods as described by Chesbrough (2018). Isolates were sub-cultured on Mannitol Salt Agar (MSA; Oxoid, Basingstoke, UK) and plates were incubated aerobically at 37°C for up 24 hours. After 24 hours, colonial morphology were carefully observed and recorded. Furthermore, Gram staining and other biochemical test such as catalase and coagulase were performed. Isolates that ferment mannitol, Gram, catalase and coagulase positive were identified as S. aureus. Pure culture of the identified S. aureus was maintained in 25% Glycerol (Sigma – Aldrich®) in Brain Heart Infusion Broth (BHB) (Oxoid, Basingstoke – UK) and stored at -4°C until required.

Antibiotics susceptibility testing (AST) of uropathogenic S. aureus

Antibiogram studies was performed using antibiotics (Oxoid, Basingstoke, UK) such as gentamicin (CN-10µg), ciprofloxacin (CIP-5µg), ofloxacin (OFX-5µg), tetracycline (TE-30µg), sulphamethoxazole-trimethoprim (SXT-25µg), ampicillin (AMP-10 µg), penicillin G (P-10 units) and nitrofurantoin (F-30 µg) by modified Kirby-Bauer disc-diffusion method as described by Hudzicki (2016). Freshly sub - cultured isolates of S. aureus were standardized to 0.5
McFarland turbidity, aseptically inoculated by flooding onto prepared Mueller-Hinton agar plates (MHA; Oxoid, Basingstoke, UK) and the antibiotic discs were placed at equidistant using flame sterilized forceps. *Staphylococcus aureus* ATCC 25923 was used as control. The MHA plates were then incubated at 37°C for 18 hours. After 18 h incubation, the clear zones around the antibiotic discs were considered as zones of inhibition, diameter was measured in millimeter and interpreted based the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017).

Furthermore, resistant category which grouped isolates as multi drug resistant (MDR), extreme drug resistant (XDR) and pan drug resistant (PDR) was done according to the recommendation of Magiorakos *et al.*, (2011) while resistant phenotypes among *S. aureus* was determined as described by Yılmaz and Aslantaş (2017).

**Screening for Beta- Lactamase producing uropathogenic *S. aureus* Isolates**

The detection of beta-lactamase production among *S. aureus* was carried out using the filter paper iodometric method as described by Izevbizua, (2022). Iodometric filter paper strips were made by preparing 2 % w/v of starch solution by dissolving 2g of starch powder in 98 mL of phosphate buffer saline (pH=7.3) and one ample of benzyl penicillin containing 1 000 000 UI in 10 mL of phosphate buffer saline. Then, the 2 % w/v of starch solution was heated gently to ensure complete dissolution. Ten (10) mL of prepared benzyl penicillin solution was added to 90 mL of 2% starch solution and vortexed properly. Aseptically, Whatman® filter paper No. 1 were cut into strip size of 7 by 4 cm, placed into sterile petri dish and soaked in prepared benzyl penicillin-starch solution for 5 minutes. After 5 minutes, prepared strips were removed, dried in Safety cabinet Class - II (Cellgard - 480) for 24 hours and stored at 4°C until required.

Freshly sub-cultured *S. aureus* isolates were smeared on prepared Iodometric filter paper strips, incubated at 37°C for 30 minutes and Gram’s Iodine solution (Pro-Lab, Canada) was added gradually using pasture pipette. Isolates were deemed positive for Beta-lactamase production when the site of inoculation turns colorless within 5 minutes and negative when it retains the blue-black color of the iodine solution.

**Detection of Methicillin Resistant *S. aureus* among uropathogenic Isolates**

Methicillin resistant *S. aureus* were detected using cefoxitin diffusion method as recommended by the Clinical and Laboratory Standards Institute (2016). Standardized turbidity of 0.5 McFarland suspension of the *S. aureus* isolates was made and inoculated on MHA plate by flooding. Cefoxitin (30 µg) disc recommended to detect mecA among MRSA was placed aseptically on MHA plates using flamed sterilized forceps, allowed to pre-diffuse for 15 minutes and plates were incubated at 37°C for 18 hours. Eighteen hours (18h) post incubation, zones of inhibition were measured. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and ≥ 22 mm was considered as methicillin susceptible.

**Data Analysis**

Obtained data were analyzed using descriptive statistics such as bar, pie charts, percentages, frequency and tabular representations.

**RESULTS**

**Identification of uropathogenic *S. aureus***

All presumptive isolates of uropathogenic *S. aureus* were Gram positive cocci, mannitol fermenters, catalase and coagulase positive.

**Antibiotics susceptibility testing (AST) of uropathogenic *S. aureus***

Based on susceptibility test results as shown in Figure 1, highest resistance was observed against Penicillins and cephalosporin; including penicillin (97.8%), ampicillin (95.5%), cefoxitin (87%) and oxacillin (82%). Other notable resistance was observed against tetracycline (77.8%) and sulphamethoxazole trimethoprim (66.4%). However, *S. aureus* isolates showed high sensitivity to nitrofurantoin (93.3%) and ofloxacin (91.1%).

