Sjournals
Scientific Journals
Scientific Journal of Microbiology (2013) 2(2) 43-52
ISSN 2322-2948

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Molecular detection of PVL, msrA genes and antibiotic susceptibility pattern of *staphylococcus aureus* from skin and soft tissue infections in Zaria, Nigeria

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ARTICLE INFO

Article history:
Received 29 January 2013
Accepted 14 February 2013
Available online 28 February 2013

Keywords:
Staphylococcus aureus
SSTIs
PVL
msrA
CA-mrsA
Zaria

ABSTRACT

Skin and soft tissue infections are infections involving the non-skeletal tissues i.e., exclusive of bone, ligaments, cartilage, and fibrous tissue. The aim of this research was to isolate, determine the antibiotic susceptibility pattern and detect molecularly, PVL and MSRA genes from *Staphylococcus aureus* isolated from skin and soft tissue infections in Zaria. Four hundred skin and soft tissue (SSTI) specimens were collected from some hospitals in Zaria and screened for the presence of *Staphylococcus aureus.* The male-to-female ratio of patients with SSTIs was approximately 2:1 (62.30% in male and 37.70% in female). The highest frequency of 30% was observed in the 51-60 years age group. Antibiotic susceptibility test was done on the isolates using Kirby Bauer's Disc Diffusion Technique and it was found that the highest level of resistance of *S. aureus* was recorded in Ceftazidime (49.28%), followed by Chloramphenicol (28.99%), Cefoxitin (27.54%), Oxacillin (26.09%) and Linezolid (7.25%). All isolates were sensitive to Vancomycin. Molecular assay was carried out on 25 selected isolates using Panton Valentine Leukocidine (PVL which codes for Community Acquired MRSA) and macrolide efflux resistance determinant (msrA, since Hospital Acquired mrsA shows high resistance to macrolides) as gene markers. PCR amplification showed 4 positive isolates (16%) for PVL genes and 7 positive isolates (28%) for msrA. There was high rate of antibiotic resistance. Vancomycin is the drug of choice while Linezolid can be considered in its absence. There was higher prevalence in HA-mrsA than CA-mrsA. Both strains showed multi-drug resistance to antibiotics. There is

need for strict antibiotic policy, continuous monitoring of antibiotic susceptibility pattern of all *S. aureus* and observation of infection control measures to curtail the evolution of these resistant strains. Further molecular study on *S. aureus* SSTIs and Methicillin-Resistant *S. aureus* epidemiology in future is desirable.

1. Introduction

Skin and soft tissue infections (SSTIs) are infections involving the non-skeletal tissues i.e., exclusive of bone, ligaments, cartilage, and fibrous tissue, resulting from either a break in the skin in surgery, bed sores, cuts, puncture, animal or insect bites, thorn and needle pricks or burns. Bacterial SSTIs range from superficial epidermal infections to life-threatening necrotizing fasciitis (Templer and Brito, 2009).

Among organisms frequently isolated from SSTIs include Gram positive bacteria such as *Staphylococcus* and *Streptococcus.* Staphylococci have a record of developing resistance quickly and successfully to antibiotics (Nwankwo and Nasiru, 2011). Many strains of *S. aureus* exhibit multidrug resistance, leading to the use of Methicillin in their management. Resistance to Methicillin (Methicillin Resistant *S. aureus* or MRSA) have also emerged and have been reported severally (Ikeh, 2003; Ghebremedhin *et al.,* 2009; Fayomi *et al.,* 2011; Shittu *et al.,* 2011). Some of such level of resistance are seen in Community-Acquired Methicillin Resistant *S. aureus* (CA-MRSA), associated with the expression of the Panton Valentine Leukocidin (PVL) gene as well as Hospital-Acquired Methicillin Resistant *S. aureus* (HA-MRSA) which can be detected using macrolide efflux resistance determinant (methionine sulfoxide reductase A or msrA) gene (Singh *et al*., 2001), as HA-MRSA are always resistant to macrolides (Harbarth *et al*., 1999). SSTIs are common in clinical practice. Empiric therapy with β-lactam drugs may no longer be adequate for treatment now that MRSA strains are being identified more frequently as the causative agents for SSTIs (Templer and Brito, 2009). In the developing world, mortality associated with severe *S. aureus* infections far exceeds that in developed countries (Nickerson *et al.,* 2009). From 1993 to 2005, the impact of CA-MRSA tripled emergency department SSTI visits in the USA (Pallin *et al.,* 2008).However, there are limited data on the incidence and characteristics of SSTIs in sub-Saharan Africa, and knowledge of pathogen-specific attributable morbidity is lacking (Truong *et al.,* 2011).

