



ISOLATION AND ANTIBIOGRAM OF METHICILLIN RESISTANT *Staphylococcus aureus* (MRSA) AMONG APPARENTLY HEALTHY STUDENTS OF A TERTIARY INSTITUTION IN BENIN CITY, EDO STATE.

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is of public health concern leading to potentially fatal diseases. The aim of this research was to isolate methicillin resistant *Staphylococcus aureus* (MRSA) from urine samples of apparently healthy students of University of Benin, Benin-City, Edo State. A total of One hundred and fifty (150) urine samples were collected and screened using cultural and biochemical methods and multiple antibiotic susceptibility was evaluated using Kirby Bauer disc diffusion technique. Organisms isolated from the urine samples were *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Klebsiella* spp. Females recorded the highest of *Staphylococcus aureus* (42.5%) than their male counterpart (22.8%), while total of 11.6% were tested as MRSA. It was observed that the MRSA isolates were resistant to Gentamicin (54.5%), Ceftriaxone (72.7%), Erythromycin (81.8%), Oxacillin (100%) and had multiple antibiotic resistant index of >2. Hence the need for antibiotic control and public health measures should be put in place towards the usage of antibiotics in other to curtail the menace of MRSA.

Keywords: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Antibiotic, susceptibility, Oxacillin, Multiple antibiotic resistant index

Introduction

Urinary tract infection (UTI) is the most common and severe health problem both in the community and hospital settings worldwide (Bano *et al.*, 2012). Incidence of UTI is second only to upper respiratory infections. Incidence of urinary tract infection is higher in women than men with 40%- 50% of whom will suffer at least one clinical episode during their lifetime (den Heijer *et al.*, 2013). Urinary tract infections and the presence of an indwelling catheter

increases the risk of *S. aureus* carriage in the urinary tract (Coll *et al.*, 1994). *Staphylococcus aureus* is a ubiquitous Gram-positive bacteria and highly adaptive pathogen that colonizes the skin and mucous membrane of the anterior areas, gastrointestinal tracts, perineum, the genitourinary tracts and pharynx (den Heijer *et al.*, 2013).

Staphylococcus aureus is a relatively uncommon cause of urinary tract infection in the general population (Cedric *et al.*, 2004; Barrett, 1999). The most frequently

encountered organisms associated with UTIs include enteric Gram-negative bacteria (with *Escherichia coli* being the most predominant), coagulase negative *Staphylococcus* spp along with *Proteus mirabilis*, *Klebsiella*, and *Enterococcus* (Orenstein and Wong 1999; Khan and Amed 2001). However, recent studies have reported the increasing prevalence of *Staphylococcus aureus* in UTIs (Nwanze *et al.*, 2007; Akortha *et al.*, 2008).

MRSA infection is usually acquired in hospitals and health care facilities. Heavy selection pressures toward acquiring MRSA infection are introduced by the intensive use of antibiotics (Chambers and DeLeo, 2009). Methicillin resistance is a major risk factor for increased morbidity and mortality in *S. aureus* infections as compared to those caused by methicillin-sensitive *S. aureus* (MSSA) (Turner *et al.*, 2019). Bacteriuria with *S. aureus* is postulated to occur through a limited number of mechanisms which include catheterization, urologic procedures, or seeding of the genitourinary tract. Bacteremia is associated with bacteriuria in patients infected with *S. aureus*, which suggests that bacteremia is an important precursor for bacteriuria (Chihara *et al.*, 2010).

The emergence and spread of MRSA strains with reduced susceptibility to non beta-lactam (glycopeptides and new therapies) has escalated the problem by shrinking treatment options (Lowy, 1998). Although, MRSA infection is a global problem, there is greater concern in low-income countries where the burden of infectious diseases is high, and effective antibiotics are not readily available (Lee *et al.*, 2011). When these antibiotics are available and accessible, they are usually expensive and unaffordable. In addition, some of these antibiotics are associated with toxicity that requires monitoring further making

management of MRSA infection difficult. The aim of this study was to evaluate the rate of methicillin resistant *Staphylococcus aureus* in urine samples of apparently healthy students of University of Benin, Benin City, Nigeria.

Materials and Methods

Study Area

This study was conducted in University of Benin, Benin City, Edo State. University of Benin was founded in 1970 as an Institute of Technology and was accorded full University status by the Nigeria National Universities Commission" (NUC) on the 1st of July, 1971. The university is located in Benin City, south-south of Nigeria on 6°20.022¹ North latitude and 5°36.009¹ East longitude. It is situated approximately 40km North of the Benin river and 320km by road East of Lagos.

Sample Collection

Informed consent was obtained from University of Benin Ethical Clearance Committee. Students were counseled about the research work and consented students were given labeled sterile universal bottles to submit their urine sample. The specimen was appropriately labeled, transported to the laboratory, and were analyzed after collection.

