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ISOLATION AND CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN SELECTED HOSPITALS OF DUTSE METROPOLIS, JIGAWA STATE, NIGERIA

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ABSTRACT

The purpose of this study was to examine the equipment used in five hospitals located in Jigawa state. Finding Methicillin-Resistant Staphylococcus aureus (MRSA) on hospital equipment in a few Dutse metropolitan hospitals was the main goal. Three hundred and fifty hospital instruments in all were swabbed and then examined. Tests for antibiotic susceptibility were conducted after *S. aureus* was isolated and identified. The isolates were most effectively treated by gentamicin and ciprofloxacin, according to the results. Primary healthcare facilities contained 31 *S. aureus* isolates and 16.67% MRSA isolates, whereas secondary healthcare hospitals had 48 *S. aureus* isolates and 55.56% MRSA isolates. There were 25 *S. aureus* isolates and 27.78% MRSA isolates from tertiary healthcare facilities. The mecA and blaZ genes' polymerase chain reaction (PCR) analyses revealed amplicons of 336 bp and 400 bp, respectively, which were in line with control samples. Six of the 11 isolates that were tested tested positive for mecA, suggesting resistance to methicillin, and nine tested positive for blaZ, indicating the synthesis of β-lactamase. In conclusion, patients, medical staff, and the general public are seriously threatened by the MRSA infection that exists in these facilities.

Keywords: Community, Infection, Sensitivity, Surveillance

INTRODUCTION

Staphylococcus aureus is a ubiquitous bacterium that colonizes approximately 30% of the general population, predominantly as an asymptomatic carrier (Wertheim *et al.*, 2005). While most *S.aureus* infection can be treated with beta-lactam antibiotics, some strains have developed resistance to these drugs, known as methicillin-resistant *S. aureus* (MRSA). Consequently, MRSA infections pose significant treatment challenges. Moreover, MRSA can acquire resistance to additional classes of antibiotics, becoming a multi-resistant bacterium (MRB) with limited therapeutic options (Nathan and Cars, 2014).

S. aureus, a coagulase-positive, gram-positive bacterium, is among the most successful human pathogens. Both methicillin-sensitive *S. aureus* (MSSA) and MRSA can cause a spectrum of diseases ranging from mild to fatal cases spread both locally and globally, colonize various human body parts, and persist in diverse environments outside the host. MRSA is often termed a "superbug" due to its acquired resistance to multiple antibiotics. Specifically, MRSA exhibits resistance to beta-lactam antibiotics, such as methicillin, penicillin, and amoxicillin. Beta-lactams act by inhibiting bacterial cell wall synthesis and are among the most commonly used antibiotics worldwide (Suarez and Gudiol, 2013).

Methicillin was introduced in the 1950s to treat *S. aureus* infections. However, resistance to methicillin in *S. aureus* was identified a few years later (Jevons, 1961). Methicillin, a beta-lactam antibiotic, disrupts the function of penicillin-binding proteins essential for peptidoglycan synthesis in *S. aureus* (Shurland *et al.*, 2007). The detection of MRSA is often benchmarked by the presence of the *mecA* gene, which encodes the penicillin-binding protein 2a (PBP2a) (Rachman *et al.*, 2017).

The impact of MRSA infections is profound, with treatments often proving ineffective, leading to increased morbidity, mortality, hospital admissions, and healthcare costs (Cosgrove *et al.*, 2005). MRSA also exhibits high resistance rates to other antibiotics such as tetracycline, clindamycin, cotrimoxazole, rifampicin, macrolides, and fluoroquinolones (Kaleem *et al.*, 2010).

In Dutse, Jigawa State, Nigeria, the prevalence and impact of MRSA present significant public health concerns, given the bacterium's ability to cause severe infections and its resistance to multiple antibiotics. Understanding the local epidemiology of MRSA is crucial for developing effective infection control strategies and treatment protocols.

The aim of this study was to detect and characterize MRSA from selected hospitals in Dutse metropolis, Jigawa State, Nigeria, to provide insights into its prevalence, resistance patterns, and potential implications for public health and clinical management.

MATERIALS AND METHODS

Sampling Site

The study was conducted in five selected hospitals within Dutse metropolis, Jigawa State, Nigeria: Dutse General Hospital (DGH), Federal University Dutse Clinic (FUDC), Dr. Sambo Hospital (DSH), Dr. Bashir Hospital (DBH), and Rasheed Shekoni Teaching Hospital (RSTH).

