Original Research Article

Prevalence of *Staphylococcus aureus* and S*taphylococcus sciuri* Isolated From Apparently Healthy and Hospitalized Patients In Ekiti State, Nigeria

Abstract

The emergence of microbial resistance to antibiotics on daily basis has become a major global challenge. The increasing prevalence of methicillin resistant Staphylococcus aureus (MRSA) infections among hospitalized and non-hospitalized patients due to emergence of unique community-associated strains has become a great problem in Nigeria in particular and the entire world in general. In this study, a total of four hundred (400) clinical specimens were collected from hospitalized patients at Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti and Federal Teaching Hospital (FETHI), Ido-Ekiti and from apparently healthy individuals from both communities. Standard bacteriological procedures were employed to isolate and identify these organisms. Antibiotic susceptibility test was carried out using the modified Kirby Bauer disc diffusion method. Polymerase chain reaction (PCR) assay was used to identify S. aureus nuc gene, as well as mecA and aac resistance genes. Seventy seven (19.3 %) S. aureus isolates were phenotypically identified; 43 (21.5 %) hospital-associated (23 from EKSUTH and 20 from FETHI) and 34 (17.0 %) community-associated (27 from Ado-Ekiti and 7 from Ido-Ekiti communities). No S. sciuri was phenotypically isolated in both locations. Fifty five (71.4 %) of the 77 S. aureus isolates were nuc gene positive, while 11 (20 %) of the 55 isolates were mecA gene positive, implying that they were MRSA. Of the 11 isolates, 6 (19.4 %) were hospital-associated MRSA while 5 (20.8 %) were community-associated MRSA. Phenotypic resistance of S. aureus to cefoxitin in the two locations ranged between 77.8 – 100 %, but 80 % of cefoxitin-resistant nuc gene positive S. aureus lacked mecA gene. S. aureus isolates exhibited high phenotypic resistance to tetracycline, erythromycin and fusidic acid but susceptible to clindamycin and gentamycin. The prevalence of clinical MRSA infection of 20% in this study is relatively high. However, there was 0% prevalence of S. sciuri colonization. Cefoxitin disc test demonstrated low specificity as a phenotypic marker of methicillin resistance.. There is need to institute control measures for MRSA infections and colonization in this environment.

INTRODUCTION

Staphylococcal infections are aused by the bacteria in the genus Staphylococcus. The spectrum of infections can range from a minor boil or skin abscess to life threatening infections such as septicaemia or endocarditis. There are many species of staphylococci but most infections are caused by *Staphylococcus aureus*. A number of studies done on *S. aureus* both in Nigeria and elsewhere have focused on nasal carriage of the organism and its impact

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on skin and soft tissue infections (Sheng-Yun *et al*., 2011). Most nosocomial pathogens associated with multiple antimicrobial resistance mechanism are Staphylococci. For many years, *S. aureus* was the only species recognized as an important human pathogen whereas coagulase-negative staphylococci (CONS) were viewed mostly as clinically non relevant contaminant or commensals. Only recently, the importance of CONS strains as a major cause of nosocomial infections began to be established (Kloss *et al*., 1999). Infections with *S. aureus* are especially difficult to treat because of evolved resistance to antimicrobial drugs such as penicillin and newer narrow spectrum drugs and β-lactamase resistance penicillin antimicrobial drug (Lowdy, 2003). The widespread incidence of antibiotics resistance across various strains of *S. aureus* has been attributed to horizontal transfer of genes encoding antibiotics resistance and virulence (Chan *et al*., 2011). Methicillin resistant *S. aureus* (MRSA) is the bacterium responsible for several difficult-to-treat infections in human. Strains of methicillin resistant *S. aureus* (MRSA) that are common causative agents of nosocomial disease world-wide often pose therapeutic dilemma to clinicians because of the nature of these strains (Taiwo *et al*., 2004). It is any strain of *S. aureus* that has developed through the process of natural selection, resistance to β-lactam antibiotics which include methicillin, dicloxacillin, nafcillin, oxacillin and cephalosporins. Strains unable to resist these antibiotics are classified as methicillin sensitive *S. aureus* (MSSA). The evolution of this resistance makes MRSA infection very difficult to treat with standard antibiotics and thus making it more dangerous. Resistance in MRSA is related to a chromosomal *mecA* gene that encodes the production of an abnormal penicillin binding protein called PBP2a. These proteins are membrane bound enzymes which are target for all β-lactam antibiotics. They have decreased affinity for binding β-lactam antibiotics resulting not only to methicilin resistance but also to all β-lactams including penicillin and cephalosporins (Weems, 2011). *Staphylococcus sciuri* strains have been found to be an important pathogen responsible for UTI (Stepnavoic *et al*.,

