PREVALENCE OF METHICILLIN RESISTANCE AMONG CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* IN ZARIA METROPOLIS, KADUNA

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Abstract

The emergence of antibiotic-resistant strains of *S. aureus* such as Methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine.This study determines the phenotypic and genotypic prevalence of MRSA (Methicillin resistant *staph aureus*) among clinical isolates in three hospitals in Zaria, Nigeria. A total of 350 suspected *staphylococcal* isolates from all specimens (blood, urine, high vaginal swab, wound swab, ear swab, urethral swab) submitted to the laboratory microbiology unit of the selected hospitals were collected, out of which 51 were identified as *Staphylococcus aureus* using microgen *staph* identification kit. Disc agar diffusion method was used for the antibiotics susceptibility test while MIC test strip was used for vancomycin susceptibility evaluation. Susceptibility of the isolates tested against various antibiotics shows that a large number of *Staph aureus isolates were generally* susceptible to *gentamycin 96.1%, chloramphenicol 82.4%, ciprofloxacin 78.4%, vancomycin 76.5%, erythromycin 58.8% and teicoplanin 52.9%,* while *generally resistant to cefoxitin 98%, cefixime 100%, amoxicillin 100% and oxacillin 100%. The high percentage 100% of MARI at ≥0.3 suggests that the isolates originate from an environment where antibiotics are often used. Phenotypic MRSA evaluation showed that 98% of isolates were completely resistant to cefoxitin antibiotic, while Phenotypic VRSA evaluation showed that 3.92% of isolates were VRSA (vancomycin resistant staph aureus). 12(twelve) isolates that were resistant to cefoxitin antibiotic were selected for genotypic evaluation for detection of 16SrRNA and mecA genes. All isolates amplified with 16SrRNA signifying all are Staph Aureus.* The result shows that 58% of the isolates harbors *mecA* gene. In conclusion, MRSA is a major cause of Hospital Acquired Infection and increasingly, community-acquired infection. It appears that there is a decline in the overall ability of different healthcare settings to stop or reduce the spread of MRSA. If we do not control MRSA, we can predict that yet more antibiotic-resistant and pathogenic strains will continue to emerge.

Keywords: *Staphylococcus aureus*, Multidrug resistant, MRSA, Zaria

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is also called oxacillin-resistant *Staphylococcus aureus* (ORSA) (McDougal *et al*, 2003).

Human morbidity and mortality in hospital settings are largely caused by

staphylococcal bacteremia (Klevens *et al*., 2007).

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital-acquired (HA) infections highlights this species as a potential pathogen that is able to cope with the antimicrobial agent (Boucher and Corey, 2008) and also in different patient populations is a major public health concern.

MRSA is a major cause of morbidity and mortality both in healthcare settings and in healthy individuals in the last two decades. MRSA is a huge clinical burden that is causing great public and political concern (Gould 2012). Additional screening for MRSA needs to be performed, not only to establish the size of the problem and to allow initiation of decolonization measures to prevent the onset of clinical disease, but also to allow implementation of infection control precautions that will be necessary to control the epidemic.

The aim of the present study was to find out the rate of MRSA and vancomycin resistant *Staphylococcus aureus* (VRSA) in hospital-acquired infections and to determine the antibiotic responsiveness pattern.

Statement of Research Problem

Antimicrobial drug resistance is a global challenge for the 21st century with the emergence of resistant bacteria strains worldwide and account for most antibiotic therapeutic failure (Slade *et al.,* 2009). The emergence of antibiotic-resistant strains of *Staph aureus* such as Methicillin-resistant *Staph aureus* (MRSA) is a worldwide problem in clinical medicine. Hospital strains of *Staph aureus* are usually resistant to a variety of different antibiotics (Todar, 2005). Methicillin resistant *Staphylococcus aureus* with its multidrug resistant profile has put pressure on agents to treat the organism (Thati *et al*., 2011). The treatment of the MRSA infections has become problematic because of the emergence of resistance to vancomycin and other antibiotics, the determination of the anti-microbial susceptibility is crucial for an optimal therapy (Mathews *et al*., 2012).