The purpose of this study was to isolate, determine the antibiotic susceptibility pattern and detect molecularly, PVL and msrA genes from *Staphylococcus aureus* isolated from skin and soft tissue infections in Zaria, Nigeria.

2. Materials and methods

2.1. Study population and ethical approval

This study was conducted in Zaria, with specimens collected from both in- and out-patients in four different hospitals. The hospitals were Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Hajiya Gambo Sawaba General Hospital, Kofan Gaya, Zaria city, St. Luke's Anglican Hospital, Wusasa and Salama Hospital, Kwangila. Ethical clearance was sought from the ethical committee of Kaduna State ministry of Health as well as the individual hospitals.

2.2. Specimen size and collection

A total of 400 Skin and soft tissue samples yielding growth of *S. aureus* from March 2012 to September 2012 were included. Samples were collected from pus, wound sites, eye, ear, pleural fluid, peritoneal fluid or aspirates by qualified laboratory scientists or medical personnel.

2.3. Media preparation

Media used for this study were mannitol salt agar medium (CM0085B-Oxoid England), Nutrient Agar (Oxoid, CM0003), Mueller-Hinton Agar (Oxoid, CM0337), Blood Agar and Luria Bertani broth. All the media were prepared according to manufacturer's instruction.

2.4. Identification of the isolates

Organisms were identified as *S. aureus* by Gram stain, catalase and coagulase test. Other supplemental tests were DNase and mannitol fermentation.

2.4.1. Gram staining

Culture smears of the isolates were made on sterile slide and then heat-fixed. The fixed smears were then covered with crystal violet for one minute and rinsed rapidly with clean water. The smears were covered with Lugol's iodine for one minute and also washed off with water. They were decolorized rapidly with alcohol for a few seconds and rinsed with water. They were then flooded with Safranin red for 30 seconds and then rinsed out. Finally, the smears were placed in a draining rack for air-drying. The smears were then examined microscopically, first with ×40 objective to obtain the level of distribution of material and then with oil immersion ×100 objective to observe the microscopic appearance of the bacterial cells. Suspected isolates showed purple-coloured cocci in clusters.

2.4.2. Catalase test

Using sterile wooden sticks, several similar colonies of the isolates were immersed in test tubes containing 2-3 ml of hydrogen peroxide solution. The test tubes were then observed for immediate bubbling. Active bubbling was recorded as positive.

2.4.3 Coagulase test

A drop of distilled water was placed on two separate slides. The isolates were then emulsified in each of the drops, forming thick suspensions. A loopful of plasma was then added to the suspensions and mixed gently, then observed for clumping. This was repeated using test tubes. Presence of clumping within ten seconds (10 secs) was recorded as positive for *S. aureus.*

2.4.4. DNase test

Isolates were incubated on DNase agar for 24 hours at 37ºC. Excess 1N HCL was then poured over the agar surface (approximately 15ml), after which excess acid was removed using vacuum pipette. Clear zone around bacterial colonies was recorded as positive for *S. aureus.*

2.4.5. Haemolysis on blood agar

Blood agar was used to test for haemolysis of the presumptive isolates. Presence of β-haemolysis on blood agar was recorded as positive.

2.5. Antibiotic susceptibility testing

Antimicrobial sensitivities against ceftazidime (30µg), chloramphenicol (30µg), oxacillin (1µg), cefoxitin (10µg), linezolid (10µg) and vancomycin (30µg) (OXOID) were performed by Kirby Bauer Disc Diffusion technique. A sterile cotton wool swab stick was used to inoculate the entire surface of Mueller-Hinton agar (MHA) plate (Oxoid, UK) with the inoculum of *Staphlococcus aureus*, turbidity matching 0.5 MacFarland Nephalometer standard, before antibiotic discs was laid on the surface. The plates were incubated overnight at 35ºC. The inhibition zone diameter (IZD) was evaluated according to the Clinical and Laboratory Standard Institute (CLSI, 2009) guidelines.