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2002), wound infection (Shittu *et al*., 2004), endocarditis (Hedin *et al*., 1998), peritonitis (Wallet *et al*., 2000), septic shock (Horii *et al*., 2001), endophthalmitis (Benz *et al*., 2004) and pelvic inflammatory diseases (Stepnavoic *et al*., 2002). *S. sciuri* may be found as a colonizing organism in humans with low carrier rates in nasopharynx, skin and urogenitals (Couto *et al*., 2000; Stepnavoic *et al*., 2003). The *mecA* gene is native to *S. sciuri* which has been found to have the same similarity with *mecA* gene in *S. aureus* (Couto *et al*., 1999). Inspite of the close sequence similarities between the *mecA* of *S. sciuri* and the antibiotic resistance *mecA* gene of *S. aureus*, *S. sciuri* strains have been found to be uniformly susceptible to β-lactam antibiotics (Couto *et al*., 1996). It has been noted that *S. sciuri* may be an evolutionary precursor of the methicillin resistance gene *mecA* of the pathogenic strains of MRSA (Wu *et al*., 2001). Though in Nigeria, *S. aureus* is one of the major nosocomial infection pathogen (Chikere *et al*., 2008), *S. sciuri* also has become another major pathogen in nososcomial infection (Ivan *et al*., 2005) which result in morbidity and mortality. The aim of this study therefore was to compare the prevalence of *Staphylococcus aureus* among hospitalized patients and apparently healthy persons in the community with that of *Staphylococcus sciuri* among hospitalized patients and apparently healthy persons in the community.

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Methodology

Study Area

This study was carried out in two hospitals (Federal Teaching Hospital FETHI, Ido Ekiti and Ekiti State Teaching Hospital EKSUTH Ado-Ekiti) and Ado-Ekiti andnIdo-Ekiti communities both in Ekiti State, Southwest, Nigeria.

Study Population

These consist of selected hospitalized patients in FETHI and EKSUTH and apparently healthy individuals from Ido and Ado-Ekiti communities from whom appropriate clinical samples were obtained.

Inclusion Criteria

The inclusion criteria were hospitalized patients with clinical symptoms and signs of infection, age range 15 to 60 years, and not less than one month of hospitalization, and apparently healthy individuals from the two communities in the same age group.

Ethical Clearance

The ethical clearance was obtained from Ekiti State University Teaching Hospital (Protocol number: EKSUTHA67/2015/08/004)

Specimen Collection and Transport

Urine, wound, blood and pus samples were obtained from hospitalized patients while nasal samples and urine were obtained from healthy individuals. Samples were collected into appropriately label specimen bottles using aseptic techniques to prevent contamination, and transported immediately to the laboratory for analysis.

Sample Analysis

Isolation of staphylococci

Wound, pus and nasal specimens were inoculated onto MacConkey agar and Chocolate agar plates and incubated at 37°C overnight to primarily isolate staphylococci species (Cheesbrough, 2006). The staphylococci colonies were then subcultured onto Mannitol salt agar (MSA) and Tryptic Soy Agar (TSA) for isolation of *S. aureus* and *S. sciuri* respectively

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(Shittu *et al*., 2004). Urine specimens were first cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated at 37°C for 24 hrs (Cheesbrough, 2006). Isolated staphylococci were then sub-cultured onto MSA and TSA for isolation of *S. aureus* and *S. sciuri* respectively (Shittu *et al.,* 2004). Pure colonies of suspected *S. aureus* and *S. sciuri* were then stored on nutrient agar slopes for further phenotypic tests.

Speciation of Isolates

S. aureus was identified based on Gram reactions, catalase positive test, coagulase positive test, fermentation of mannitol (Chessbrough, 2006) while *S. sciuri* was identified based on catalase positive test, tube coagulase negative, oxidase test positive, urease test positive, esculin hydrolysis, resistance to novobiocin and bacitracin, acid production from galactose and sucrose but not raffinose fermentation (Kloss *et al*., 1997)

Molecular Analysis

DNA Extraction

The DNA of the isolates was extracted by suspending 4-5 bacteria colonies in 500 µl of Tris Borate EDTA (TBE) buffer in Eppendorf tubes appropriately labeled. The cells were boiled at 100°C for10minutes and were cooled rapidly on ice for 30minutes. 3ul of Proteinase K was added to the lysed cells and the mixture was incubated for 15-20minutes at 55-60°c. the enzyme was denatured by boiling at 100^{0}c for 10minutes and was centrifuge at 13, 400 rpm. The supernatant containing the DNA was collected for PCR and was stored at -20°C for further use.

Amplification of *nuc, mecA* and *aac*-genes

Polymerase chain reaction (PCR) was used to detect *nuc* gene that is common to all *S. aureus* and resistance genes; *mecA* that encodes methicillin resistance and *aac* that encodes aminoglycoside resistance.

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Table 1. Primers used for the amplification of genes

Primers	Sequence 5 ^{1}-3^{1}	gene	Product size(bp)	Annealing temp.(^{o}c)	Reference
SANuc F SANuc R	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	SA Nuc	276	58	Brakstad *et al*, 1992
MecA F MecA R	GTAGAAATGACTGAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	MecA	310	52	Geha *et al*, 1994
AAC-3-IV F AAC-3-IV R	AGTTGACCCAGGGC GTGTGCTGCTGGTCCACAGC	AAC-3-IV	286	55	

PCR Procedural Steps

Gel Electrophoresis

Preparation of agarose gel: At the completion of the amplification, PCR products were resolved on 1 % agarose gel prepared by dissolving 1g of agarose powder in 100 ml of 1X TBE buffer solution inside a clean conical flask. The 1% agarose solution was heated in a microwave oven for 2-3 minutes and was observed for clarity which was an indication of complete dissolution. The mixture was then allowed to cool to about 50 ^{0}C after which 0.5 µl

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of ethidium bromide was then added. It was allowed to cool further and then poured into a tray sealed at both ends with support to form a mould with special combs placed in it to create wells. The comb was carefully removed after the gel had set.