Methodology

Study Area

The study was carried out using three selected hospitals within Zaria metropolis. The following hospitals were selected for this study base on patients' population, distance apart, good representation of Zaria metropolis: Gambo Sawaba General Hospital Kofan gayan, (MIBA) Major Ibrahim B. Abdullahi memorial hospital Sabon Gari Zaria, St. Luke Anglican Hospital Wusasa Zaria, Kaduna State.

Sample size;

Three hundred and fifty (350) isolates were used for the research.

Ethical approval

Ethical clearance was obtained from Ethical committee approval management Board, Ministry of Health, Kaduna State.

Collection of Isolates

A total of Three hundred and fifty (350) suspected *staphylococcal* isolates from all specimens (blood, urine, high vaginal swab, wound swab, ear swab, urethral

swab) submitted to the microbiology laboratory unit of the selected hospitals were collected over a period of 3 months and transported in a sterile ice park to Pharm. Microbiology lab A.B.U. Zaria.

Identification of *Staph aureus* isolates

Microgen *Staph* identifcation kit (bioMerieux, Inc, Durham, USA) was used to identify the *Staph. aureus* isolates. The procedure was carried out according to the manufacturer's instructions.

Antibiotic susceptibility test

Kirby–Bauer Disk diffusion tests was performed for each of the isolates previously identified as *Staph. aureus* following the method recommended by the of Clinical Laboratory Standard Institute [3]. List of antibiotics used are: Cefoxitin 30 µg, Vancomycin 30 µg, chloramphenicol (30µg), Amoxicillin (30µg), Gentamicin 10 µg , ciprofloxacin 5 µg, Teicoplanin 30 µg, Erythromycin 15 µg, oxacillin 30 µg , Clindamycin 30 µg and cefixime 30 µg, (Oxoid Ltd. Basingstoke, London).

Determination of multiple antibiotics resistance (MAR) index

The Multiple Antibiotic Resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the organisms is resistant to by the total number of antibiotics tested (Paul *et al*., 1997; Christopher *et al*., 2013).

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

Determination of Methicillin (Oxacillin) Resistance Using Cefoxitin Disc

CLSI recommends using cefoxitin instead of oxacillin when using disk diffusion method to determine methicillin resistance in *Staph.aureus* (CLSI, 2014). Cefoxitin results are easier to interpret and are thus more sensitive and enhance induction of PBP2a for the detection of *mecA* mediated resistance than oxacillin results. The gene is located on the *staphylococcal* chromosome cassette *mec* and encodes penicillin binding protein (PBP2a) (BergerBachi and Rohrer 2002).

Bacteria Cell Preparation

The preparation of the bacteria cell was carried out using the method described by Dubey (2009)

DNA extraction

Genomic DNA extraction was carried out using the method described by DNA extraction kit (zymogen^{R}) manufacturer protocols.

Detection Of *mecA* Gene by PCR

16SrRNA and *mecA* (Strommenger *et al*., 2003), PCR was carried out using their respective primers. PCR was performed with the following thermal settings: 5 min at 94°C for initial enzyme activation, followed by 40 cycles of amplification consisting of denaturation at 94°C for 30 s for *mecA*, annealing at 57°C for 45s for *mecA* and extension at 72°C for 30 s for *mecA* with a final extension at 72°C for 5 min. (DNeasy Blood and Tissue Handbook 2006).