2.6. Molecular characterization of the Isolates

2.6.1. DNA extraction

Genomic DNA were obtained from a 10ml overnight culture grown in Luria-Bertani (LB) broth using a DNA extraction kit with lysostaphin (100 µg/ml) to achieve bacterial lysis.

2.6.2. Detection of antibiotic resistance genes

The detection of the msrA genes (macrolide efflux resistance determinants) were investigated using the following primers msrA f: GAA GCA CTT GAG CGT TCT; msrA r: CCT TGT ATC GTG TGA TGT (Shittu et al., 2011).

2.6.3. Molecular detection of Panton-Valentine leukocidin (PVL) genes

Polymerase chain reaction (PCR) amplification of PVL genes (*lukF-PV* and *lukS-PV*) was performed on CA-MRSA strains and methicillin-susceptible *S. aureus* (MSSA) strains. The following PCR primers and the procedures used were those described previously by Lina *et al* (1999): luk-PV-F (ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A) and luk-PV-R (GCA TCA AST GTA TTG GAT AGC AAA AGC).

The PCR conditions were as follows: initial denaturation at 95°C for 2 minutes, amplification at 94 °C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72 °C for 5 minutes.

2.7. Statistical analysis

Results obtained are represented in chart, tables and figures. Statistical analysis (Chi-Square) was done using a computer software program, SPSS.17 version. Categorical variables were described using counts and percentages. The calculated value was compared with the table value by tracing the degree of freedom to the probability, p (0.05).Two tailed P- values >0.05 was considered statistically non-significant.

3. Results

Table 1 shows the number of isolates positive to the conventional tests conducted after isolation using mannitol salt agar (MSA). All isolates were positive to Gram staining (Gram positive cocci in clusters), Haemolysis on blood agar, DNase and catalase tests. Only three isolates were coagulase negative. Fig. 1 shows the gender distribution of *Staphylococcus aureus* showed that 43 (62.30%) out of the 69 isolates were from male patients while 26 (37.70%) were from female patients. There was however, no significant difference between the isolates and gender of the patients sampled (p>0.05, P=0.567). Table 2 shows the different specimens from which *Staphylococcus aureus* was isolated. Ear swabs had the highest incidence of 18.82%, followed by pus, with an incidence of 18.6%, whereas no incidence was recorded in pleural fluid and ascitic fluid. The difference was not statistically significant (χ^2=4.236, df=6, P= 0.645). Table 3 shows percentage sensitivity of *Staphylococcus aureus* isolates from hospitals sampled**.** All the isolates were sensitive to Vancomycin while the highest resistance was recorded in Ceftazidime. Table 4 shows the antibiotic sensitivity pattern of *Staphylococcus aureus* from the various clinical specimens. The highest frequency of sensitivity was observed with Vancomycin(100%) while the least was observed with Ceftazidime (wound=48.39%, Ear swabs=50%, Eye swabs=42.86%, Aspirates=25%, Pus=72.73%).

Table 1
Isolation of *S. aureus* using conventional tests.

Tests conducted	Number of isolates screened	Number of positive isolates	Percentage positive (%)
Gram Staining	69	69	100.00
Catalase Test	69	69	100.00
Coagulase Test	69	66	95.65
Haemolysis on Blood Agar	69	69	100.00
Clear zone on DNase Agar	69	69	100.00

Figure 2 (a and b) show the gene amplicons coding for Panton-Valentine leukocidin (PVL) genes. A total of 4 different isolates were found to be positive among 25 selected isolates, mainly including GEY142, GER127, AAS67, and AER 05. Two of the isolates were found in aspirate (AAS67) and ear (AER 05) isolated from Ahmadu Bello University Teaching Hospital, Shika, while the other two were found in eye (GEY142) and ear (GER127) isolated from Hajiya Gambo Sawaba General Hospital, Kofan Gaya, Zaria City. Figure 3(a and b) show the gene amplicons coding for macrolide efflux resistance determinants (mrsA genes). The result showed a total of 7 isolates positive for mrsA genes among 25 selected isolates. The isolates include AAS67, GER127, AW27, AW11, GP194, GEY148, and AW327 rerepectively. Three of the isolates were isolated from wound in Ahmadu Bello University Teaching

Hospital, Shika, (AW27, AW11, AW327), and one from aspirate (AAS67), while one each (GER127, GP194, GEY148) was isolated from ear, pus and eye respectively in Hajiya Gambo Sawaba General Hospital, Kofan Gaya, Zaria City.