Analysis and Interpretation of Gel Pattern

The DNA fragment of 276bp and 310bp were separated for *nuc* and *mecA* gene respectively on a 1% agarose gel with a 100bp DNA ladder.

Data Entry and Statistical Analysis

All data were analysed using statistical package for the social sciences (SPSS) version 16.0. Data were presented using frequency tables as appropriate and cross tabulation to study relationships associated between variables. Statistical significance was set at 5% using chi-square.

RESULTS

A total of 400 clinical samples were collected from hospitalized patients in two hospitals and apparently healthy individuals in two communities in Ekiti State, Nigeria. Table 1 shows the distribution of organisms isolated from the study areas. Seventy seven (19.25%) *S. aureus* was phenotypically isolated from the 400 samples collected; 23 (5.75 %) from Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti, 20 (5 %) from Federal Teaching Hospital (FETHI), Ido-Ekiti, 27 (6.75 %) from Ado-Ekiti community and 7 (1.75 %) from Ido-Ekiti community. In this study, no *S. sciuri* was isolated phenotypically in all the locations. The prevalence of *S. sciuri* in these study areas by the standard phenotypic detection method is therefore 0 %

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The comparison of Cefoxitin resistance to all other antibiotics of all the isolate obtained in all the location is shown in table 2 only Erythromycin and Gentamicin have no statistical significant.

Table 3 shows the distribution *S. aureus* isolates with *nuc* and *mecA* genes in the study areas. Aminoglycoside resistance, *aac* gene, was not detected in any isolate. Fifty five (71.43%) of the 77 phenotypically confirmed *S. aureus* isolates were positive for *nuc* gene, while 11 (20%) of these were positive for *mecA* gene. The prevalence of MRSA in these study areas using the gold standard PCR detection method is therefore 20 %. .

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Table2: Distribution of staphylococcus isolates detected by phenotypic method in two locations in Ekiti State, Nigeria

Staphylococcus isolate	Hospital (%)		Community (%)		Total (%)	95% CI	P value
	FETHI	EKSUTH	Ido-Ekiti	Ado-Ekiti			
No of samples	100	100	100	100	400		
No of *S. aureus*	20 (20)	23 (23)	7 (7)	27 (27)	77 (19.3)		
No of *S. sciuri*	0	0	0	0	0		
Total isolate	43 (21.5)		34 (17.0)		77 (19.3	0.3851 –	0.2449

	)	1.211

FETHI = Federal Teaching Hospital; EKSUTH = Ekiti State University Teaching Hospital, CI = Confidence Interval

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Table 3: Comparative antibiotic susceptibility of hospital and community associated *Staphylococcus aureus* isolates in Ekiti State, Nigeria

Antibiotics	*S. aureus* isolates						P value
	Hospital-associated (n=43)			Community-associated (n=34)			
	S	I	R	S	I	R	
Cefoxitin	0	-	43	6	-	28	0.0057*
Fusidic acid	11	-	32	17	-	17	0.0336*

Clindamycin	25	13	5	30	1	3	0.0049*
Erythromycin	5	14	24	10	12	12	0.0806
Gentamicin	37	2	4	31	-	3	0.7233
Tetracycline	4	4	35	12	2	20	0.0095*
Novobiocin	22	1	20	31	-	3	0.0002*

* Statistically significant difference. S = sensitive, I = intermediate, R = resistant. Note that for statistical analysis, intermediate isolates were considered resistant.

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Table 4.: Distribution of *Staphylococcus aureus* isolates detected by molecular method in two locations in Ekiti State, Nigeria

Staphylococcus aureus isolate	Hospital (%)		Community (%)		Total (%)	95% CI	P value
	FSH, Ido-Ekiti	EKSUTH Ado-Ekiti	Ido-Ekiti	Ado-Ekiti			
No positive for *nuc* gene	16	15	5	19	55		
No positive for *mecA* gene (MRSA isolate)	3 (18.8)	3 (20)	1 (20)	4 (21.1)	11		

Total MRSA isolate	6 (19.4)	5 (20.8)	11 (20)	0.5279 - 1.746	1.000

FSH = Federal Teaching Hospital; EKSUTH = Ekiti State University Teaching Hospital, MRSA = methicillin resistant *Staphylococcus aureus*

