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Molecular Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Catheterized Patients at the Urology Clinic of Usmanu Danfodiyo University Danfodiyo Teaching Hospital (UDUTH), Sokoto

Fatima G Inuwa[1*], Ummu-Khulthum Muhammad[2], Aminu Y. Fardami[3], Nuhu Tanko[4], Sabitu M. Zainu[5] and Salwa K. Muhammad

[1]Department of Microbiology, Sokoto State University, Sokoto State, Nigeria.
[2]Department of Microbiology, Usman Danfodiyo University Sokoto.
[3]Department of Pharmaceutical Microbiology and Biotechnology, Usmanu Danfodiyo University Sokoto.

***Corresponding author's email:**

phertyber2l44@gmai.com

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Catheter-associated urinary tract infections (CAUTIs) are a major cause of morbidity and mortality among hospitalized patients, with Staphylococcus aureus particularly methicillin-resistant *Staphylococcus aureus* (MRSA) emerging as a significant pathogen. This study investigated the molecular detection and antibiotic resistance profile of MRSA isolated from urine samples of catheterized patients. A total of 191 urine samples were collected and processed using standard microbiological methods. *S. aureus* was isolated on mannitol salt agar and confirmed by catalase and coagulase tests. Antibiotic susceptibility testing was carried out using the disc diffusion method, and molecular identification of MRSA was performed by PCR detection of the mecA gene. Out of the 191 samples, 123 (64.4%) yielded *S. aureus*, with a higher prevalence among females (65.9%) compared to males (34.1%). Socio-demographic analysis revealed higher isolation rates in the 29–39 age group and among married individuals. Of the 123 *S. aureus* isolates, 63 (51.2%) were confirmed as MRSA, with females, rural residents, and married individuals being the most affected. Antibiotic susceptibility testing showed universal resistance to cefoxitin, high resistance to ciprofloxacin (55.6%), ofloxacin (52.4%), and penicillin (93.7%), while comparatively better activity was observed with levofloxacin (46.0% sensitivity) and ceftriaxone (42.9% sensitivity). Molecular detection revealed that 49 (77.8%) of the 63 MRSA isolates carried the mecA gene. The high prevalence of MRSA and multidrug resistance observed underscores the need for continuous surveillance, rational antibiotic use, and implementation of molecular diagnostics in routine hospital laboratories. The findings highlight the urgent necessity for effective infection control strategies, particularly in catheterized patients who are at greater risk of MRSA infections.

Keywords: *Staphylococcus aureus*, Antibiotic resistance, Catheterized patients.

1. Introduction

Staphylococcus aureus (*S. aureus*) is one of the major pathogens causing infections in humans ranging from mild, minor infections to severe life-threatening conditions. *S. aureus* is a human commensal and opportunistic pathogen constitutes the major causative agent of several bacterial infections (Medina & Castilo-Pino, 2019). Despite the advancement and availability of several antibiotic therapies, staphylococcal infection still remains as one of the most frequent infections in hospitalized patients causing a wide variety of clinical manifestations ranging in severity from superficial infections such as cutaneous infections to severe invasive diseases like bacteraemia (Chakolwa *et al*., 2019).

Antimicrobial resistance (AMR) is one of the world's most urgent public health concerns. The term "Methicillin-Resistant *Staphylococcus aureus*" (MRSA) refers to the *S. aureus* that can resist classes of antimicrobials, including aminoglycosides, macrolides, and quinolones. Because of its resistance to antibiotics, management of MRSA infections requires more complicated, toxic, and expensive treatment (Kahsay *et al.,* 2014). The presence of an indwelling catheter increases the risk of *S. aureus* carriage in the urinary tract. MRSA is an emerging cause of CAUTI, which frequently progresses to more serious invasive infections representing 1% of uncomplicated cases and 3% of complicated cases associated with the physical obstruction of the urinary tract (Medina &Castilo-Pino, 2019).

Thus, this study was aim to determine the prevalence and detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from urine sample

of catheterized patients attending Usmanu Danfodiyo University Teaching Hospital Sokoto.

