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Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Hospital Instruments

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Abstract
Methicillin resistant Staphylococcus aureus (MRSA) is a threat to both the hospitalized patients and community. This work aimed at detecting MRSA from commonly used hospital instruments. It is a descriptive hospital based study and 74 samples were randomly collected from swabbed instruments from five Hospitals in Kano, Nigeria. Staphylococcus aureusisolates were identified by culture and biochemical tests. Susceptibility test was carried out using disc agar diffusion method and MRSA was detected phenotypically using cefoxitin 30 µg discs. Also mecAand blaZgene were detected from some of the samples. A total of 33/74 (44.5%) isolates were identified as S. aureus with 16/33 (48.5%) being MRSA. The results further revealed thatinvasive hospital instruments had the highest number of S. aureus and MRSA isolates of 18/33 (54.5%) and 11/16 (68.8%) respectively, while instruments used for superficial assessment of patient body had the least number of S. aureus and MRSA isolates of 6 (18.2%) and 2 (12.5%) respectively. Ciprofloxacin had the greatest activity on the isolates ranging from 75% to 100%, followed by ofloxacin(71.4% to 100%)and gentamicin (66.67% to 90.9%) respectively. The greatest level of resistance was observed with ceftazidime (33.3% to 75%) followed by cefoxitin (33.3% to 72.75) and ceftriaxone (33.3% to 66.7%). Furthermore, the 16 MRSA isolates were generally resistant to the beta-lactam antibiotics used with 7/16 (44%) being multi-drug resistant.Also2/10 (20%) and 4/10 (40%) of the MRSA isolates were positive for mecA and blaZ gene respectively. The study detects a high level contamination of hospital instruments and recommends strict adherence to aseptic procedures and regular screening of hospital workers for the presence of MRSA to control colonization and infection. Further studies are also needed to define the optimum use of ciprofloxacin and gentamicin against MRSA infection.
***Keywords**: Staphylococcus aureus, MRSA, Detection, Hospital instruments, Kano.*

INTRODUCTION

The emergence of methicillin–resistant *Staphylococcus aureus*(MRSA) strains becomes an important public health issue both in the hospital and the community presenting with severe skin and soft tissue infection, necrotizing pneumonia and other complications that include endocarditis, meningitis as well as toxic shock syndrome (TSS) (Mims *et al.*, 2009; Hayani *et al.*, 2008; Boyce *et al.*, 2004; Livermore, 2000). *Staphylococcus aureus* form part of the normal flora of the skin, intestine, upper respiratory tract and vagina but can become pathogenic when condition become favorable for overgrowth (Mims *et al.*, 2009; Lowy, 1998). In the healthy individual, the carrier rate of *S. aureus* range from 15% to 35% with a risk of 38% of individuals developing infection followed by a further 3% risk of infection when colonized with methicillin-susceptible *S. aureus* (MSSA) (File, 2008). Methicillin-resistant *S. aureus*(MRSA) isolates came into existence soon after the introduction of methicillin and have been associated with nosocomial infections and rapidly developed resistance to multiple drug classes (El-Gayar *et al.*, 2014).

Chromosomes or plasmid can mediate antibiotic resistance in *S. aureus* through various mechanisms, including transduction and conjugation, as well as other resistance mechanisms that include; a) enzymatic inactivation of the antibiotic; b) alteration of the target with decreased affinity for the antibiotic (e.g. vancomycin-resistant strains); c) trapping of the antibiotic (for vancomycin and possibly daptomycin) and; d) efflux pumps (fluoroquinolones and tetracycline) (Costa *et al.*, 2013).

The *mecA* gene present in MRSA strains is associated with the resistance and resides on the staphylococcal cassette chromosome *mec* (SCC*mec*) and is expressed by the regulator genes *mecR1* and *mec1* and further encodes the altered protein-penicillin-binding protein(PBP2a), which is not inactivated by methicillin (Gaze *et al.*, 2008; Lowy 1998; Berger-Bech, 1994).The regulator gene *mecR1* is activated by beta-lactam antibiotics such as penicillin and methicillin and serve as a signal transducer that inactivates the *mec1* repressor gene product (Lowy, 1998). Furthermore, some *SCC mec* types contain genetic element for other antibiotic resistance, such as *tn554,* a transposon responsible for the resistance to macrolides, clindamycin, while the *pT181* plamid accounts for tetracycline resistance (Oliviera *et al.*, 2006).