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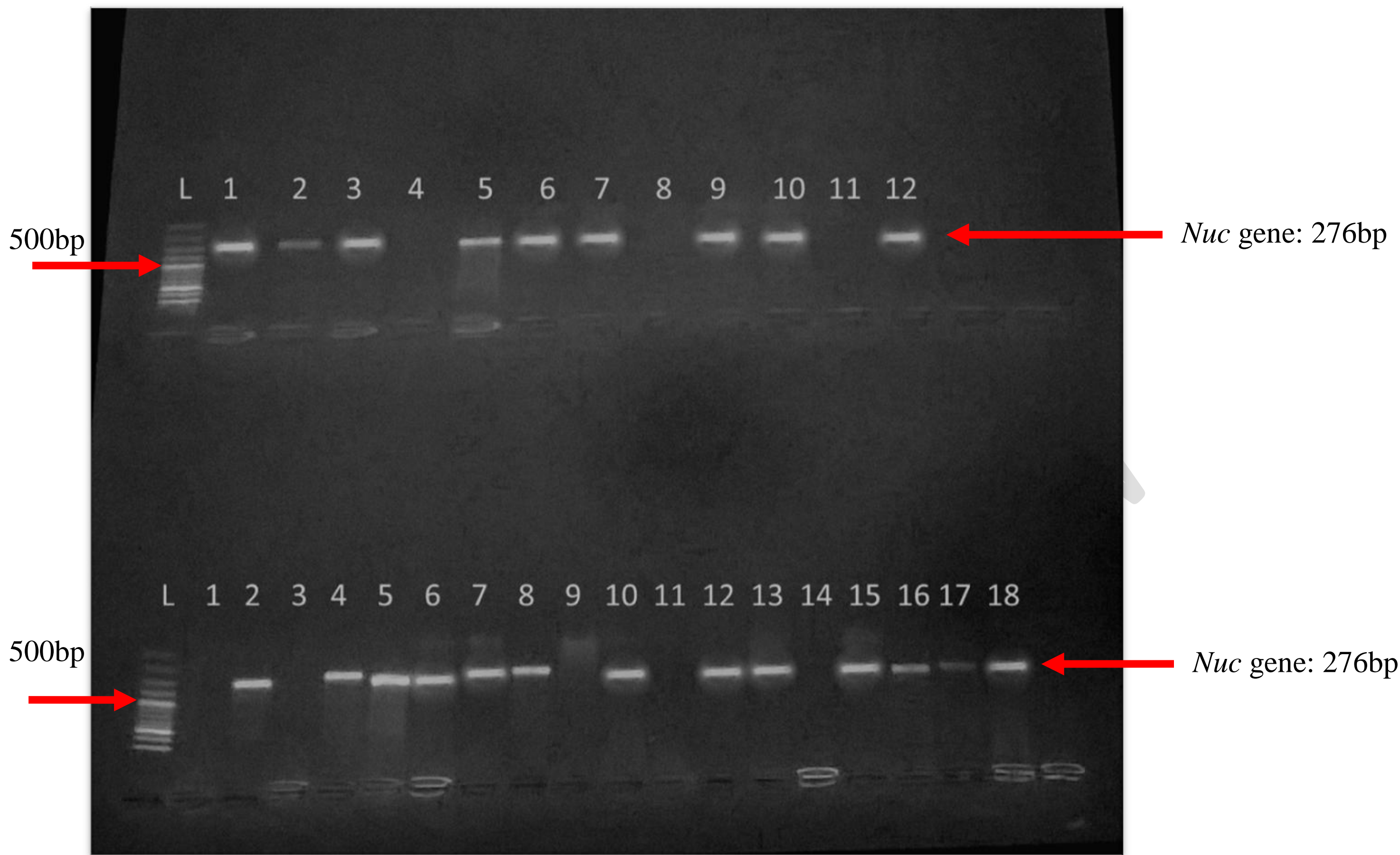
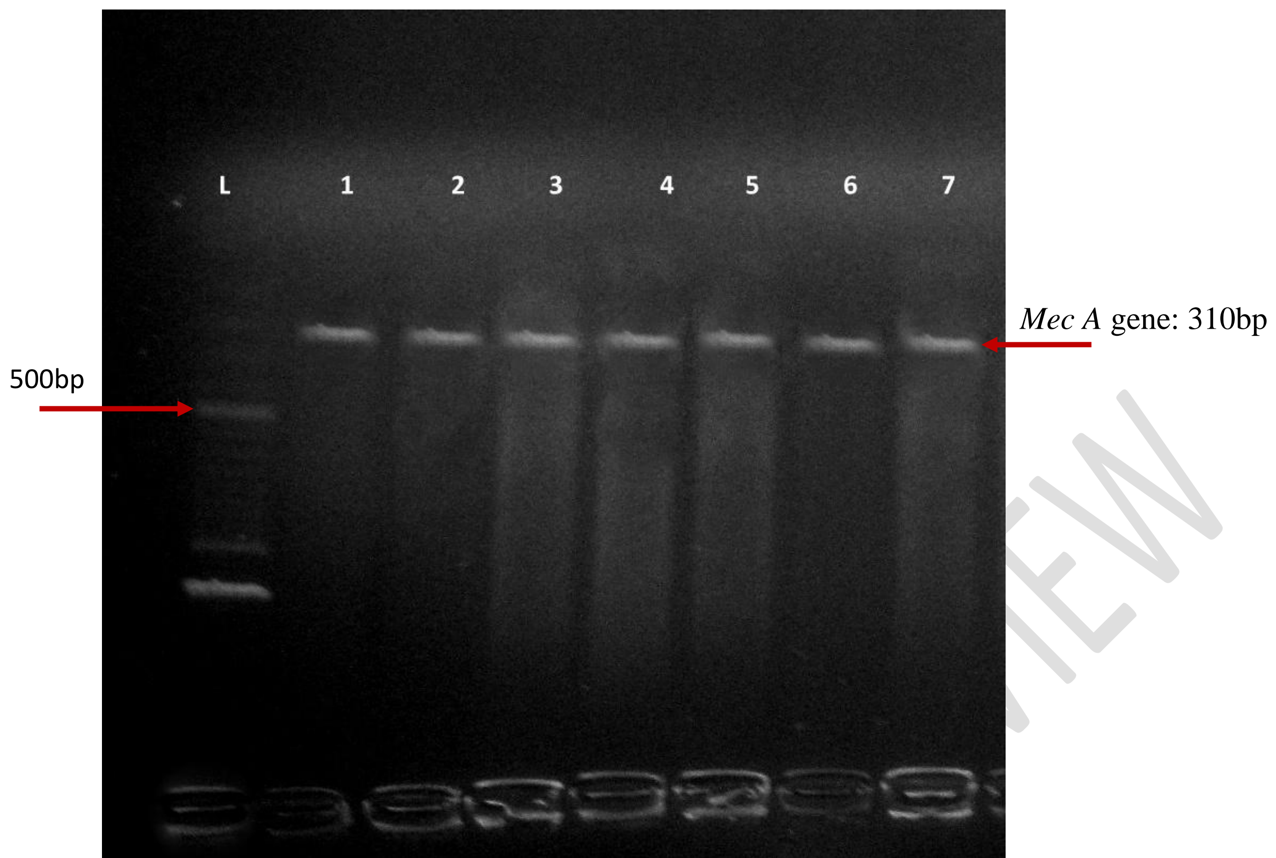