2. Materials and Methods

2.1 Isolation and Identification

This study was conducted from June- December 2024. Urine samples from catheterized patients was obtained aseptically by disinfecting the sample port thoroughly with 70% alcohol-impregnated swabs and aspirates 10-20 mL into a sterile container. Samples were taken and processed in Microbiology laboratory within 30 minutes. A sterile wire-loop was dipped vertically into the urine sample and streaked onto the surface of the Mannitol Salt Agar plate using semi quantitative streaking technique as described by Chesbrough (2006) to ensure proper distribution of the sample. All the streaked culture plates were then incubated at 37 °C for 24h to 48h. The plates were checked for growth of bacteria colony macroscopically (Lunacek *et al.,* 2014).

Identification of *S. aureus* was performed based on the typical characteristics of the organism, such as round colony, convex, golden yellow, mannitol fermenting colonies on MSA plates. In order to achieve a pure culture, golden yellow colony were further cultured using streak plate method on nutrient agar, catalase and coagulase positive tests were used to ensure an organism of *S. aureus* (Singh *et al.,* 2019).

2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities of the bacterial isolates were performed according to the criteria of the Clinical and Laboratory Standards Institute (CLSI), 2023 using the Kirby–Bauer disc diffusion method on Muller-Hinton Agar (Oxoid Ltd, UK). A 3–4 colony of bacteria was taken from a pure culture colony and transferred to a tube containing 5 mL sterile normal saline (0.85% NaCl) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 standard in a tube. Using disposable pipette 0.5ml of suspension was inoculated on Muller Hinton Agar and then spread using a sterile bend glass rod (Hussain *et al.,* 2013).

The antibiotics used were Ciprofloxacin (10 ug), Amoxicillin clavulanate (30 µg), Erythromycin (15 µg), Nitrofurantoin (30 µg), Penicillin (30 µg), Tetracycline (10 ug), Ceftriaxone (25 µg), Gentamycin (10 ug), Ofloxacin (10 ug), and cefoxitin (30 µg). These antimicrobial drug discs were selected based on CLSI and also by considering the availability of these drugs in the study area for the treatment of UTI. The antibiotic discs were placed on cultured MHA plates, then left at room temperature to dry for 3 to 5 minutes and incubated at 37 °C for about 18 to 24 hours and the zones of inhibition were measured using a ruler. The results of the antimicrobial susceptibility test were interpreted according to the CLSI, 2023 guidelines (Dilness & Bitew, 2016).

2.3 Detection of Methicillin Resistance

Detection of MRSA was performed using the Cefoxitin disc as a turning point. Isolates were subjected to Cefoxitin disc diffusion test using a 30 µg disc. A 0.5 McFarland standard suspension of the isolates was done. Using disposable micropipette 0.5ml of suspension was inoculated on Muller Hinton Agar and then spread using a sterile bend glass rod. Then Cefoxitin disc were placed on the cultured plates and incubated at optimum temperature and time I.e., 35 °C for 18–24 hours, and then a zone of inhibition in mm was measured. If the zone of inhibition was ≤21 mm in diameter, it was reported as methicillin resistant and if it was ≥22 mm in diameter, it was considered as methicillin sensitive (Looney *et al.,*2017).

2.4 Molecular Detection

2.4.1 DNA Extraction

Genomic DNA was extracted from liquid-broth cultures of *S. aureus* grown from catheter urine isolates. Extraction was performed using DNA extraction kit (Qiagen, USA), with appropriate pretreatment for Gram-positive bacteria (to ensure efficient cell wall lysis), following the manufacturer's recommended workflow for bacterial samples. Extracted DNA was quantified and checked for purity by spectrophotometric methods and stored at appropriate temperatures pending PCR analysis (Momtaz *et al.*, 2020).

2.4.2 PCR Amplification of the mecA Gene

Amplification of the mecA gene encoding Methicillin resistant *S. aureus* (MRSA) was performed according to the method described by Momtaz *et al.* using primers previously designed. Amplification was performed using published primer sets and a commercial PCR master mix in a thermal cycler following the manufacturer's instructions and previously described protocols for mecA detection. Each run included a positive control (a mecA-positive *S.aureus* strain), a negative extraction control (mecA-negative *S.aureus* or equivalent), and a no-template control to monitor contamination. Amplicons were resolved by agarose gel electrophoresis alongside an appropriate molecular weight marker and visualized using an intercalating nucleic acid stain, band sizes were compared with the expected mecA amplicon. Representative positive amplicons were verified by comparison with the positive control and by size conformity (Vatansever *et al.,* 2016).