In Nigeria there had been reported cases of MRSA of 12.5% from clinical specimens from six tertiary hospitals in North Western Nigeria (Okon *et al.*, 2013). Olowe *et al.* (2013) also reported a prevalence of 19.2% MRSA from clinical isolates in Medical Microbiology Laboratory of University Teaching Hospital, Ado-Ekiti. Another study at ObafemiAwolowo University Teaching Hospitals Complex (OAUTHC) showed that 40.2% of the isolates were methicillin-resistant while 59.8% were methicillin-sensitive (Obianju*et al.*, 2015). Other studies revealed detection of *mecA*gene in 1.5% of *S. aureus*isolates from clinical samples in South Western Nigeria (Shittu *et al.*, 2006).

Generally, fomites such as stethoscopes and neckties associated with health care providers as well as basic hospital equipments such as IV drip tubes, catheters, and life support equipment are potential carriers of hospital-acquired infections and serve as possible routes to pass pathogens between patients. Thus, rapid and accurate detection of methicillin resistance in *S. aureus*particularly from these fomites as well as detecting their drug resistant pattern is important for controlling the nosocomial spread of MRSA strains especially through strict adherence to the basic aseptic precautions and the use of appropriate antimicrobial therapy. This study was conducted to isolate and identify *Staphylococcus aureus* and detect MRSA among the isolates. It also identifies the resistant gene (*mecA and blaZ*) associated with MRSA present on the commonly used hospital instrument.

Materials and methods

Sample collection and processing

A total number of 74 hospital instruments surface-swabbed samples were randomly collected from the five purposely selected hospitals; Murtala Mohammed Special Hospital (MMSH), Aminu Kano Teaching Hospital (AKTH), Bayero University Kano Clinic (BUK), Premier Hospital (PH) and AbdullahiWase (Nasarawa) Hospital (MAWH), Kano from May 2015 to August 2015.

The swabbed samples were streaked on prepared plates of mannitol salt agar and incubated at 37^0C for 24 hours. After incubation, isolates that produced colonies exhibiting characteristic deep golden yellow colouration were confirmed as *S. aureus* using Grams staining and biochemical tests according to Cheesbrough (2002). Furthermore, the confirmed colonies were streaked on nutrient agar slants and incubated at 37^0C for 24 hours and later stored in the refrigerator until required for further analysis.

Antibiotic susceptibility tests were performed using the disk diffusion technique for each of the identified isolates using Mueller Hinton agar (MHA) as described by Clinical Laboratory Standard Institute (CLSI) (2013). The inoculated plates were allowed to dry for 10 minutes and the commercially obtained antibiotic discs (ciprofloxacin-5µg, gentamicin-10µg, cefuroxime-30µg, ceftazidime-30µg, ofloxacin-5µg, ceftriaxone-30µg, amoxicillin-20µg, chloramphenicol-30µg, cefoxitin-30µg and erythromycin-5µg) were applied aseptically to the surface of the agar and after 30 minutes, the plates were inverted, and incubated at 35^0C for 24 hours.

Cefoxitin disc diffusionmethod for the detection of MRSA was also done according to CLSI (2013). A 0.5 McFarland standard suspension of the isolate was made and a lawn culture was done on Mueller Hinton agar (MHA) plate. Cefoxitin 30µg discs were placed and plates were incubated at 37^0C for 18 hours and zone diameter was measured in reflected light. An inhibition zone diameter ≤21 mm was reported as methicillin resistant and ≥22 mm was considered as methicillin susceptible.

Staphylococcal cassette chromosome *mecA*and *blaZ* gene were detected from the MRSA isolates employing standard molecular protocol methods using DNA extraction, amplification of the extracted DNA using PCR and detection using gel electrophoresis as described bySambrook (1989).

i) DNA extraction

Genomic DNA isolation from the isolates was carried out using solution-based DNA extraction methods that employed organic solvents (phenol and chloroform).Accordingly, cell lysis, denaturation of nucleoproteins and inactivation of cellular enzymes, removal of contaminants and DNA precipitation were carried out as explained in the following steps. First, 2mls of Phosphate buffer was added into bijou bottles containing 1.5ml of the *Staphylococcus aureus* culture and the washed isolates were then poured into appendorf tube (i.e. in duplicate) and then centrifuge at 14,000 RPM for 5minutes and 400µl of buffer and 25µl of protein K were added into the appendorftube containing the isolate and mixed well by vortexing and was then incubated for 1 hour and vortex every 20 minutes. Then 400µl of phenol chloroform was then added into the mixture and vortex briefly and centrifuged at 1300rpm for 10 minutes and using a pipette, the upper layer of the mixture was then collected and transferred to a new appendorftube. Then 100% of ethanol and 40µl of 3M sodium acetate ware added into the tube and stored at -20^{0}C for overnight. The mixture was then centrifuged for 14000 rpm at 4^{0}C for 10 min and the upper layer was discarded. Finally, 400µl of 70% ethanol was then added to the content and spinned at 12,000rpm for 5mintutes, and the upper layer was then discarded and the contents allowed to air dry. The extracted DNA was stored at -20^{0}C in distilled water until required.