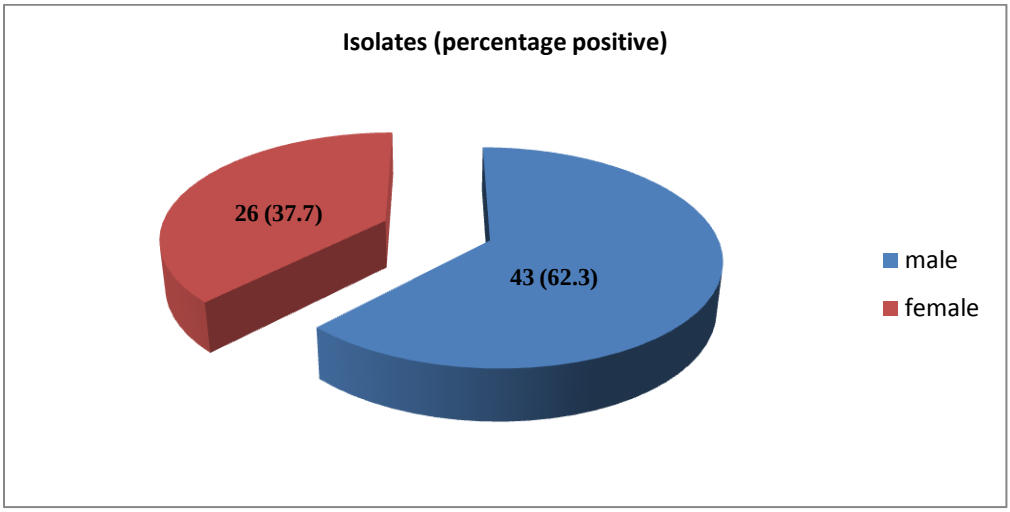

Fig. 1. Distribution of *Staphylococcus aureus* according to gender.

Table 2
Distribution of *Staphylococcus aureus* from various clinical specimens.

Specimen type	Number of specimens screened	Number of positive specimens	Percentage positive (%)
Wound	169	31	18.34
Ear	85	16	18.82
Eye	44	7	15.90
Aspirate	26	4	15.39
Pleural fluid	8	0	0.00
Pus	59	11	18.60
Ascitic fluid	9	0	0.00
Total	400	69	17.25

(χ^2=4.236, df=6, P= 0.645)

Table 3
Percentage sensitivity of *Staphylococcus aureus* isolates from hospitals sampled.

Hospitals sampled	Number of isolates	Number of susceptible isolates (percentage positive)					
		CAZ (30µg)	C (30µg)	OX (1µg)	FOX (10 µg)	LZD (10µg)	VA (30µg)
ABUTH	40	22(31.88)	30(43.48)	29(42.02)	32(46.37)	37(53.62)	40(57.97)
GAMBO	24	9(13.04)	16(23.19)	18(26.29)	14(20.29)	22(31.88)	24(34.78)
ST. LUKE	5	4(5.80)	3(4.35)	4(5.80)	4(5.80)	5(7.25)	5(7.25)
SALAMA	0	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
TOTAL	69	35(50.72)	49(71.01)	51(73.91)	50(72.46)	64(92.75)	69(100.00)

ABUTH= Ahmadu Bello University Teaching Hospital, GAMBO= Hajiya Gambo Sawaba General Hospital, ST. LUKE= St. Luke's Anglican Hospital, SALAMA= Salama Hospital, CAZ=Ceftazidime, C= Chloramphenicol, OX= Oxacillin, FOX= Cefoxitin, LZD= Linezolid, VA= Vancomycin

Table 4
antibiotics sensitivity pattern of *Staphylococcus aureus* isolates from various clinical specimens.

Antibiotics tested	Specimens (percentage sensitive)						
	Wound (N=31)	Ear (N=16)	Eye (N=7)	Aspirate (N=4)	Pleural fluid (N=0)	Pus (N=11)	Ascitic luid (N=0)
CAZ (30µg)	15(48.39)	8(50.00)	3(42.86)	1(25.00)	0	8(72.73)	0
C (30µg)	21(67.74)	11(68.75)	5(71.43)	3(75.00)	0	9(81.82)	0
OX (1µg)	21(67.74)	12(75.00)	5(71.43)	3(75.00)	0	10(90.91)	0
FOX (10µg)	23(74.19)	10(62.50)	6(85.71)	3(75.00)	0	8(72.73)	0
LZD (10µg)	28(90.32)	14(87.50)	7(100.00)	4(100.00)	0	11(100.00)	0
VA (30µg)	31(100.00)	16(100.00)	7(100.00)	4(100.00)	0	11(100.00)	0

N=number of isolates, CAZ=Ceftazidime, C= Chloramphenicol, OX= Oxacillin, FOX= Cefoxitin, LZD= Linezolid, VA= Vancomycin.