Study Equipment

This study utilized various hospital equipment from the selected hospitals, including stethoscopes, thermometers, intravenous drip tubes, catheters, forceps, sphygmomanometers, syringes, disposable gloves, microscopes, electrophoresis machines, colorimeters, burettes, ventilators, stopwatches, weighing scales, and

general surgery tools such as scalpels, scissors, and forceps. These items serve as potential vectors for pathogen transmission between patients.

Sample Collection

A total of 350 hospital equipment surface swab samples were randomly collected from the five selected hospitals. Sterile cotton swabs were moistened in sterile water and firmly applied, rotating slowly to thoroughly cover the surface of the hospital equipment. The swabs were then placed in a cooler with ice packs and transported to the laboratory as described by Ayokunmi et al. (2014) and Bale and Mukhtar (2021).

Isolation, Identification, and Confirmation of MRSA

MRSA was isolated by direct plating of the swabs onto Mannitol Salt Agar (MSA) containing 2 mg/L oxacillin (Oxoid), followed by incubation for 48 hours at 35°C. Suspected colonies, characterized by deep golden-yellow coloration, were confirmed as *S. aureus* using Gram staining and biochemical tests according to CLSI (2021). The confirmed colonies were streaked on Nutrient agar slants, incubated at 37°C for 24 hours, and stored in the refrigerator until further analysis (Bale and Mukhtar, 2021; Sani *et al.*, 2023).

Biochemical Characterization

All isolated organisms were gram-stained and subjected to standard biochemical tests based on their gram reaction, including catalase, indole, citrate, oxidase, urease, and motility tests (Bale and Mukhtar, 2023).

Antibiotic Susceptibility Testing

Antibiotic susceptibility tests were performed using the disk diffusion technique for each identified isolate on Mŭeller-Hinton agar (MHA) as described by the Clinical Laboratory Standard Institute (CLSI, 2013). The inoculated plates were allowed to dry for 10 minutes, after which commercially obtained antibiotic discs were aseptically applied onto the surface of the agar. After 30 minutes, the plates were inverted and incubated at 35°C for 24 hours.

Screening for Methicillin Resistance Using Cefoxitin Disc Diffusion Method

This method followed the Clinical Laboratory Standards Institute guidelines (CLSI, 2021) to confirm suspected resistance using a Cefoxitin disc (FOX, 30µg). Isolates were categorized based on the inhibition zone diameter: susceptible (S; >22mm), low or borderline resistance (B; =21mm), intermediate (I; <21mm), or highly resistant (R; no inhibition zone). Detection of the *mecA* gene is considered the reference method for determining methicillin resistance (Chambers, 1997). Resistance to Cefoxitin is indicative of MRSA (Van Enk and Thompson, 1992). The Cefoxitin disc diffusion method is the most sensitive for detecting MRSA, with negative and positive predictive values of 100% and 98%, respectively (Valesco *et al.*, 2005).

Molecular Identification of Resistance Genes

The MRSA strains were confirmed using Polymerase Chain Reaction (PCR) to detect the virulence genes *blaZ* and *mecA*. These genes are associated with methicillin resistance, residing on the staphylococcal cassette chromosome mec (SCCmec), and expressed by the regulatory genes mecR1 and mec1. The *mecA* gene encodes the altered penicillin-binding protein (PBP2a), which is not inactivated by methicillin (Gaze *et al.*, 2008).

Polymerase Chain Reaction (PCR) Assay

After DNA extraction, the templates were subjected to PCR amplification to detect the *blaZ* and *mecA* genes (Ayepola, 2012).

RESULTS AND DISCUSSION

The highest occurrence of *S. aureus* was on laboratory equipment (50.96%), followed by 23.08% from theatre equipment. Equipment from OPD had 13.46%, while equipment in wards had the least occurrence of 12.50% of *S. aureus* as shown in Table 1.

In Table 2, Laboratory has highest prevalence of MRSA of 33.33% while wards has the least occurrence of 16.67% MRSA. Out of the Three healthcare; Secondary Healthcare has highest pevalence of 48 *S. aureus* 55.56% MRSA while Tertiary healthcare has the least occurrence of 25 *S. aureus* and 27.78% MRSA and Primary healthcare has 31 *S. aureus* with 16.67% MRSA shown in Table 3.