ii) PCR amplification

The extracted DNA from above was amplified and used for the detection of *blaZ* and *mecA* gene. The primer sequences and predicted sizes used in the PCR were shown in Table 1. The PCR amplification process was achieved as follows: (i) For the *blaZ* gene, 2.0µl of the isolated genomic DNA sample was added to 18 µl of PCR mixture (1µl of primers and 17µl of distilled water) and for the *mecA* another 2.0µl of the isolated genomic DNA sample was added to 18µl of PCR mixture (1µl of primers and 17µl of distilled water). Each cycle of the amplification process consisted of three steps and each PCR reaction consisted of 35 cycles of amplification (Figure 1).

iii) Detection of *mecA and blaZ* gene by agarose gel electrophoresis

After amplification the extracted DNA was analyzed for the presence of *mecA and blaZ* gene by DNA agarose gel electrophoresis where 8µl of the PCR products obtained from above and 8µl of molecular weight marker were loaded directly onto a 1.5% agarose gel in 1 x Tris-acetic acid -EDTA buffer (TAE) containing 10 µl Green nucleic acid stain (Figure 1). The DNA bands corresponding *mecA and blaZ* gene were of the molecular weight 400 and 336 base pairs when compared with molecular weight marker. DNA amplicons were visualized using a gel imaging system.

Statistical analysis

Data generated from the study was presented using descriptive statistics in form of percentages.

RESULTS

Table 2 reveals that out of the 74 samples collected from five different hospitals in Kano, 33 (44.6%) were *S. aureus* and the highest number of isolates of 11 (33.3%) was from Murtala Muhammad Specialist Hospital, while Premier Hospital has the lowest number of isolates of 3 (9.1%). Table 2 further revealed that 16 (48.5%) out of the 33 *S. aureus*isolated were MRSA isolates. Similarly, samples from Murtala Muhammad Specialist Hospital were found to harbor the highest number of MRSA isolates of 50% (8/16), while samples from Premear Clinic had the least 6.3% (1/16).

Table 3 showed that invasive hospital instruments such as blade, tower cliff, sponge holding and blade holder had the highest number of *S. aureus* and MRSA isolates of 18 (54.5%) and 11 (68.8%) respectively, while instruments used for superficial assessment of patient body such as thermometer, stethoscope and meter ruler had the least number of *S. aureus* and MRSA isolates of 6 (18.2%) and 2 (12.5%) respectively. However, MRSA isolates were not isolated from any of the instruments used in assessing and analyzing samples from patients body such as microscope, autoclave, incubator and weighing balance, although 3 (9.1%)of them had *S. aureus* isolates (Table 3).

The susceptibility test shows that ciprofloxacin had the greatest activity of 75%, 81.8%, 100%, 100%, and 83.3% on *S. aureus*isolates identified from hospital instruments from BUK, MMSH, PH, MAWH, and AKTH, followed by ofloxacin (71.4% to 100%) and gentamicin (66.67% to 90.9%) respectively (Table 4).

However, the greatest level of resistance by the isolates was observed with ceftazidime (75%, 63.6%, 66.7%, 42.95% and 50% from BUK, MMSH, PH, MAWH and AKTH respectively), followed by cefoxitin (33.3% to 72.75) and ceftriaxone (33.3% to 66.7%) respectively.

The drug resistant pattern for the 16 MRSA isolates showed that 7(44%) of the MRSA isolates were multi-drug resistant (MDRSA) as they were resistant to more than two different classes of the antibiotics used in the study (i.e. aminoglycoside, chloramphenicol, macrolides, quinolones and beta-lactams) (Table 5). Also, all the MRSA isolates were generally resistant to beta-lactam antibiotics.

The result of PCR products shows that, out of the 10 MRSA isolates 4(40%) (AKTH-AF, MMSH-NTDF, BUK-SC and MAWH-RT) amplified at 400bp (Plates 1 and 2) indicating the presence of *blaZ* gene. Whereas, only 2(20%) isolates (MMSH-AF and MAWH-TS) amplified at 336bp indicating the presence of *mecA*gene (Plates 3 and 4).

Table 1: The primer sequences and predicted sizes used in the multiplex PCRs

Gene	Oligonucleotide sequence(5'-3)	Expected amplicon size (bp)
MecA	5'-GTTGTAGTTGTCGGGTTTGG-3 5'CTTCCACATACCATCTTCTTTAAC'3	336
BlaZ	5'CAAAGATGATATAGTTGCTTATTC'3 5'TGCTTGACCACTTTTATCAGC'3	Compared with control

Adapted from Ayepola (2012) and Deneeling*et al*. (1998)

Table 2: Distribution of *S. aureus* and MRSA isolates identified from different hospital instruments among five hospitals studied in Kano.