L = 100bp ladder

→ Top Lane: 11- negative control, 12- positive control

Fig 1: PCR amplification of Nuc gene for *staphylococcus aureus*



L= 100bp ladder

L: 1=Ado2, 2 =Ado4, 3 =EK1, 4 =Ido2, 5=Ido3,6=FETHI8,7=FETHI9,

Fig 2: PCR amplification of mecA gene of some of the Cefoxitin resistant isolates.

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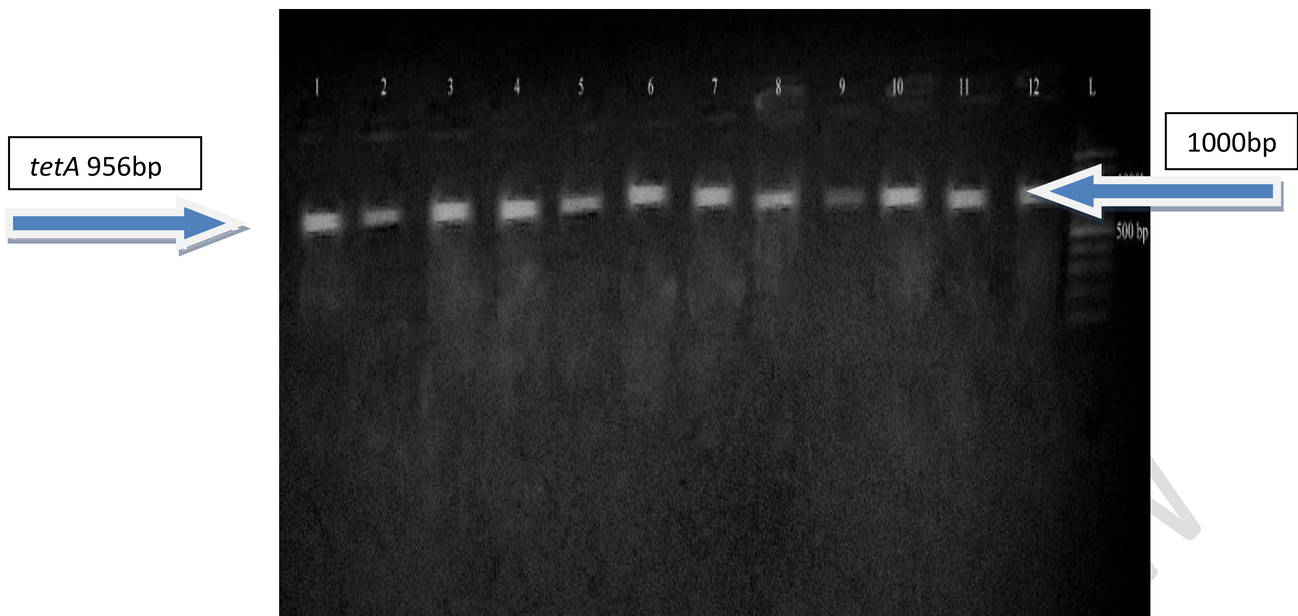

L=100bp, 1=EK1, 2=Ek2, 3=Ek3, 4=Ek5, 5=FETHI2, 6=FETHI3, 7=FETHI6,8=ADO1, 9=ADO2, 10=IDO2

11=IDO3, 12= POSITIVE CONTROL

Fig 3: PCR amplification of *tetA* gene for *staphylococcus aureus*

DISCUSSION

Emergence of resistance to antibiotics on daily basis has become a serious global health challenge. The increasing prevalence of MRSA infection among non hospitalized patients due to emergence of unique community associated *S. aureus* and hospitalized patients has become a great problem in Nigeria in particular and the globe in general. MRSA can be found in any individual regardless of their age. Isolates from the hospitalized patients were from urine, wound and aspirate while from the community the isolation was from urine and nasal specimen. Fifty-six percent of the S.aureus isolates was hospital associated while 44% were community associated. In this study, phenotypic resistance of *S. aureus* to cefoxitin in