2.5 Statistical Analysis

Data obtained from microbiological testing and PCR analysis were compiled and analysed using descriptive statistics. Microsoft Excel (Version 2010) was used for data tabulation and basic analysis. Frequencies and percentages were calculated for categorical variables. Graphical representation of PCR-positive samples was presented using bar charts. No inferential statistics (e.g., chi-square, t-test) were applied due to the descriptive nature and limited sample size of the study.

2.6 Ethical Considerations

Ethical clearance was obtained from the institutional review board of Usmanu Danfodiyo University Teaching Hospital, Sokoto with No.: UDUTH/HREC/2023/1348/V2. The objective of the study was clearly described to the study participants including the benefits and risk, and confidentiality was maintained using identification codes. Considering the objective of the study and the demographic characteristics of the participants, the institutional review board gave a permission to take both verbal and/or written consent. So, both verbal and/or written informed consents were taken from respondents prior to enrolment and collection of data. Verbal consent was taken from some respondents who are not able to read and write, and written consent was taken from those who can put their signature.

3. Results and Discussion

3.1 Results

3.1.1 Socio-Demographic Characteristics

A total of 191 catheterized adult patients were investigated during the study period, of which 88 (46%) were male and 103 (54%) were females. The ages ranged from 18 to 65 years with the mean ± SD age of participants being 37.5 ± 11.5 years (Table 1).

Table 1: Socio-Demographic characteristics among catheterized patients attending Usmanu Danfodiyo University Teaching Hospital Sokoto

Variables	Categories	Number (n=191)	Percentage (100%)
Sex	Male	88	46.1
	Female	103	53.9
Age	18-28	48	25.1
	29-39	63	33.0
	40-50	42	22.0
	51-61	29	15.2
	62-72	09	4.7
Residence	Urban	97	50.8
	Rural	94	49.2
Marital status	Single	52	27.2
	Married	73	38.2
	Divorced	66	34.6

3.1.2 Isolation of *Staphylococcus aureus*

Out of a total 191 study participants' urine cultures, 64.4% (123/191) *S. aureus* were isolated, and 34.1% (n = 42/123) of the culture positives were from males and 65.9% (n = 81/123) were from females. The isolation rate of *S. aureus* was highest in the 29-39 years group (32.5% (40/123)) whereas 42.3% (52/123) was isolated from married study participants (Table 2).

Table 2: Number and Percentage of *S. aureus* isolated from catheterized patients attending Usmanu Danfodiyo University Teaching Hospital Sokoto according to their socio-demographic characteristics

Variables	Categories	Number (n=123)	Percentage (100%)
Sex	Male	42	34.1
	Female	81	65.9
Age	18-28	37	30.1
	29-39	40	32.5
	40-50	22	17.9
	51-61	17	13.8
	62-72	07	5.7
Residence	Urban	69	56.1
	Rural	54	43.9
Marital status	Single	30	24.4
	Married	52	42.3
	Divorced/widowed	41	33.3

3.1.3 Antimicrobial Susceptibility Pattern

Among 123 *S.aureus* 63 isolates were resistant to cefoxitin, confirming their identity as MRSA strains. Nearly all isolates were resistant to penicillin (93.7%), reflecting the well-known ineffectiveness of β-lactam antibiotics against MRSA. High resistance was also observed against ciprofloxacin (55.6%), ofloxacin (52.4%), azithromycin (49.2%), and cefuroxime (58.7%), indicating widespread multidrug resistance. Gentamicin showed a balanced profile (38.1% sensitive, 28.6% intermediate, and 33.3% resistant). Amoxicillin and cefotaxime also showed moderate resistance levels (~41-43%). Erythromycin and levofloxacin (46.0%sensitive) showed comparatively better sensitivity rates among MRSA isolates. Ceftriaxone showed the highest effectiveness, with 42.9% sensitive and only 28.6% resistant isolates, suggesting it may still be useful in treatment (Table 3).