Primers sequence and base pair

Primer	Forward	Reverse	Bp	Reference
16Sr RNA	AATCTT TGTCGG TAC ACG ATATTCT TC ACG	CGTAAT GAG ATT TCA GTA GAT AATACA ACA	107	Martineau *et al*., 2001
mecA	AAA ATCGAT GGTAAA GGT TGGC	AGT TCT GCA GTACCG GAT TTG C	532	Strommenger *et al*., 2003

Results

Rate of Occurrence of *Staph aureus* among the collected isolates from the hospitals,

A total of 350 isolates were collected from three Hospitals in Zaria and were analysed for *Staph aureus.* 31(20.67%) were positive for *Staph aureus* in HGSGH, 11(11%) were positive for *Staph aureus* in MIBA, 9(9%)were positive for *Staph aureus* in SLAH as shown in table 1.It also shows the prevalence of *Staph aureus* (14.6%) from the clinical isolates collected from this hospitals.

Distribution of *Staph aureus* isolates by source.

The result shows that 20(39%) of the 51 positive *staph aureus* were found from high vaginal swab (HVS), 16 (31%) of the 51 positive *staph aureus* were found from urine.

Table 1; Rate of Occurrence of *Staph aureus* among the collected isolates from the hospitals

Hospital	No. of Isolates screened	No of *S. aureus* isolated	% Prevalence of *S. aureus*
SLAH	100	11	11.00
MIBA	100	9	9.00
HGSGH	150	31	20.67
Total	350	51	14.6

SLAH= St. Luke Anglican Hospital. Wusasa, MIBA= Major Ibrahim B. Abdullahi memorial hospital Sabon Gari Zaria, HGSGH= Hajiya Gambo Sawaba General Hospital, Kofan-Gayan.

Table 2; Distribution of *Staph aureus* isolates by source.

S/No	**SOURCE**	**NUMBER (%)**
I	High Vagina Swab	20 (39%)
II	Urine	16 (31%)
III	Wound	9 (17%)
IV	Ear Swab	5 (9.8%)
V	Urethral Swab	1 (2%)
	TOTAL	**51**

Table 3*;* Antibiotics susceptibility profile of *Staph aureus* isolates

S/No	ANTIBIOTIC (µg)	SENSITIVE No. **(%)**	INTERMEDIATE No. (%)	RESISTANT No.(%)
I	Vancomycin (30)	39(76.5)	0(0)	12(23.5%)
II	Tecoiplanin (30)	27(52.9)	18(35.3)	6(11.8%)
III	Erythromycin (15)	30(58.8)	8(17.6)	13(23.5%)
IV	Gentamycin (30)	49(96.1)	0(0)	2(3.92%)
V	Amoxicillin(10)	0(0)	0(0)	51(100%)
VI	Chloramphenicol(30)	42(82.4)	3(5.9)	6(11.8%)
VII	Cefoxitin(30)	1(1.96)	0(0)	50(98%)
VIII	Clindamycin(2)	18(35.3)	21(43.1)	12(21.63%)
IX	Ciprofloxacine(5)	40(78.4)	2(3.9)	9(17.65%)
X	Oxacillin(30)	0(0)	0(0)	51(100%)
XI	Cefixime(30)	0(0)	0(0)	51(100%)

About 9(17%) of the 51 positive *staph aureus* were found from wound, 5(9.8%) of the 51 positive *staph aureus* were found from ear swab, 1(2%) of the 51 positive *staph aureus* were found from urethral swab as shown in table 2.

Antibiotics susceptibility profile of *Staph aureus* isolates

The isolates were generally *susceptible to* gentamycin 96.1%, chloramphenicol 82.4%, ciprofloxacin 78.4%, vancomycin 76.5%, erythromycin 58.8% and teicoplanin 52.9%, *while* generally resistant to cefoxitin 98%, cefixime 100%, amoxicillin 100% and oxacillin 100%. As shown in table 3.

Multiple Antibiotic Resistance (MAR) Index of Isolates

The multiple antibiotic resistant indexes (MARI) for each of the 51 isolates were determined as:

MARI =Number of antibiotics to which isolate is resistant/ Total number of antibiotics used

MARI showed that all 51(100 %) isolates were resistant to three or more antibiotics. MARI $\geq$ 0.3 indicated that the isolates originated from an environment where antibiotics were frequently used. Two isolates showed 90 % resistance to the eleven antibiotics tested. All the isolates were consistently resistant to amoxicillin, cefixime and oxacillin (Table 4).