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the two locations used ranges between 77.8% - 100% which when compared to previous work done in Ekiti by Olowe *et al*., (2013) shows 46.5% resistance. The antimicrobial susceptibility of cefoxitin resistance to *S. aureus* in this study shows that *S. aureus* is multi-resistant to tetracycline as described previously by Yanagihara *et al*., (2012). *S. aureus* isolates in the hospitals were found to show more resistance to erythromycin while fusidic acid shows a high rate of resistance to isolates of *S. aureus* in the community. Gentamycin shows a high rate of sensitivity ranging between 73.9-79.3% in Ado Ekiti, while it shows 100% sensitive in Ido-Ekiti. Macrolides and aminoglycosides show a very low resistance in this study as against Taiwo *et al*., (2004). The high rate of clindamycin and gentamycin susceptibility in all isolates from both the hospital and community in this study does not support previous work done by Gould *et al*, (2010) and Yanagihara *et al.,* (2012). This suggests that the susceptibility of clindamycin and gentamycin varies with location and frequency of use of antibiotics, likewise fusidic acid and erythromycin. The susceptibility of clindamycin to MRSA in this study shows that clindamycin can be used as drugs of choice in the treatment of MRSA in combination with gentamycin. Hospital acquired MRSA still remains a major challenge in the medical health care in Nigeria. Hospital acquired *S. aureus'* genetic resistance to Cefoxitin of 27% in each location is very high compared to the Community acquired resistance of 5.4-9% and1.8-5.4% in each location as shown in this study. The distribution of MRSA detection in both the hospital and community in Ekiti shows that genetically hospital associated MRSA is 19.4% and community associated, 20.8%. Compared to previous studies by Olowe *et al*., (2013) of 19.2%, 1.4% by Adesida *et al*., (2005), 1.5% by Shittu *et al*., (2006) in Southwest Nigeria appears to increase as the year goes by. But compared to studies by Terry, (2011) of 22.2% and Taiwo *et al*., (2004) of 34.7% in other part of the country, the prevalence high rate of MRSA varies according to location and time which supports Okon *et al*., studies in (2013). 38.8% by Fayomi *et al*.,

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(2009) in Ido Ekiti compared to the 27% prevalent rate in this study shows that the rate has reduced but still on the high side. The MRSA isolated in this studies was found to be multi-resistant to tetracycline and fusidic acid as earlier reported by Vaez *et al*., (2010). Cefoxitin has greatly shown a high rate of resistance of 95% phenotypically in this study, this shows that cefoxitin has develop a high rate of resistance when compare to studies done by Olowe *et al.*, (2013).

MSSA (methicillin sensitive staphylococcus aureus) also shows multiresistance to tetracycline, erythromycin, and fuscidic acid in this study. According to this study, 80% of phenotypically cefoxitin resistant *S.aureus* does not possess the resistance gene mecA (MSSA- *mecA* negative). This suggests that disc diffusion test for the detection of MRSA gives a high false prevalence rate as described by Syed *et al*., (2011). PCR amplification still remains the gold standard for the detection of MRSA and MSSA. According to this study, isolation of MRSA in relation to gender, occupation and age group shows no relationship. Phenotypically, MRSA was found to be high in urine 92.9%, wound swab 100% and nasal swab of 87% but shows no statistically significant between the isolation of MRSA and sample site. The high rate of detection of MRSA in the hospitalized patient shows that HA-MRSA still remains a very big challenge.

The route of *S. sciuri* is usually transmitted from animal to human and in this study, the individuals involved had no close contact with animals frequently. The absence of *S. sciuri* in the locations of this study might be due to the low population density as well as the requirement of enriched environment for isolation as previously described by Ivana *et al*., (2005) or no contact with infected animal and animal dairy. The frequently colonization of the hospital environment which makes rate of isolation to be sporadic and most probably transient as described by Ahoyo *et al.,* (2013) might also be a contributing factor to why *S. sciuri* was not isolated during the cause of this study. Non isolation of *S. sciuri* does not

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primarily indicate that the organism does not exist in the area of study and not necessarily means that it does exist. This study suggests that for the detection of interaction between *S. aureus* and *S. sciuri* based on the presence of *mecA* gene will greatly depend on the isolation of both organisms in the same location and individual to clearly give the better understanding and analysis of the nucleotides of the amino acid sequences as done previously by Couto *et al*., (2000). Though, previous study done already shows that there is a relationship in the nucleotide sequence of the *mecA* in MRSA and *S. sciuri*.

There is a limited study on the isolation and prevalence of *S. sciuri* in Ekiti State therefore more studies are needed in the area of isolation and detection of *S. sciuri* in humans in this region, and Also the interaction and relationship between *S. aureus* and *S. sciuri* based on their *mecA*. Until these two organisms are isolated together in this region, the study of their interactions will still be difficult.