Sampling sites	No of samples Collected	No of samples positive for *S. aureus*	No of samples positive for MRSA
AKTH	10	6(18.2%)	2(12.5%)
MAWH	18	8(24.2%)	3(18.8%)
PH	10	3(9.1%)	1(6.3%)
BUK	18	5(15.2%)	2(12.5%)
MMSH	18	11(33.3%)	8(50%)
Total	**74**	**33**	**16**

KEY: AKTH: Aminu Kano Teaching Hospital, MAWH: Mohd Abdullahi Wase Hospital, PH: Premear Clinic, BUK: Bayero University Kano, MMSH: Murtala Mohd Specialist Hospital.

Table 3: Distribution of *S. aureus* and MRSA isolates identified from different hospital instruments

Type of Devices	No of device Screened	No of *S. aureus* isolated (%)		No of MRSA Isolated (%)
		Positive (n=33)	Negative (n=41)	
i)Instruments used in assessing and analyzing sample for patients[a]	10	3(9.1)	7(17.1)	0
ii)Instruments used for superficial assessment of patient body[b]	27	6(18.2)	21(63.6)	2 (12.5)
iii)Semi-invasive instruments[c]	11	6(18.2)	5(12.2)	3 (18.8)
iv)Invasive instrument[d]	26	18(54.5)	8(19.5)	11(68.8)
Total	**74**	**33**	**41**	**16**

Note: a: Microscope, autoclave, incubator, weighing balance.
b: Thermometer, stethoscope, meter ruler, bed.
c: Tonisil Smear, Lid wire, Conial Loop, Screw.
d: Blade, Tower Cliff, Sponge Holding, Blade Holder.

Table 4: Susceptibility profile of *S. aureus*isolates to the various antibiotics used.

ANTIBIOTICS	SITES OF COLLECTION (HOSPITALS)									
	BUK		MMSH		PH		MAWH		AKTH	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Cefoxitin	50	50	72.7	27.3	33.3	66.7	42.9	57.1	33.3	66.7
Ceftriaxone	50	50	63.6	45.5	33.3	66.7	42.9	57.1	50	33.3
Ceftazidime	75	25	63.6	18.2	66.7	33.33	42.9	42.9	33.3	50
Amoxicillin	50	50	54.5	45.5	33.3	66.7	28.6	71.4	50	50
Cefuroxime	50	50	54.5	36.4	33.3	33.3	28.6	71.4	33.3	66.7
Erythromycin	50	50	27.3	45.5	33.3	66.7	28.6	57.1	33.3	66.7
Chloramphenicol	25	75	36.4	45.5	0	100	28.6	57.1	33.3	50
Ofloxacin	25	75	9.1	90	0	100	28.6	71.4	33.3	50
Gentamicin	0	75	0	90.9	0	66.67	14.3	85.7	33.3	50
Ciprofloxacin	0	75	9.1	81.8	0	100	0	100	16.7	83.3

KEY: BUK: Bayero University Kano, MMSH: Murtala Mohd Specialist Hospital, PH: Premear Clinic, MAWH: Mohd Abdullahi Wase Hospital, AKTH: Aminu Kano Teaching Hospital, R: Resistance, S: Sensitive

Table 5: Drug resistant pattern of the 16 MRSA isolates

Frequency of drug resistance	Number (n=16)	Resistant pattern	Remarks
Resistant to 2 antibac. Drugs	1	i-E^{M}, CE^{B}	-MRSA
Resistant to 3 antibac. drugs	4	i-AML^{B}, CH^{C},CE^{B}	-MRSA
		ii-AML^{B},CE^{B}, CRX^{B}	-MRSA
		iii-CH^{C},CXM^{B},CRX^{B}	-MRSA
		iv-OFL^{Q}, AML^{B},CN^{T}	-MDRSA
Resistant to 4 antibac. drugs	5	i- AML^{B},CXM^{B},CE^{B},CRX^{B}	-MRSA
		ii-AML^{B},CXM^{B},CE^{B},CRX^{B}	-MRSA
		iii-E^{M},CXM^{B},CE^{B},CRX^{B}	-MRSA
		iv-AML^{B},CH^{C}, CE^{B}, CRX^{B}	-MRSA
		v-CH^{C}, CXM^{B}, OFL^{Q}, CRX^{B}	-MDRSA
	2	i-AML^{B}, E^{M}, CXM^{B}, OFL^{Q},CRX^{B}	-MDRSA
		ii-AML^{B},E^{M},CXM^{B},CE^{B},CRX^{B}	-MRSA
Resistant to 5 antibac. drugs	1	i-AML^{B}, E^{M}, CH^{C}, CXM^{B}, CE^{B},CRX^{B}	-MDRSA
Resistant to 6 antibac. drugs	3	i-CPX^{Q}, E^{M}, CH^{C}, CXM^{B}, CE^{B}, OFL^{Q},CRX^{B}	-MDRSA
		ii- CN^{T}, AML^{B},E^{M}, CH^{C}, CXM^{B}, CE^{B}, OFL^{Q}	-MDRSA
Resistant to 7 antibac. drugs		iii- CPX^{Q}, CN^{T}, AML^{B}, E^{M}, CXM^{B}, CE^{B},CRX^{B}	-MDRSA