Phenotypic prevalence of MRSA among *Staph aureus* isolates

The result shows that 50 of the 51 positive Staph aureus were found resistant to cefoxitin antibiotic (zone diameter $\leq$ 21mm) which are Methicillin resistant *Staphylococcus aureus*(MRSA), this gives a prevalence rate of phenotypic MRSA among the *Staph aureus* isolates studied to be 98% as shown in pie chart.

Discussion

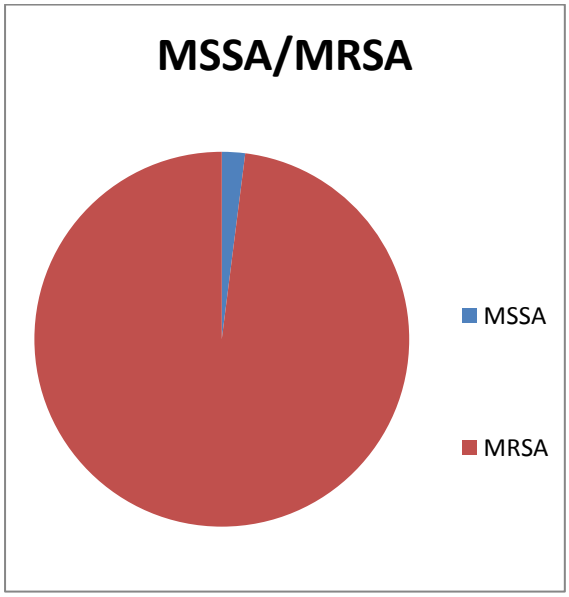
Phenotypic evaluation for the prevalence rate of MRSA in this study shows that 98% (50) of *Staph aureus* isolates were resistant to cefoxitin antibiotic (zone diameter ≤ 21mm). This figure is higher than 20.6% and 47.8% reported from Southwestern Nigeria [Terry *et al.*, 2011; Olowe *et al*., 2007) and 69% reported in Zaria Northern Nigeria (Onanuga *et al.*, 2006). Also higher than (32.8%) reported from Samaru Zaria (Salau *et al*., 2015) The differences in rates of MRSA occurrence in this study may be due to lack of strict measures involve in the use of antibiotics in this hospitals by both health practitioners and also in the community since development of antibiotics resistance in the organisms is thought to be because of unjustified, irrational and irregular use of antibiotics by the human population, over the counter accessibility without recommendations and unrestricted use of antimicrobials in poultry, farm animals, and fisheries.

It also appears that there is a decline in the overall ability of different healthcare settings to stop or reduce the spread of MRSA. In developing countries, it has always been contended that the inappropriate use of antibiotics for community infections may increase the prevalence of resistant bacteria infection (Agwu *et al.,* 2005) including MRSA. *12(twelve) isolates that were resistant to cefoxitin antibiotic were selected for genotypic evaluation for detection of 16SrRNA and mecA genes. All isolates amplified with 16SrRNA* signifying that all isolates are *Staph. Aureus.* The result also shows that 58% of the isolates harbors *mecA* gene. Most of the MRSA isolates were also resistant to other antibiotics.

Table 4; Multiple Antibiotic Resistance (MAR) Index of Isolates

No. of antibiotic to which resistant	Resistant isolates	MAR index	% of Staph to MARI
I	0	0	0
II	0	0.1	0
III	1	0.2	1.96
IV	14	0.3	27.45
V	21	0.4	41.17
VI	10	0.5	19.6
VII	1	0.6	1.96
VIII	1	0.7	1.96
IX	1	0.8	1.96
X	2	0.9	3.92
XI	0	1.0	0

Fig 1; Pie chart showing the occurrence of MRSA isolates in this Hospitals**.**



Molecular Characterization of *StaphA 30* (16S rRNA)