Table 4 showed the distribution of MRSA isolates between each hospitals in the three healthcare. In Primary healthcare , FUDC has highest occurrence of *S. aureus* and MRSA of 8 and 2 respectively while DSH has 13 nd 1 respectively. In Secondary healthcare, DGH has highest number of *S. aureus* and MRSA of 33 and 7 respectively while DBH has 15 and 3 respectively. Lastly, at Tertiary healthcare, RSTH has prevalence of 25 isolates of *S. aureus* and 5 MRSA.

Antibiotic susceptibility testing was conducted for the 13 MRSA isolates phenotypically detected with cefoxitin discs, and the resistance patterns are shown in Table 5. It was observed that 5 (27.80%) of the MRSA isolates were resistant to three or more classes of antibiotics, including macrolides, quinolones, β-lactams, chloramphenicol, and aminoglycosides, indicating multi-drug resistance. Additionally, all MRSA isolates exhibited resistance to one or more β-lactam antibiotics, such as ceftazidime, cefepime, amoxicillin, and cefuroxime.

The results of the PCR analysis of *mecA* and *blaZ* genes on a 1.5% agarose gel are shown in Figure 1. The amplicon sizes for *mecA* and *blaZ* corresponded to the controls, measuring 336 bp and 400 bp, respectively, as represented by the mass ruler DNA ladder. Of the 11 isolates tested, 6 (lane 1,2,4,5,10 and 12) amplified at 336 bp, confirming them as *mecA* positive, indicating methicillin resistance. Additionally, 9 (lane 1,2,3,5,6,7,8,10 and 12) isolates amplified at 400 bp, confirming them as *blaZ* positive, indicating β-lactamase production.

Table 5 details the resistance patterns of the isolates, distinguishing between MRSA and multi-drug-resistant MRSA (MDMR) isolates. Isolates resistant to two antibiotic agents are classified as MRSA, while those resistant to three or more agents are classified as MDMR.

Table 1: Distribution of *S. aureus* among Different Units in the Selected Hospitals

UNIT	No of Samples	*S. aureus* Isolated (%)
Laboratory	165	53(50.96%)
Theatre	94	24(23.08%)
OPD	45	14(13.46%)
Wards	46	13(12.50%)
Total	350	104(100%)

Table 2: Distribution of MRSA among Different Units in the Selected Hospitals

UNIT	No of Samples	*S. aureus* Isolates (%)
Laboratory	165	6(33.33%)
Theatre	94	5(27.78%)
OPD	45	4(22.22%)
Wards	46	3(16.67%)
Total	350	18 (100%)

Table 3: Distribution of *S. aureus* and MRSA among Primary, Secondary and Tertiary Hospitals in Dutse Metropolis

Study Site	No of Samples Collected	No of *S.aureus* Isolates	No MRSA Isolates (%)
Primary	120	31	3(16.67%)
Secondary	150	48	10(55.56%)
Tertiary	80	25	5(27.78%)
Total	350	104	18(100%)

Table 4: Distribution of MRSA Isolates Between Primary, Secondary and Tertiary Health Care Hospitals in Dutse:

Hospitals	No of *S. Aureus*	No. of MRSA
Primary Healthcare		
DSH	13	1
FUDC	18	2
TOTAL	**31**	**3**
SecondaryHealthcare		
DGH	33	7
DBH	15	3
TOTAL	**48**	**10**
Tertiary Healthcare		
RSTH	25	5
TOTAL	**25**	**5**

Key: DGH: Dutse General Hospital, FUDC: Federal University Dutse Clinic, RSTH: Rasheed Shekoni Teaching Hospital, DSH: Dr.Sambo Hospital, DBH: Dr.Bashir Hospital.