Table 3: Antibiotic sensitivity and resistance pattern of *Staphylococcus aureus* from the urine samples of catheterized patients attending UDUTH Sokoto

Antibiotics (µg)	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Cefoxitin (30)	0 (0.0)	0 (0.0)	63 (100)
Erythromycin (15)	29(46.0)	13(20.64)	21(33.33)
Ciprofloxacin (5)	19 (30.15)	09(14.3)	35 (55.55)
Ofloxacin (5)	24(38.1)	06(9.5)	33(52.4)

Levofloxacin (5)	29 (46.0)	12 (19.1)	22 (34.9)
Gentamycin (10)	24(38.1)	18(28.6)	21(33.33)
Amoxicillin clavulanate (30)	19 (30.1)	17 (27)	27 (42.9)
Azithromycin (10)	23(36.5)	09(14.3)	31(49.2)
Ceftriaxone(45)	27(42.8)	18(28.6)	18(28.6)
Penicillin(10)	0(0.0)	04(6.35)	59(93.65)
Cefotaxime(25)	20(31.7)	17(27.0)	26(41.3)
Cefuroxime (30)	15(23.8)	11(17.5)	37(58.7)

3.1.4 Socio- Demographic Distribution of MRSA Isolates

Out of the 123 *S. aureus* recovered, 63 (51.22%) were resistant. The overall prevalence of MRSA among study participants was 33%. MRSA was more prevalent among females (73.0%) than in males (27.0%), which is consistent with the higher number of *S.aureus* infections observed in females. This may be due to anatomical predisposition to UTIs and higher rates of catheterization in women. The isolation rate of MRSA was highest in the 29-39 years group (33.3%) followed by 18-28 years (25.4%). This indicates that young and middle-aged adults are significantly affected, possibly due to higher hospital admission in this age range. Interestingly, MRSA was more common among rural residents (57.1%) than the urban residents (41.3%). This could suggest differences in hygiene, healthcare access, and antibiotic use patterns between rural and urban populations. Married individuals accounted for the highest proportion of MRSA isolates (46.0%), followed by divorced (33.3%) and single (20.6%). Overall, the results indicate that MRSA is widespread across all demographic groups, but females, young-to-middle-aged adults, rural residents, and married individuals appear to be the most affected groups (Table 4).

Table 4: Distribution of MRSA isolates from urine sample of catheterized patients by socio-demographic characteristics (n=63).

Variables	Categories	Number (n=53)	Percentage (100%)
Sex	Male	17	**27.0**
	Female	46	**73.0**
Age	18-28	16	**25.4**
	29-39	21	**33.3**
	40-50	11	**17.5**
	51-61	09	**14.3**
	62-72	06	**9.5**
Residence	Urban	27	**42.9**
	Rural	36	**57.1**
Marital status	Single	13	**20.6**
	Married	29	**46.1**
	Divorced/widowed	21	**33.3**

3.1.5 Molecular identification of *Staphylococcus aureus*

Molecular confirmation of the 63 identified MRSA isolates was carried out using PCR amplification of the mecA gene, the genetic determinant responsible for the methicillin resistance. Out of the 63 MRSA isolates mecA was detected in 49 isolates (77.8%). Amplification of the mecA gene resulted in a distinct band on 310 base pairs (bp) on agarose gel electrophoresis, consistent with the expected product size. *Figure 1* below shows a representative gel image indicating positive and negative amplification of the mecA gene.

The remaining isolates that were antibiotic resistant but PCR-negative for mecA may be due to alternative resistance mechanisms (e.g., mecC or overexpression of β-lactamase) or technical limitations. No amplification was observed in the negative control samples.

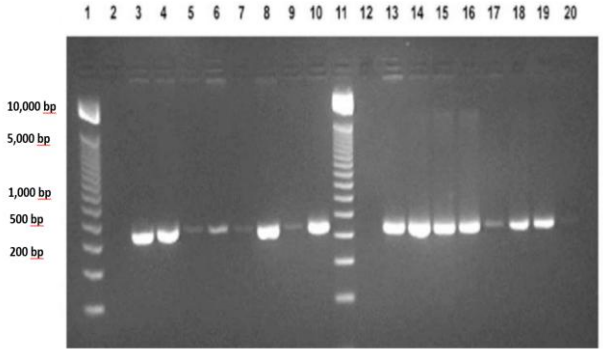

Figure 1: Showing Agarose gel (2%) Electrophoresis of PCR Amplification of the MecA Gene from Methicillin-Resistant *Staphylococcus aureus* Detection within Eighteen Isolates with Ten (10 positive) isolates on Lane 3,4,8,10,13,14,15,16,18 and 19 Expressed at 310 Base Pair.