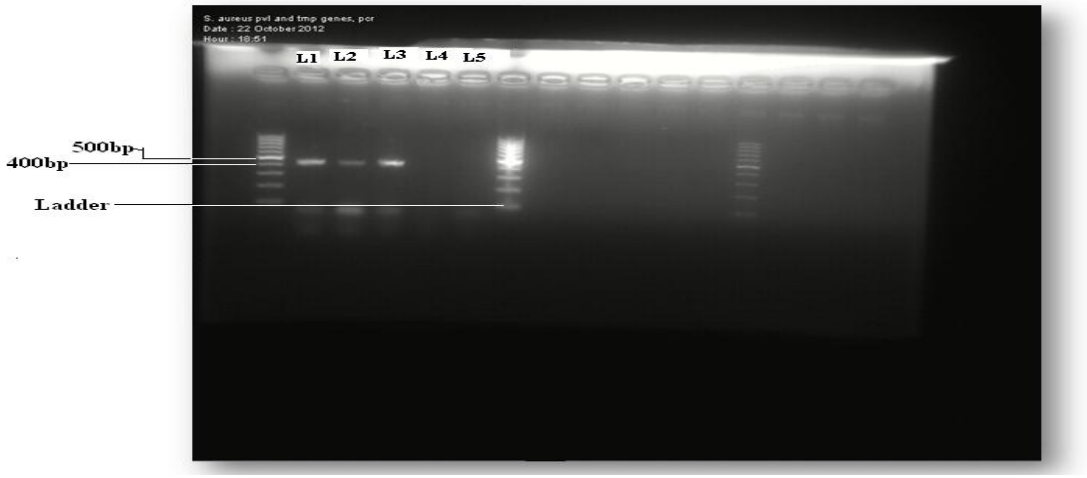

Fig. 2(a). Gene amplicons coding for PVL genes.
bp= base pairs, L1= Lane 1 (GEY142, 400bp), L2= Lane 2 (GER127, 400bp), L3= Lane 3 (AAS67, 400bp), GEY142= Hajiya Gambo Sawaba Hospital, Eye swab, specimen 142; GER127= Hajiya Gambo Sawaba Hospital, Ear swab, specimen 127; AAS67= Ahmadu Bello University Teaching Hospital, Aspirate, specimen 67.

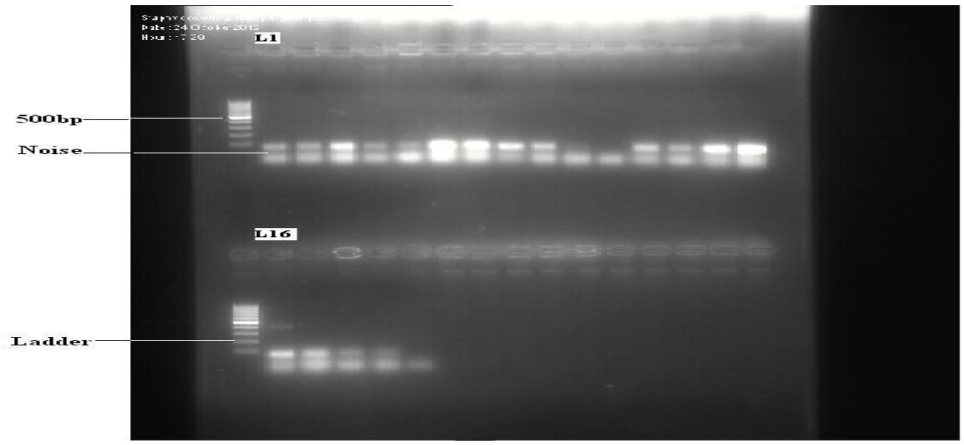

Fig. 2(b). Gene amplicon coding for PVL gene.
bp= base pair, L16= Lane 16 (AER05, 400bp), Noise= Excess primers bound to each other; AER05= Ahmadu Bello University Teaching Hospital, Ear swab, specimen 05.