Bacterial Enumeration and Identification

The media used were prepared following manufacturer's specification. Sterilization of glassware and other autoclavable materials was done at 121°C for 15 min. Urine samples were cultured using Manitol Salt Agar and Cysteine Lactose Electrolyte Deficient (CLED) Agar with Andrade Indicator and incubated for 24 hours at 37 °C. Upon incubation, total bacterial count (TBC) was carried out. The isolates were further identified using cultural and biochemical methods as described by Cheesbrough (2000).

Isolated *Staphylococcus aureus* were further screened for Methicillin Resistant *Staphylococcus aureus* (MRSA). This was done by sub-culturing the bacterial colony on Mueller Hinton and Oxacillin resistant

antibiotic disc (Oxiod) was placed on the cultured plate and incubated for 24 hours at 37 °C to check for susceptibility or resistance to methicillin.

Antibiotics Susceptibility Test

Few colonies of overnight culture plates were re-suspended in sterile normal saline to match the turbidity of 0.5 McFarland standard for sensitivity testing as described by Cheesbrough (2000). The antibiotic susceptibility of the isolates was determined by the Kirby-Bauer disc diffusion method on Mueller Hilton agar following the guidelines of the clinical and laboratory standards institute (CLSI, 2017). The sensitivity of standard inocula of the isolates to the following antibiotics Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Ceftriaxone (5µg), Erythromycin (5 µg), Cloxacillin (5µg), Ofloxacin (5µg), Augmentin (30µg) (Abtek UK) was carried out. The plates were incubated at 37°C for 24 hours. After incubation, the diameter zone of inhibition was measured in millimeters and interpreted using the CLSI (2017) interpretative chart, and results were taken as resistant, intermediate and sensitive. The multiple antibiotic resistance (MAR) index was determined using the method described by Krumperman (1983) using the formula: a/b , with “a” being the number of antibiotics to which an organism

is resistant to and “b” being the total number of antibiotics tested.

Results

The percentage occurrence of bacteria isolated from apparently healthy male and female students is shown on Table 1. The organisms isolated were; *Staphylococcus aureus*, *Klebsiella* spp, *Proteus mirabilis* and *Escherichia coli*. *S. aureus* had a total occurrence of 26.8% among men and 34.3% among women. Figure 1 is the frequency of occurrence of *S. aureus* and Methicillin Resistant *S. aureus* from urine samples of the study population. Out of the total of isolated *S. aureus*, 33(31.25%) were tested for Methicillin Resistant *S. aureus* (MRSA) and 11(11.6%) were resistant to Oxacillin antibiotic. The antibiotic Susceptibility pattern of the Methicillin Resistant *Staphylococcus aureus* isolated from urine samples is presented on Table 2. It was observed that all the isolates were resistant to Oxacillin 11(100%), while 6(54.5%) of the isolates were susceptible to Ofloxacin. Also Table 3 shows the Multiple Antibiotics Resistant (MAR) index of the isolated Methicillin Resistant *Staphylococcus aureus* (MRSA) from the study population. All the isolates were resistant to more than two antibiotics with MAR index of greater than 2 (>2).

Table 1: Percentage frequency of occurrence of bacteria isolated from urinary tract based on gender of student of the study population.

Bacteria Isolates	Male		Female	
	Occurrence (n)	Frequency (%)	Occurrence (n)	Frequency (%)
<i>E. coli</i>	28	22.8	77	42.5
<i>S. aureus</i>	33	26.8	62	34.3
<i>P. mirabilis</i>	14	11.4	23	12.7
<i>Klebsiella</i> spp	48	39.0	19	10.5
Total	123	100	181	100



Figure 1: Frequency of occurrence of *S. aureus* and Methicillin Resistant *S. aureus* from urine samples of the study population.

Table 2: Antibiotic Susceptibility pattern of the Methicillin Resistant *Staphylococcus aureus* isolated from urine samples

Class	Antibiotics	Code	I (%)	R (%)	S (%)
B lactams	Oxacillin	OXC	-	11(100)	
	Augmentin	AUG	2(18.2)	2(18.2)	7(63.3)
	Cloxacillin	CXC	1(9.1)	10(90.9)	-
Cephalosporins	Ceftazidime	CAZ	2(18.2)	7(63.3)	2(18.25)
	Cefuroxime	CRX	3(27.3)	5(45.5)	3(27.3)
	Ceftriaxone	CTR	3(27.3)	8(72.7)	-
Fluoroquinolones	Ofloxacin	OFL	1(9.1)	4(36.4)	6(54.5)
Aminoglycosides	Gentamicin	GEN	1(9.1)	6(54.5)	4(36.4)
Macrolide	Erythromycin	ERY	2(18.2)	9(81.8)	-
Glycopeptide	Vancomycin	VAN	4(36.4)	6(54.5)	1(9.1)

Intermediate, R-Resistance, S-Susceptible

Table 3: Antibiotic Susceptibility Profile and MAR index of Methicillin Resistant *Staphylococcus aureus* isolated from urine samples