Conclusion

The high prevalent rate of MRSA in this study shows that MRSA continues to be a major challenge in our society and nation and disc diffusion test is not 100% precise in detecting MSSA. PCR remains the gold standard to detect and isolate both MRSA and MSSA. On the isolation and detection of *S. sciuri* in human, more work needs to be done in order to have the complete understanding and knowledge about the organism, then the interaction between *S. aureus* and *S. sciuri* can be fully understand. There is a need to put more control measures in place to eradicate MRSA to reduce one of the major healthcare challenges. Therefore, everyday awareness on proper hygiene and usage of antibiotics should be put in place.

Recommendation

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Since MRSA still pose a lot of threat to the medical services, the healthcare unit and the general public should be exposed to more education on proper hygiene and sanitation periodically. Proper handwashing techniques and procedures should henceforth be in place in all healthcare service provider units. Further studies should be encouraged in the aspect of identifying *S. sciuri* in clinical specimens using molecular methods, and to understand its interaction with *S.aureus* in human infection or colonization.

References

Adesida S, Boelen H, Babajide B, Kehinde A, Snijders S, van Leeuwen W, Coker A, Verburgh H, van Belkum A(2005): Major epidemic clones of *Staphylococcus aureus* in Nigeria. *Microbial. Drugs Resistance*. 11(2), 115-121.

Ahoyo TA, Yehouenou Pazou E, Baba-Moussa L, Attolou Gbohou A, Boco M, Dramane KL and Aminou T (2013): *Staphylococcus sciuri* outbreak at Tertiary Hospital in Benin. *J Med Microb Diagn* 2:126

Annad KB, Agrawal P, Kumar S, Kapila K (2009): Comparison of cefoxitin disc diffusion test, oxacillin screen agar and PCR for mecA gene for detection of MRSA. *Indian J. Med Microbiol*. 27:27-29

Brakstad, O.G., Aasbakk, K., Maeland, J.A. (1992): Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J. Clin. Microbiol*., 30: 1654-1660.

Chan CX, Beiko RG, Ragan MA (2011): "Lateral transfer of genes and gene fragments in *Staphylococcus* extends beyond mobile elements". *J Bacteriol* 193 (15):

Clinical and Laboratory Standard Institutes (CLSI) (2012): Performance Standard for Antimicrobial Disk Susceptibility Test. Approved Standard. Eleventh Edition. CLSI document M02-A11, 32:1.

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Clinical and Laboratory Standard Institutes (CLSI) (2013): Disc diffusion supplementary table. Performance Standard for Antimicrobial Disk Susceptibility Test. 940 West Valley Road Suite 1400, Wayne, PA19807

Couto, I., H. de Lencastre, E. Severina, W. Kloos, J. Webster, I. Santos Sanches, and A. Tomasz. (1996): Ubiquitous presence of a *mecA* homologue in natural isolates of *Staphylococcus sciuri*. *Microb. Drug Resist*. 2:377–391.

Couto, I., I. S. Sanches, R. Sa-Leao, and H. de Lencastre. (2000): Molecular characterization of *Staphylococcus sciuri* strains isolated from humans. J. Clin. Microbiol. 38:1136–1143.

Couto, I., S. W. Wu, A. Tomasz, and H. de Lencastre. (2003): Development of methicillin resistance in clinical isolates of *Staphylococcus sciuri* by transcriptional activation of the *mecA* homologue native to *S. sciuri*. *J. Bacteriol*. 185:645–653.

Fayomi OD, Oyediran EIO, Adeyemo AT, Oyekale OT. (2009): Prevalence and antibiotics resistance of Methicillin-Resistance Staphylococcus aureus among inpatients at a tertiary health facility in Ido-Ekiti Nigeria. *Int. J. Lab. Med*. 4:2

Geha DJ., Katayama Y., Hiramatsu K. (1994): Cloning and nucleotide sequence determination of the entire *mec* DNA of premethicillin-resistant *Staphylococcus aureus* N315. *Antimicrob Agt.& Chemo* 43: 1449-1458.

Gould, SWJ, Cuschieri, P, Rollason, J, Hilton, AC, Easmon, S, Fielder, MD. (2010): The need for continued monitoring of antibiotic resistance patterns in clinical isolates of *Staphylococcus aureus* from London and Malta.;9:1-7.

Hedin, G. and Widerstrom, M. (1998): Endocarditis due to Staphylococcus sciuri. *Eur. J. Clin. Microbiol. Infect. Dis*. 17, 673-675.

Ivana D., Donald M, Dragana V, Branislava S, Adebayo S. Petr J, Tomasz H. and Srdjan S, (2005): Isolation and Molecular Characterization of *Staphylococcus sciuri* in the Hospital Environment. *J Clin. Microbiol*, 43: 2782–2785.

UNDER PEER REVIEW

Kloos WE, Schleifer KH, Smith RF (1976): Characterization of *Staphylococcus sciuri* sp. nov. and its subspecies. *Int J Syst Bacteriol*. 26:22-37.

Kloos, W.E., Ballard, D.N., Webster, J.A., Hubner, R.J., Tomasz, A., Couto, I., Sloan, G.L., Dehart, H.P., Fiedler, F., Schubert, K., De Lencastre, H., Santos Sanches, I., Heath, H.E., Leblanc, P.A. and Ljungh, A. (1997): Ribotype delineation and description of Staphy-lococcus sciuri subspecies and their potential as reservoirs of methicillin resistance and staphylolytic enzyme genes. *Int. J. Syst. Bacteriol*. 47: 313-323.