Figure 2 shows their antibiotics resistance category, 56 % of *S. aureus* were multi drug resistant (MDR) and 8.9 % were extended drug resistant (XDR) isolates. Thirty – six percent were none multi drug resistant (NMDR) while none of the uropatogncir *S. aureus* isolates were pan- drug resistant (PDR).
Fig 1: Antibiotic resistance profiles of uropathogenic S. aureus isolates.

Fig 2: Antibiotics Resistance category of uropathogenic S. aureus isolates.

NMDR: Not Multi-drug resistant isolates; MDR: Multi-drug resistant isolates; XDR: Extended drug resistant isolates; PDR: Pan-drug resistant isolates

In addition, 20 different phenotypic resistant patterns were identified with CN-P-SXT-TE-AMP-FOX (15.6%) being the highest occurring phenotype. Other resistance phenotypes were observed in very low frequencies as indicated in Table 1.
Table 1: Phenotypic resistance pattern of uropathogenic *S. aureus* isolates from pregnant women

<table>
<thead>
<tr>
<th>S/No</th>
<th>Phenotypic Resistant Pattern</th>
<th>Frequency</th>
<th>%</th>
</tr>
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<tr>
<td>1</td>
<td>CN-P-SXT-TE-AMP-OX-FOX</td>
<td>7</td>
<td>15.60</td>
</tr>
<tr>
<td>2</td>
<td>CIP-P-SXT-TE-AMP-OX-FOX</td>
<td>5</td>
<td>11.10</td>
</tr>
<tr>
<td>3</td>
<td>P-SXT-TE-AMP-OX-FOX</td>
<td>5</td>
<td>11.10</td>
</tr>
<tr>
<td>4</td>
<td>P-TE-AMP-OX-FOX</td>
<td>4</td>
<td>8.90</td>
</tr>
<tr>
<td>5</td>
<td>CIP-P-SXT-TE-AMP-OX-FOX</td>
<td>4</td>
<td>8.90</td>
</tr>
<tr>
<td>6</td>
<td>P-SXT-TE-AMP-FOX</td>
<td>3</td>
<td>6.67</td>
</tr>
<tr>
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<td>6.67</td>
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<tr>
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</tr>
<tr>
<td>10</td>
<td>OFX-CN-P-SXT-TE-AMP-FOX</td>
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</tr>
<tr>
<td>11</td>
<td>OFX-CN-P-SXT-AMP-FOX-F</td>
<td>1</td>
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</tr>
<tr>
<td>12</td>
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</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>45</td>
<td>100</td>
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</table>

*Gentamicin: CN; Penicillin: P; Ampicillin: AMP; Oxacillin: OX; Nitrofurantoin: F; Ciprofloxacin: CIP; Ofloxacin: OFX; Tetracycline: TE; Sulphamethaxazole-trimethoprim: SXT.*

**Screening for Beta-lactamase producing uropathogenic *S. aureus* (BL-PSA) and detection of MRSA isolates**

Figure 3 shows the proportion of BL-PSA among uropathogenic *S. aureus*. Eighty percent (80%) of isolates were BL-PSA and 87% were methicillin resistant *S. aureus* as indicated in Figure 4.

![Figure 3: Proportion of BL-PSA among uropathogenic S. aureus.](image)
DISCUSSION

This antimicrobial resistance surveillance study in UTI associated with pregnant women within the study area shows the involvement of \textit{S. aureus} as a complicating etiologic agent which could exacerbate an overwhelming colonization of the urethra by \textit{S. aureus}. Although, the involvement of \textit{S. aureus} in UTI is rare which only accounts for about 0.5 - 6 % cases (Alshomrani \textit{et al.}, 2023), with 1.3 % \textit{S. aureus} isolates from urine samples been previously reported in France (Goldstein, 2000). In addition, several other reports documented the isolation of \textit{S. aureus} from urine samples among pregnant women (Sibi \textit{et al.}, 2014; Simon-Oke \textit{et al.}, 2019), however, these studies isolated both BL-PSA and MRSA that were resistant to a plethora of antibiotics.

This susceptibility profile indicates highest resistance to Penicillins, namely penicillin, ampicillin and oxacillin suggesting a decline in the effectiveness of this class of antibiotic in the empiric and non-empiric treatment of UTI caused by \textit{S. aureus} within the study area. This concurs with several reports over the years (Musbau \textit{et al.}, 2017; Yılmaz and Aslantaş, 2017; Majumder \textit{et al.}, 2022; Assouma \textit{et al.}, 2023). But oppose the finding of Rohini \textit{et al.} (2017) which indicate moderate resistance of 40 % against penicillin among pregnant women attending a tertiary health care in India. However, Iheanacho, \textit{et al.} (2018) reported 100 % resistance of \textit{S. aureus} against ampicillin in a tertiary health care in Cross Rivers, Nigeria. This high resistance to beta-lactam antibiotics such as the penicillin, ampicillin and oxacillin could be due to beta-lactamase production and methicillin resistance among uropathogenic \textit{S. aureus} (Foster, 2017 and Guo \textit{et al.}, 2020) and possibly be responsible for the high proportion of multidrug resistance among uropathogenic \textit{S. aureus} in this study.