KEY: MRSA: Mathicillin resistance *S. aureus,* MDRSA: Multidrug resistance methicillin resistance *S. aureus*, CFX: Cefoxitin, CXM: Cefuroxime, CA: Ceftriaxone, CRX: Ceftazidime, AML: Amoxicillin, CH: Chloromphenicol, CPX: Ciprofloxacin, CN: Gentamicin, E : Erythromycin, T: Aminoglycoside, C: Chloramphenicol, M: Macrolides, Q: Quinolones, B: Betalactam,antibac: Antibacterial.

Note: Resistance to at least any 3 or more of the different classes of antibiotics (Aminoglycoside, Chloramphenicol, Macrolides, Quinolones and Beta-lactam) used in the study indicates Multidrug resistance methicillin resistance *S. aureus*.

1-Preparation of mixture for PCR

2.0µl of Genomic DNA + PCR mixture (i.e. 1µl of primers and 17µl of distilled water)

2-Amplification process

Amplification Process	***blaZ***	***MecA***
Pre-denaturation	94^0C for 5 minutes	94^0C for 5 minutes
Denaturation	94^0C for 1 minute	94^0C for 1 minute
Annealing	47^0C for 1 minute	50.5^0C for 1 minute
Extension	72^0C for 1 minutes	72^0C for 1 minutes
Final extension	72^0C for 5 minutes	72^0C for 5 minutes

3-Identification process by gel electrophoresis

8µl of PCR products + 8µl of molecular weight marker

▼

Loaded into 1.5% agarose gel in 1 x Tri-S-acetic acid EDTA buffer (TAE) (contain Green nucleic stain)

▼

DNA amplicons were visualized using gel imaging system

Figure 1: PCR protocol

Plate 1: PCR for detection of *blaZ*gene from methicillin resistant *S. aureus*isolates

Legend: Lane 7=AKTH-AF**;** Lane 8=MMSH-NTDF; Lane 9=BUK-SC; Lane 10=MMSH-AF; Lane 11=Negative ControlLane 12=MAWHTS

Note: MRSA isolates that amplified at 400bp (i.e. **Lane 7=AKTH-AF; Lane 8=MMSH-NTDF;Lane 9= BUK-SC)** were identified as those having *blaZ* positive gene.