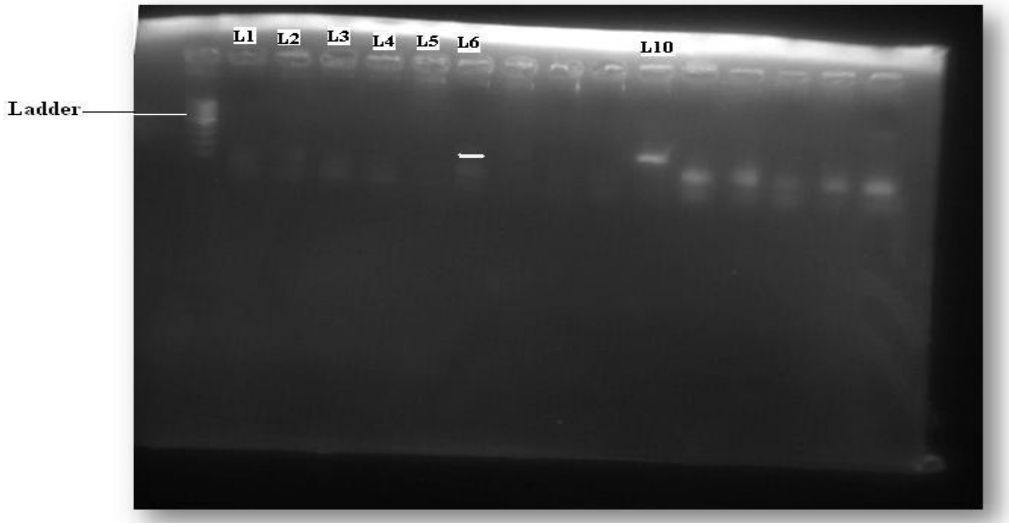

Fig. 3(a). Gene amplicons coding for MSRA genes.
bp= base pairs, L6= Lane 6 (AAS67, 200bp), L10= Lane 10 (GER127, 200bp), AAS67= Ahmadu Bello University Teaching Hospital, Aspirate, specimen 67; GER127= Hajiya Gambo Sawaba Hospital, Ear swab, specimen 127.

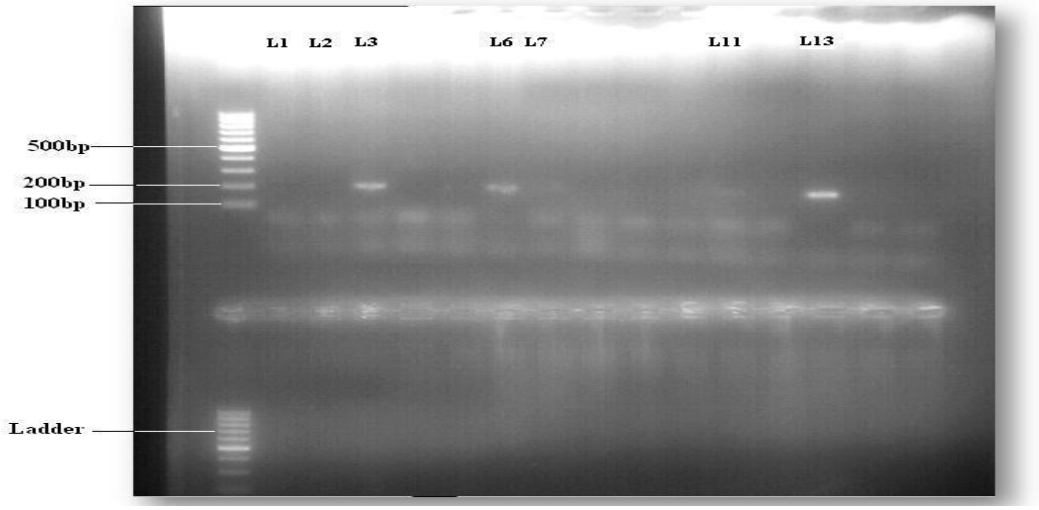

Fig. 3(b). Gene amplicons coding for MSRA genes.

bp= base pairs; L3= Lane 3 (AW27, 200bp); L6= Lane 6 (AW11, 200bp); L7= Lane 7 (GP194, 200bp); L11= Lane 11 (GEY148, 200bp); L13= Lane 13 (AW327, 200bp); AW27= Ahmadu Bello University Teaching Hospital, Wound, specimen 27; AW11= Ahmadu Bello University Teaching Hospital, Wound, specimen 11; GP194= Hajiya Gambo Sawaba Hospital, Pus, specimen 194; GEY148= Hajiya Gambo Sawaba Hospital, Eye swab, specimen 148; AW327= Ahmadu Bello University Teaching Hospital, Wound, specimen 327.