Lowy FD (2003): "Antimicrobial resistance: the example of *Staphylococcus aureus*". *J. Clin. Invest.* 111 (9): 1265–73.

Okon K.O, Basset P, Uba A, Lin J, Oyawoye B, Shittu A.O, Blanc D.S (2009): Co-occurrence of predominant PVL-positive (ST152) and multidrug-resistant (ST241) *Staphylococcus aureus* clones in Nigerian hospitals. *J. Clin. Microbiol*. 47: 3000-3003.8

Olowe OA, Eniola KIT, Olowe RA, Olayemi AM (2007) Antimicrobial susceptibility and betalactamase detection of MRSA in Osogbo, S.W. Nigeria. Nat Sci 5:44-48.

Olowe OA, Kukoyi OO TaiwoS.S, Ojurongbe OO, Opaleye OO, Bolaji OS, Abiodun AA, Makanjuola OB, Ogbolu DO, Alli OT (2013): Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from Ekiti State, Nigeria

Rushdy, A.A., Salama, M.S., Othman, A.S. (2007): Detection of Methicillin/oxacillin resistant *S. aureus* isolated from some clinical hospitals in Cairo using mecA/nuc genes and antibiotic susceptibility profile. *Int J. Agric and Biol*, 9(6): 800–806

Sheng-Yun L, Fang-Yu C, Ching-Chung C, Keong-Diong L, Yhu-Chering H (2011): Methicicllin-resistant staphylococcus aureus nasal colonization among adult patients visiting emergency department in a medical centre in taiwan. *PLoS One* 6: e18620.

UNDER PEER REVIEW

Shittu A.O, Lin J, Kolawole D.O (2006): Antimicrobial susceptibility patterns of *Staphylococcus aureus* and characterization of MRSA in Southwestern Nigeria. Wounds 18: 77-84.

Shittu, A., J. Lin, D. Morrison, and D. Kolawole. (2004): Isolation and molecular characterization of multiresistant *Staphylococcus sciuri* and *Staphylococcus haemolyticus* associated with skin and soft-tissue infections. *J. Med. Microbiol*. 53:51–55.

Stepanovic, S., I. Dakic, N. Opavski, P. Jezek, and L. Ranin. (2003): Influence of the growth medium composition on biofilm formation by *Staphylococcus sciuri*. *Ann. Microbiol*. 53:63–74.

Stepanovic, S., I. Dakic, S. Djukic, B. Lozuk, and M. Svabic-Vlahovic. (2002): Surgical wound infection associated with *Staphylococcus sciuri*. *Scand. J. Infect*. Dis. 34:685–686.

Stepanovic, S., P. Jezek, D. Vukovic, I. Dakic, and P. Petras. (2003): Isolation of members of the *Staphylococcus sciuri* group from urine and their relationship to urinary tract infections. *J. Clin. Microbiol*. 41:5262–5264.

Syed ZB, Safia A, Naheed Z (2011): Antimicrobial susceptibility pattern of staphylococcus aureus on clinical isolates and efficacy of Laboratory test to diagnose MRSA: A multicentre study. *J Ayub Med Coll Abbottabad* 23(1): 139-142.

Taiwo SS, Onile BA, Akanbi AA., II (2004): Methicillin-resistant Staphylococcus aureus (MRSA) isolates in Ilorin, Nigeria. *African Journal of Clinical and Experimental Microbiology.* 5(2):189–197

Vaez, H., Tabaraei, A., Moradi, A., Ghaemi, E.A. (2011): Evaluation of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients in Golestan, Province-North of Iran. *Afr. J. Microbiol. Res*.5 (4): 432-436.

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Valones MAA, Guimarães RL, Brandão LAC, Eleutério de Souza PR, Carvalho AT,Crovela S (2009): Principles and application of polymerase chain reaction in medical diagnostic fields: A Review. *Brazil J of microbiol*. 40:1-11

Weems JJ (2001): The many faces of *Staphylococcus aureus* infections. Postgraduate Med. 110(4): 24-36.

Wu SW, DE Lencastre H, Tomasz A. (2001): Recruitment of the *mecA* gene homologue of *Staphylococcus sciuri* into a resistance determinant and expression of the resistant phenotype in *Staphylococcus aureus.* J Bacteriol. 183:2417–24.

Yanagihara, K, Araki, N, Watanabe, S, Kinebuchi, T, Kaku, M, Maesaki, S, Yamaguchi, K, Matsumoto, T, Mikamo, H, Takesue, Y, Kadota, JI, Fujita, J, Iwatsuki, K, Hino, H, Kaneko, T, Asagoe, K, Ikeda, M, Yasuoka, A, Kohno, S. (2012): Antimicrobial susceptibility and molecular characteristics of 857 methicillin-resistant *Staphylococcus aureus* isolates from 16 medical centers in Japan (2008-2009): nationwide survey of community-acquired and nosocomial MRSA. *Diagnostic Microbiology and Infectious Disease*.;72:253-257.

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