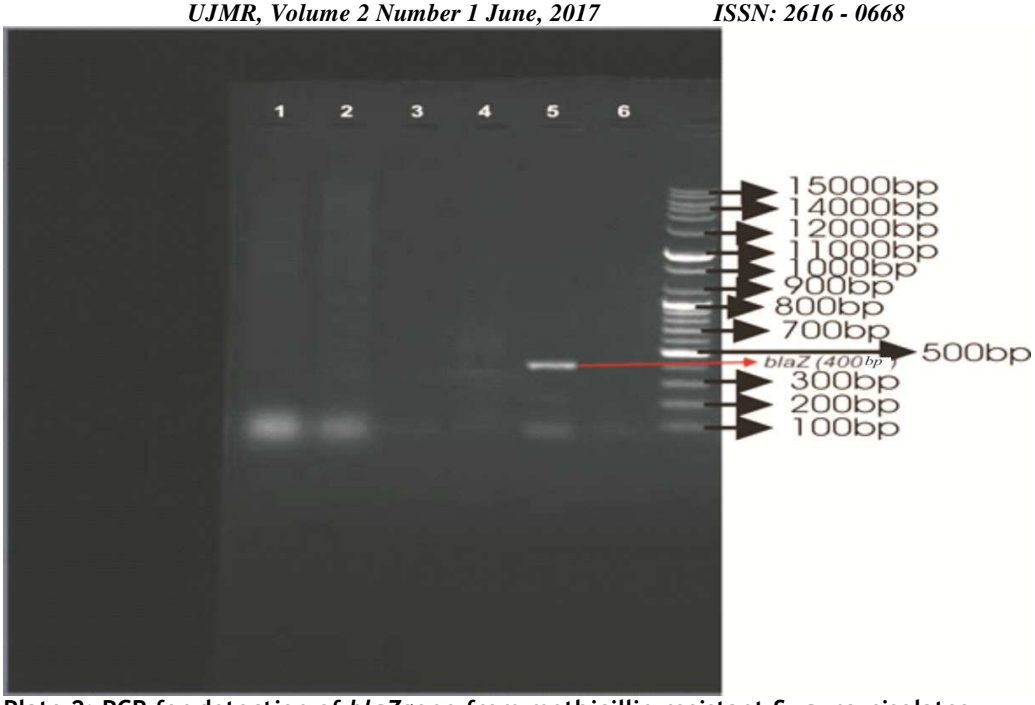

Plate 2: PCR for detection of *blaZ*gene from methicillin resistant *S. aureus*isolates
Legend:Lane 1=Negative Control; Lane 2=MMSH-CR; Lane 3=AKTH-DF; Lane 4=MAWH-TS; Lane 5= MAWH-RT**;** Lane 6= Positive control
Note: MRSA isolates that amplified at 400bp (i.e. **Lane 5= MAWH-RT)** were confirmed as those having *blaZ* positive gene.

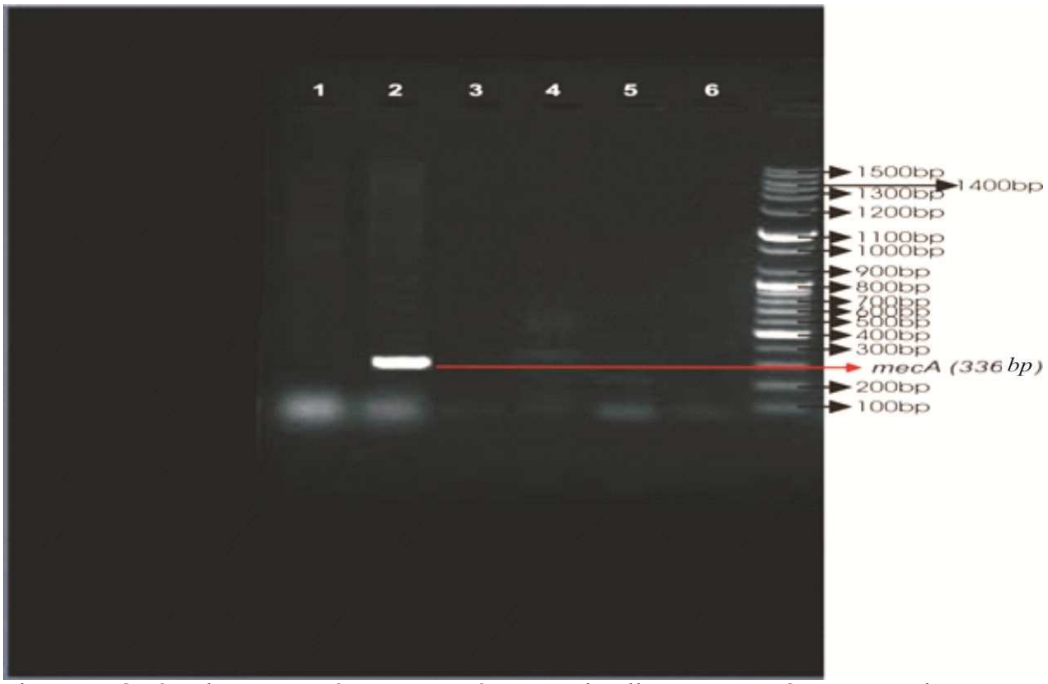

Plate 3: PCR for detection of *mecA*gene from methicillin resistant *S. aureus*isolates
Legend: Lane 1= Negative Control; Lane 2=MMSH-AF; Lane 3=BUK-SC; Lane 4=MMSH-NTDF; Lane 5=MMSH-BD; Lane 6=AKTH-AF
Note: MRSA isolates that amplified at 336bp (i.e. **Lane 2=MMSH-AF**) was identified as having *mecA*gene.