3.2 Discussion

MRSA is an emerging cause of hospital-associated UTI, especially in catheterized individuals. Despite being rare, MRSA CAUTI is prone to potentially life-threatening exacerbations such as bacteremia that can be refractory to routine antibiotic therapy. The incidence of urinary tract infection caused by MRSA is increasing because patients are more frequently fitted with various urinary catheters (Rahimi *et al.,* 2016).

The prevalence rate of MRSA among catheterized patients with *S. aureus* was 51.2% in our study, which is higher than that of a study conducted by Olarinde *et al.,* in Abeokuta which report the prevalence of MRSA to be 41.1% and Muzzammil *et al.,* in Kano with prevalence of 17.3%. In similar

study conducted by Ushie *et al.,* in Lagos, MRSA prevalence was found to be 58.4% and that of Bashir *et al.,* and Amisu *et al.,* from Yola with prevalence rate of 94.0% and 52.2% respectively, which is higher than that of our study. In contrast, the prevalence of MRSA was found to be low in previous studies conducted in Iran (25.8%) by Rahimi *et al.,* (2016), Nepal (30.8%) by Shrestha *et al.,* (2009), Uganda (33.3%) by Bahati *et al.,* (*2020*).
Unauthorized prescription of antibiotics and self-treatment with antibiotics are likely explanations for the boost of antibiotic resistance in the present survey. Boost resistance rate of MRSA recovered from human clinical infections toward Penicillin, Erythromycin, Gentamycin, Ceftaroline, and Ciprofloxacin was also reported from Portugal (Mottola *et al.,* 2016), and United States (Watkins *et al.,* 2019). Onanuga *et al.,* in Nigeria harboured severe resistance toward Ampicillin (100%), Tetracycline (97.8%), Chloramphenicol (80.4%), Cotrimoxazole (73.9%), Gentamycin (73.9%), Cefuroxime (54.3%), and Ciprofloxacin (32.6%) antibiotics. Sina *et al.,* designated that the UTIs *S. aureus* isolates from Benin displayed a high prevalence of resistance toward Penicillin (100%), Amoxicillin (83.3%), Gentamycin (54.1%), Erythromycin (50.0%), Ciprofloxacin (54.1%), and Tetracycline (83.33%), which are similar to our findings. The persistence of partial sensitivity to Ceftriaxone, Levofloxacin, and Erythromycin suggests these may remain treatment options, though caution is warranted due to the risk of rapid resistance development.

The molecular analysis revealed that 49 out of 63 MRSA isolates (77.8%) harbored the mecA gene, which is widely regarded as the gold standard marker for MRSA identification. The detection rate in this study aligns with findings from Nigeria and other African countries where mecA prevalence among MRSA ranged between 70–85% (Akindele *et al.,* 2012; Shittu *et al.,* 2018). This molecular confirmation establishes an MRSA prevalence of 25.7% (49 out of 191) in the total patient population which appears lower than rates observed in other sokoto-based research by Shuaibu *et al.,* 2020 on nasal colonization studies report an MRSA prevalence of 46.9% among healthcare personnel and inpatients, while in a general clinical specimen surveys showed 44.2% MRSA among 95 *S.aureus* isolates from various clinical samples reported by Asiya *et al.,* 2022.

Prevalence of MRSA in catheterized patients is mainly because of not adopting meticulous aseptic precautions during catheter insertion, infrequent change of catheter and improper catheter care. This study highlights the need for continuous surveillance of antibiotic sensitivity pattern of *Staphylococcus aureus* and this supports the combined use of phenotypic cefoxitin testing and molecular PCR-based methods for robust MRSA surveillance.

4. Conclusion

This study investigated the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in catheterized patients using both conventional and molecular diagnostic methods. *Staphylococcus aureus* was isolated in 64% of catheterized patients, confirming its role as a significant pathogen in hospital settings. More than half of these isolates were MRSA, indicating a high prevalence of methicillin resistance. PCR serves as a reliable and rapid method for molecular detection of mecA and accurate identification of MRSA. The use of PCR in routine diagnostics could minimize misdiagnosis, reduce unnecessary antibiotic use, and enhance infection control efforts in healthcare settings.

Conflict of interest

The authors declare no conflict of interest.

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