Table 5: Drug Resistant Pattern of the 13 MRSA Isolates

Frequency of Drug Resistance	Number of Isolates (n = 18)	Resistant Pattern	Remarks
Resistant to 2 Agents	4	i- AML^{B}, CXM^{B}	MRSA
		ii- CE^{B}, OFL^{Q}	MRSA
		iii- E^{M}, CE^{B}	MRSA
		iv-CXM^{B}, CE^{B}	MRSA
Resistant to 3 Agents	5	i- AML^{B}, CE^{B}, CRX^{B}	MRSA
		ii- CPX^{Q}, OFL^{Q}, AML^{B}	MRSA
		iii- CH^{C},E^{M}, CRX^{B}	MDMR
		iv- OFL^{Q}, AML^{B},CE^{B}	MRSA
		v- CXM^{B}, OFL^{Q},CPX^{Q}	MRSA
Resistant to 4 Agents	3	i- CH^{C}, CE^{B}, CRX^{B}, CXM^{B}	MRSA
		ii- AML^{B}, CXM^{B}, CE^{B}, CRX^{B}	MRSA
		iii- CXM^{B}, AML^{B}, CRX^{B}, CH^{C}	MRSA
Resistant to 5 Agents	3	i- AML^{B}, CXM^{B}, CE^{B}, CRX^{B}, OFL^{Q}	MRSA
		ii- CPX^{Q}, CE^{B}, CPX^{B}, CE^{B},OFL^{Q}	MRSA
		iii- CE^{B}, CRX^{B}, AML^{B}, E^{M}, CH^{C}	MDMR
Resistant to 6 Agents	1	i- CRX^{B}, AML^{B}, CH^{C}, OFL^{Q}, CN^{T}, CE^{B}	MDMR
Resistant to 7 Agents	2	i- CRX^{Q}, CPX^{B}, CN^{T}, CXM^{B}, CE^{B}, OFL^{Q}, E^{M}	MDMR
		ii- OFL^{Q}, CRX^{B}, AML^{B}, CH^{C}, E^{M}, CN^{T}, CE^{B},	MDMR

Key: T: Aminoglycoside, C: Chloramphenicol, M: Macrolides, Q: Quinolones, B-Lactam, CFX: Cefoxitin, CXM: Cefuroxime, CE: Ceftriaxone, CRX: Ceftazidime, AML: Amoxicillin, CH: Chloramphenicol, CPX: Ciprofloxacin, OFL: Ofloxacin, CN: Gentamicin, E: Erythromycin, MDMR: Multi Drug Methicillin Resistance, MRSA: Methicillin Resistant *Staphylococcus aureus.*

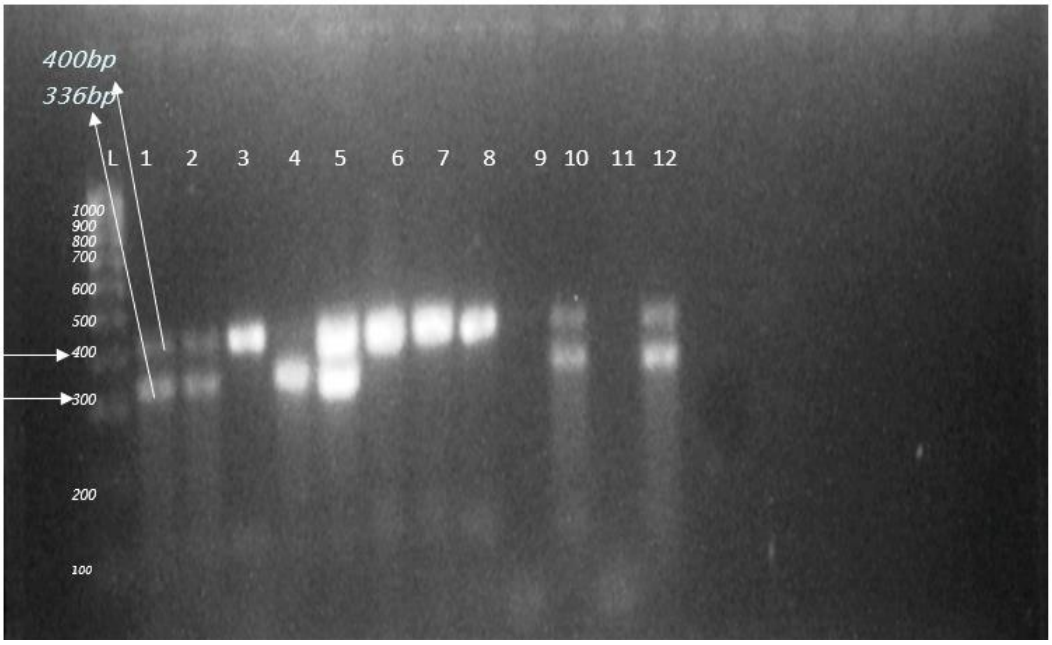

Figure 1: Gel-electrophorogram of *S. aureus mecA*(336bp) and *blaZ*(400bp) genes