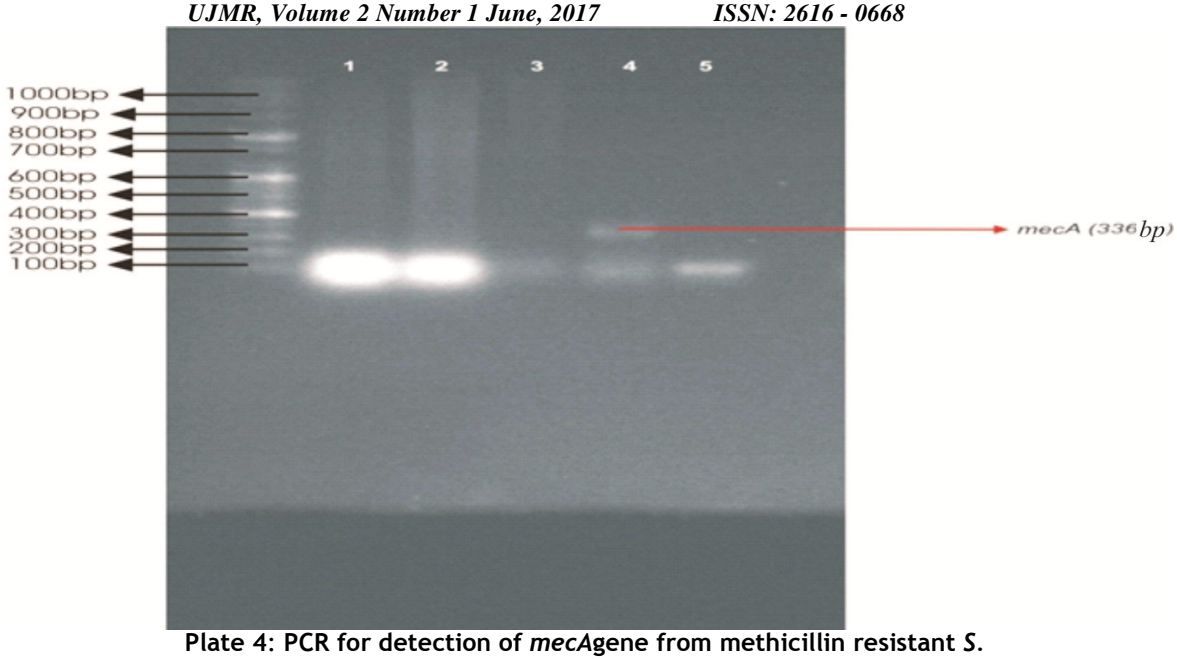

Plate 4: PCR for detection of *mecA*gene from methicillin resistant *S. aureus*isolates

Legend: Lane 1=BUK-FC ; Lane 2=MMSH-CR; Lane 3=AKTH-DF; Lane 4=MAWH-TS; Lane 5=Negative Control

Note: MRSA isolates that amplified at 336bp (i.e. **Lane 4=MAWH-TS**) was identified as having *mecA*gene.

DISCUSSION

The evolution of methicillin resistant *S. aureus*still remains a major significant health problem and hospitals instruments has been recognized as major potential carriers that transmit the pathogen between patients. This study reveals a high contamination rate of hospital instruments with *S. aureus*and MRSA Most importantly, the study further revealed that invasive hospital instruments such as blade, tower cliff, sponge holding and blade holder had the highest number of *S. aureus* and MRSA isolates compared to instruments used for assessing and analyzing samples for patients. This implies a serious concern as the possibility of biofilm formation on these instruments has been documented to cause serious illness and failure of medical devices (Høiby *et al*., 2011;Donlan, 2001).

The results of this study are also comparable to studies reported by some workers. For example, studies by Eugene and Erdoo(2011) and Edosa (2014) reported a MRSA prevalence rate of 44.3% from hospital instruments (Cus-Cus, S/greed, A/macker) in Ibadan and 62% from surgical instruments of government hospitals in Addis Ababa, Ethiopia respectively. The study supports earlier findings that *S. aureus* is one of the most common cause of nosocomial infections (Narezkina *et al*., 2006). Arif *et al*. (2007) further expounded that the majority of nosocomial infection is caused by a patient's own endogenous microbial flora present upon admission to the hospital. A study by Obianju *et al*. (2015) at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), showed that 30 (73.2%) methicillin-resistant *S. aureus* isolates were obtained from inpatients while 11 (26.8%) was from outpatients. Other studies revealed that health-care workers accounted for 93% of personnel to patient transmission of MRSA (Albrich and Harbath, 2008)

The study indicated that ciprofloxacin had the greatest activity (75% t0 100%) against *S. aureus* isolates followed by gentimicin. And this is comparable to 83.5% reported from patients at Federal Teaching Hospital (FETHA) Abakaliki (Iroha *et al*., 2013). The little resistance level to ciprofloxacin observed in this study might not be unconnected with the increasing rate of availability of different cheap brands of generic ciprofloxacin in the market which might have probably led to its misuse. It was further expounded that ciprofloxacin (an example of quinolone) is a potent inhibitor of nucleic acid synthesis and the exposure to quinolones may have selected for spontaneous mutants that alter the target protein or increase the level of efflux pump expression (Rogues *et al*., 2007; Hooper, 2002).