Other notable resistance was observed with tetracycline (77.8 %) and sulphamethoxazole-trimethoprim (64.4 %) against \textit{S. aureus} isolates thus limiting their use in the empirical treatment of UTI within the study area. This is comparative to the review findings of Abubakar and Sulaiman, 2018 in Nigeria which reported greater than 85% of \textit{S. aureus} resistance to sulphonamethoxazole-trimethoprim and tetracycline. However, Ochada \textit{et al.} (2015) reports indicates 100% resistance of uropathogenic \textit{S. aureus} to tetracycline and sulphamethoxazole-trimethoprim from two tertiary hospitals in southwest, Nigeria.

Sequent findings indicate significant percentage of uropathogenic \textit{S. aureus} isolates were multidrug (56%) and extended drug resistant (8.9 %), showing varying multiple antibiotics resistance phenotypes. This concur with reports of both MDR and XDR uropathogenic \textit{S. aureus} isolates by Khalaf \textit{et al.} (2022) although, with a lower occurrence rate of 29.1% and 1.9% respectively. This however differs from 100% MDR among uropathogenic \textit{S. aureus} reported by Ghaima \textit{et al.} (2018) among pregnant women in Baghdad and a lower rate of 6.6% reported by Kasew \textit{et al.} (2022) in a 10 years retrospective study conducted in northwest Ethiopia.

\textit{Staphylococcus aureus} has a well-defined mechanisms of resistance to the beta-lactam antibiotics thereby rendering empirical treatment of UTI ineffective through \textit{β}-lactamase production (BL-PSA) or penicillin-binding-protein 2a (PBP-2a) modification among the methicillin-resistant \textit{S. aureus} (MRSA) (Turner \textit{et al.}, 2019). Eighty percent (80%) and 87% of uropathogenic \textit{S. aureus} in this study were \textit{β}-lactamase producing and methicillin-resistant \textit{S. aureus} using standard phenotypic detection and confirmatory methods (Shrestha and Shamser, 2014; Basak \textit{et al.}, 2016). Interestingly, not all penicillin, ampicillin and oxacillin resistant isolates were \textit{β}-lactamase or MRSA positive. This further highlights the possibility that other mechanism of resistance maybe involved (Guo \textit{et al.}, 2020). Hence, suggesting the further use of molecular assessment.
This study shows that 80% of the MDR *S. aureus* were β-lactamase producers and 87% were MRSA which differ from the 10% (Hamza and Kumurya, 2016), 36.36% (Singh et al., 2019) and 45.5% (Basak et al., 2016) reported in Kano- Nigeria and India respectively. However, Baral et al., (2012) reported a 100% MDR among uropathogenic *S. aureus*. These β-lactamases are enzymes capable of hydrolyzing beta-lactam ring in beta-lactam antibiotics (Bonomo, 2017) mediated by the *bla*Z among the BLP-SA and MecA (Munita and Arias, 2016) mobile genetic element and are spread from one bacteria to the other by horizontal gene transfer via plasmid, transposon or staphylococcal cassette chromosome mec (SCCmec) (Richter et al., 2016).

Most of the isolates (80%) were MDR uropathogenic *S. aureus* representing 20 different observed resistant phenotypes with CN-SXT-TE-AMP-OX-FOX pattern being the most common and comprising antibiotics in the class of aminoglycoside, sulfonamide, tetracycline and beta-lactam antibiotics. This differ from the report of Silago et al., (2022) which indicates four (4) different resistant phenotypes among the MDR uropathogenic *S. aureus* with CIP-GEN-TCY being the highest in two locations in Tanzania. This indicates variations in the circulation of uropathogenic *S. aureus* phenotypes in different study locations and geographical regions as previously suggested (Öztürk and Murt, 2020). The resistant phenotypes in this study could be of attributed to the high antibiotics misuse probably due to their cheap nature, easy route of administration, accessibility and lack of adherence to treatment regimen in the management of UTI (Majumder et al., 2022) leading to significant human and economic burden.

**CONCLUSION**

This study established an increasing prevalence of MDR uropathogenic *S. aureus* isolates especially BL-PSA and MRSA among pregnant women in Ilorin. These isolates were highly sensitive to ciprofloxacin, ofloxacin and nitrofurantoin which may be useful as empirical antimicrobial therapeutic agents. Thus, there is need for constant Antimicrobial Resistance Surveillance of *S. aureus* to track MDR isolates spread and routine screening for BL-PSA and MRSA among pregnant women with UTI in order to reduce therapeutic complications.

**ETHICAL CONSIDERATIONS**

Ethical approval with the reference number MOH/KS/EU/777/206 was granted by the Ethical Review Committee, Ministry of Health, Kwara State, Nigeria. In addition, specific permission for approval to conduct this study in the selected clinics, was obtained from each Chief Medical Directors (CMDs), informed and oral consent were also sought from the pregnant women willing to participate in the study.

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