Discussion

According to these studies, secondary healthcare hospitals have high rates of MRSA and *S. aureus* isolates, which suggests that hospital equipment is seriously contaminated. The greatest number of isolates was specifically reported by secondary healthcare centers, which was followed by tertiary healthcare centers (25 *S. aureus* and 5 MRSA isolates) and primary healthcare centers (31 *S. aureus* and 3 MRSA isolates). These discrepancies can be linked to the hospitals' varying adherence to work ethics and hygienic procedures. For example, a lot of primary care facilities are private, which may account for why *S. aureus* and MRSA are less common there.

In addition, compared to secondary healthcare facilities, tertiary healthcare centers usually use better standards, cutting-edge buildings, labs, and technology, as well as a more planned and coordinated operating system. Moreover, the frequency of *S. aureus* on hospital equipment may be impacted by the intake rate of patients. The human-facility ratio, staffing levels, and patient volume are some of the variables that can impact the rate at which *S. aureus* and MRSA spread throughout hospital departments. According to this study, secondary healthcare facilities had a much greater prevalence of *S. aureus* than main and tertiary healthcare facilities.

Additionally, the investigations revealed a high rate of MRSA contamination in secondary healthcare centers due to the high frequency of MRSA. Samples taken from the secondary hospitals had a significant number of *S. aureus* and MRSA isolates, indicating high levels of contamination on medical equipment. The discrepancies in isolate counts can be ascribed to variations in work ethics and hygienic measures among the hospitals. Furthermore, the frequency of *S. aureus* infections on hospital equipment may be impacted by the intake rate of patients. The carriage and transmission rate of *S. aureus* and MRSA inside a department and the hospital as a whole can be determined by factors like staffing levels, patient counts, and the ratio of humans to facilities.

Compared to bigger general hospitals, private hospitals like Dr. Sambo Hospital (DSH) and Dr. Bashir Hospital (DBH) see fewer patients and visitors since they specialize in treating particular clinical diseases. The spread and evolution of MRSA infections are probably greatly influenced by hospital size as well as other elements, such as antibiotic use and hospital administration. According to other research, 93% of MRSA transmissions between people and patients occur among healthcare workers (Albrich and Harbath, 2008).

This result is consistent with a research by Aminu et al. (2017) that found 48.5% (16 out of 33) of the samples had methicillin resistance phenotypic prevalence. Clinical specimens from Ilorin (34.7%) and the University of Calabar Teaching Hospital (37.5%) showed comparable prevalence rates (Azeez-Akande et al.,2008). Furthermore, 206 S. aureus clinical isolates were examined by Atif Asghar (2014) using multiplex PCR and normal microbiological techniques. Of these, 114 (55.3%) were MRSA, and 100 of them had the mecA gene. In addition, this study showed that hospital tools, especially invasive hospital devices, were more frequently contaminated with S. aureus and MRSA than were instruments used for evaluating and analyzing patient samples. This is a worrying discovery because biofilm growth on these devices has been shown to result in major illnesses and medical device malfunctions (Hoiby *et al.,* 2011). The significant MRSA prevalence in Dutse secondary healthcare facilities points to the urgent need for better infection control practices. Mitigating the spread of MRSA requires strategies including improved antibiotic stewardship programs, strict adherence to hygiene procedures, and routine disinfection of hospital equipment. By lowering the risk of MRSA infections in both patients and healthcare personnel, these steps will eventually improve patient outcomes and lower healthcare costs.

CONCLUSION

The high contamination rates of hospital equipment underscore the urgent need for secondary healthcare hospitals to implement robust infection control strategies to curb the spread of *S. aureus* infections. The PCR results revealed that 8 of the isolates were positive for the *blaZ* gene, and 5 were positive for the *mecA* gene, confirming the presence of methicillin-resistant strains. This finding is crucial as it indicates that these hospitals harbor MRSA, posing a threat to patients, medical practitioners, and the community at large.

Conclusively, the presence of MRSA in hospital environments within Dutse metropolis emphasizes the importance of stringent hygiene practices, regular disinfection protocols, and effective antibiotic stewardship programs. These measures are essential to mitigate the spread of MRSA, protect public health, and improve patient outcomes.

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