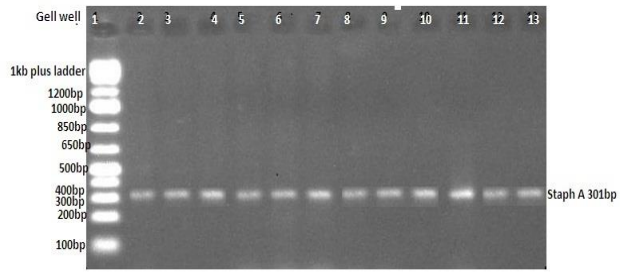


Plate 1; Electrophoretic gel of *Staph A*(301bp) amplified from *Staphylococcus aureus* **isolates.** Keys: Lane 1= 1kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Molecular Characterization of *mecA* gene

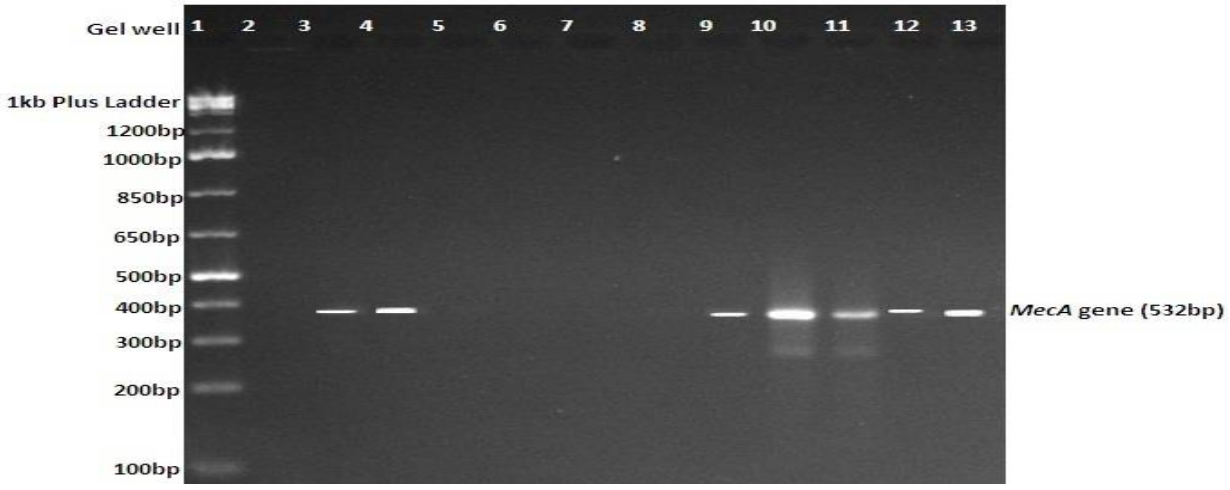


Plate 2; Electrophoretic gel of *mecA* (532bp) amplified from *Staphylococcus aureus* isolates. Keys: Lane 1= 1kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17

The presence of *mecA* gene which specifies the production of an abnormal penicillin binding protein PBP2a that has a decreased affinity for binding β- lactam antibiotics results in resistance to methicillin and also to all β-lactams including penicillins and cephalosporins also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, cross-resistance to non-β-lactam antibiotics such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin is common (Chambers, 2001).

Conclusion

In conclusion, MRSA is a major cause of Hospital Acquired Infection and increasingly, community-acquired infection. Most importantly, it is an additional burden of Hospital Acquired Infection on top of MSSA and of many other organisms, and has increased the transmissibility, morbidity, mortality and costs of infection. Provided that carriers are identified on admission and barrier nursed, control seems to be possible and is likely to be highly cost effective. Moreover, it will allow us to reassure patients that their hospital stay is less likely to end in death or severe morbidity and/or permanent disability from MRSA infection. If we do not control MRSA, we can predict that yet more antibiotic-resistant and pathogenic strains will appear.

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