The susceptibility level of *S. aureus* isolates to gentimicin in this study ranged from 50% to 90% and was a bit higher than the 67% and 72% susceptibility to gentamicin reported in other studies by Kumurya and Ado (2015) and Zerfi *et al*. (2014) respectively.

The high level of resistance exhibited by the isolates of the study to the ß-lactam antibiotics particularly ceftazidime, ceftriaxone and cefoxitin is not surprising, as this is consistent with the observation that clinical Staphylococcal isolates are resistant to a large number of commonly prescribed antimicrobial agents and particularly to ß-lactams, although it is believed that more than 80% of Staphylococcal isolates produce penicillinase regardless of the clinical setting (Pantoshi*et al*., 2007; Olukoya *et al*., 2005; Lowy, 2003).

Our study demonstrated that the prevalence of phenotypic methicillin resistance of 48.5% (16/33) was comparable to a prevalence rate of 37.5% from clinical specimens at University of Calabar Teaching Hospital and 34.7% from Ilorin (Azeez-Akande*et al*., 2008). Other studies reported a higher MRSA prevalence rate of 71.1% from urine of healthy women in Abuja and 92.6% frombacterial flora on the hands of nursing service workers in Jos University Teaching Hospitals respectively (Onanuga*et al*., 2006; Ikeh and Yakeu, 2006).However, other studies revealed lower prevalence rates of 12.5% and 19.2% from clinical specimens from six tertiary hospitals in North Eastern Nigeria and from clinical isolates in Medical Microbiology Laboratory of University Teaching Hospital, Ado-Erkiti respectively (Okon *et al*., 2013; Olowe *et al*., 2013).

The study indicates that a large proportion of the bacterial isolates have been exposed to several antibiotics as 7 (44%) of the 16 MRSA isolates exhibited multidrug resistance pattern. Paul *et al*. (1997) previously explained that in such a situation the isolates likely originated from a high risk source of contamination where antibiotics are often used. Another reason for the high resistance could be due to increase in an irrational consumption rate of antibiotics in form of self-medication and non-compliance with medication, transmission of resistant isolates between people, and sales of substandard drugs.

In this study 2/10 (20%) of the phenotypically identified MRSA isolates were confirmed as methicillin resistant *S. aureus*by the detection of *mecA* gene. Similar studies reported detection of *mecA* gene in four, two and five MRSA isolates in Benin City, Ile-Ife and Maiduguri (Obasuyi, 2013; Shittu*et al*., 2011). However, some studies reported the absence of *mecA* gene in MRSA isolates obtained from clinical isolates from Medical Microbiology laboratory of Ahmadu Bello University Teaching Hospital Zaria and from non-hospital sources in Zaria (Olayinka *et al*., 2009; Olanitola*et al*., 2007). Kumurya (2013) explained that the inability to detect *mecA gene* in some studies may not be unconnected with the fact that some *mecA*-containing isolates might have lost the gene on prolong storage and probably due to higher temperatures (>-80^{o}C) between the preliminary characterization to the time of final molecular characterization as a result of inconsistent power supply in the environment as supported by some studies.

In this study, *bla Z* gene, the gene coding for ß – lactamase was detected in 4/10 (33.3%) of the MRSAisolates. It was further expounded that many of these ß-lactamases are encoded by transposons, some of which may also carry resistance determinants to several other antibiotics: quartenary ammonium compounds, dyes (acriflavine and ethidium bromide) or heavy metals (lead, mercury and cadmium) (Pantoshi*et al*, 2007; Massidda*et al*., 2006).

CONCLUSION AND RECOMMENDATION

The study detects a high level contamination of hospital instruments in Kano with 44.6% and 44% of the isolates as *S. aureus* and MRSA respectively. The study further revealed that invasive hospital instruments had the highest number of *S. aureus* and MRSA isolates respectively. Ciprofloxacin, gentamicin and ofloxacin were the most active antibiotics against both the MRSA and methicillin sensitive isolates, however some of the isolates were resistant to ceftazideme, ceftriaxone and amoxicillin. Also 44% of the MRSA isolates had multiple antibiotic resistant. *MecA*gene was detected in 12.5% of the MRSA isolates and *blaZ* was detected in 25% of them. The study recommends strict adherence to aseptic procedures and regular screening of hospital workers for the presence of MRSA to control colonization and infection. Further studies are needed to define the optimum use of ciprofloxacin and gentamicin against MRSA infection.

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