Isolate Id	Resistance profile	Number of resistance	MAR index
S1	OFL, GEN, CTR, ERY, CXC, VAN, OXC	7	0.7
S2	OFL, CAZ, GEN, CTR, ERY, CXC, VAN, OXC	8	0.8
S3	OFL, GEN, ERY, CXC, OXC	5	0.5
S5	AUG, GEN, ERY, CXC, OXC	5	0.5
S6	AUG, CAZ, CRX, CTR, CXC, VAN, OXC	7	0.7
S7	AUG, CRX, CTR, ERY, CXC, OXC	6	0.6
S8	AUG, CAZ, CRX, GEN, CTR, ERY, CXC, VAN, OXC	9	0.9
S9	AUG, CAZ, CRX, CTR, ERY, CXC, OXC	7	0.7
S10	AUG, CAZ, GEN, VAN, OXC	5	0.5
S11	AUG, CAZ, GEN, CTR, ERY, CXC, VAN, OXC	8	0.8

KEY: CAZ-Ceftazidime (30µg), CRX-Cefuroxime (30µg), GEN-Gentamicin (10µg), CXM-Ceftriaxone (5µg), ERY-Erythromycin (5 µg), CXC-Cloxacillin (5µg), OFL-Ofloxacin (5µg), AUG-Augmentin (30µg)

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-recognized public health problem throughout the world. In this study the prevalence of *Staphylococcus aureus* and MRSA was 31.25% and 11.3% respectively. The prevalence of MRSA (11.3%) was seen to be lower than 32.7% as reported by Olorunfemi *et al.* (2020), Bunza *et al.* (2019) and Ajoke *et al.* (2020). Studies conducted in different locations within in Nigeria and other countries have had different prevalence rates. This could be attributed to different sampling size, sites, the length of study period and laboratory procedures employed. It is reported that carriers of MRSA are at risk of septicemia, wound infections and occasionally toxic

shock syndrome (Ike, *et al.*, 2016). Also, MRSA infections have been linked to higher hospital costs, increased mortality rates and prolong hospital stays (Nelson *et al.*, 2015; Thampi *et al.*, 2015; Rubio-Terres, 2010). It was observed that Augmentin demonstrated the highest susceptibility activity against the isolates (63.3%) while the Cephalosporins recorded a resistance of 72.7% to Ceftriaxone, 81.8% to Erythromycin, 54.5% to both vancomycin and gentamicin and 36.4% to Ofloxacin. This is in contrast to the study of Onanuga and Awhowho (2021) in Yenagoa, Nigeria, the study of *S. aureus* from urine samples indicated resistance of 80.4% to Co-trimoxazole, 73.9% to Gentamicin, 69.6% to Vancomycin, 34.8% to Ofloxacin and 32.6%

to Ciprofloxacin, even though the above-mentioned studies were obtained from patients and not healthy volunteers. Generally, a high level of resistance is expected from hospital isolates than in apparently healthy individuals and selective pressure of antibiotic use is higher in hospitals. Oxacillin antibiotic which is used as a marker of Methicillin resistance was used in this study to screened for MRSA and a 100% resistant was obtained. This was found to be higher than 46.3% and 52,4% as reported by Ibadin *et al.* (2017) and Garoy *et al.* (2019). This may be due to variation in prior antibiotic exposure, pattern of prescriptions and policies of health facilities in the various locations (Olorunfemi *et al.*, 2020).

It was found that all the isolates were resistant to more than two antibiotics and recorded MAR index ≥ 0.2 . This could be attributed to the isolates probably from high-risk contamination source, where antibiotics are heavily used, or the isolates have had previous exposure to antibiotics. World Health Organization had invited all countries to adopt strategies to control, monitor and prevent antimicrobial resistance (Okeke *et al.*, 1999). Nigeria needs to set up a national agency on antimicrobial resistance to collect, track and report antimicrobial resistance trends to monitor the free access to antibiotics in Nigeria. To contain antibiotic resistance,

culture and susceptibility test should be undertaken, before prescriptions are given, with strict national quality assurance program for reporting laboratories and educating health professionals and patients on the public health effect of antibiotics resistance.

Conclusion.

A high percentage of apparently healthy student in this study were observed to be carriers of multi-drug resistant *Staphylococcus aureus* placing them at risk of severe infections. Antimicrobial resistance is a major threat to public health that requires coordinated efforts of the government and the public. The problem of multi-antibiotic resistant makes selection of antibiotic for treatment of MRSA infections a challenge. For antimicrobial resistance to be contained, there must be a commitment from the healthcare professionals on antibiotic stewardship. Preventative measures and antimicrobial stewardship are required to control and minimize the spread of MRSA.

Acknowledgment

The authors wish to acknowledge the students who participated in the research and also the assistance of the laboratory staff of Department of Microbiology as well as the amiable HOD of the Department toward the success of this work.

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