4. Discussion

The prevalence rate of 17.25% detected in this study is higher than the rate noted by Winstead *et al.,* 2010 in the Emergency department of a suburban Philadelphia hospital and the prevalence rates of 1.35% and 2.98% noted by Pallin *et al* (2008) in 1993 and 2005 respectively in United States Emergency departments. This puts the prevalence rate in Zaria at the range of 15-20%. A study carried out in a tertiary hospital in Botwana (Truong *et al.*,

2011), recorded a prevalence rate of 9.09% comparatively lower than that obtained in this study. However, Ki and Rotstein, 2008 pointed out that due to the fact that majority of SSTIs tend to resolve within seven to ten days, an estimate of prevalence is highly variable; hence, among hospitalized patients, the estimated prevalence would be 7% to 10%. The high prevalence of *S. aureus* SSTIs rate in this study may not be unconnected to the poor infection control program in our hospitals and community at large, where policy and guidelines on preventing transmission of infectious agents are disregarded, poorly documented or non-existent.

The male-to-female ratio of SSTIs in this study is approximately 2:1 (62.30% in male and 37.70% in female). This is slightly different from the ratio of approximately 1:1 reported by Winstead *et al*., 2010. It is not clearly understood why males were more infected than females in the present study. However, it could be mostly because men are more likely to experience traumatic events because of the nature of their physical activity, in conjunction with being more carefree to hygiene. The presence of *S. aureus* was not gender dependent. No incidence was recorded in pleural fluid and ascitic fluid, but *Staphylococcus aureus* was found to be a frequent cause of burns, abscesses and wound sepsis in the present study (owing to its level of occurrence in wound, pus and aspirates). The absence of *S. aureus* in pleural and ascitic fluids is a pointer that severe cases of complicated skin and soft tissue infections (cSSTIs) may be non-existent or negligible in Zaria. *Truong et al*., 2011 reported that the most common sites of *S. aureus* infection or colonization were wound (N=42, 4.9%) and eyes (N=36, 4.2%). This does not agree with the result in the present study where *S. aureus* had the highest isolates of 18.82% in ear swabs, followed by pus, with an incidence of 18.6%, and then wound (18.34%). However, in the vast majority of *S. aureus* isolates, the anatomical site was unspecified 'pus' and unspecified 'aspirates', yielding 18.6% and 15.39% respectively. The highest frequency of *S. aureus* in this study occurred with susceptibility to the antibiotic Vancomycin (100%), followed by Linezolid (92.75%) while the least was Ceftazidime (50.72%). Vancomycin is a glycopeptide and is currently a drug of choice for *S. aureus* infections, especially MRSA infections (Stevens *et al*., 2005). Oral therapy with linezolid, a bacteriostatic oxazolidinone, is effective in treating patients with an SSTI; resistance to linezolid among MRSA isolates has been reported with prolonged use (Besier *et al*., 2008) but is rare with short-term use. Ceftazidime is a third generation cephalosporin. Compared with first-generation cephalosporins, the second-and third-generation cephalosporins in general have inferior in vitro activity against *S. aureus*. Due to the present unavailability of Methicillin, Oxacillin was used in this study. Oxacillin, like Methicillin, is also a penicillinase-resistant penicillin (isoxazoyl penicillin) and there is minor variation in their relative beta-lactamase stability, though this does not appear to be of clinical importance (Turnidge et al., 2008). Wound swabs recorded the highest rate of resistance to Oxacillin (32.26%) while the least resistance was observed in pus (9.09%). In previous studies (Ikeh, 2003; Nwakwo et al., 2010; Saikia et al., 2009; Fayomi et al., 2011), Methicillin-resistant *S. aureus* have mostly been isolated from wound swabs while MSSA were mostly isolated from pus/aspirates by Fayomi et al., 2011. In Nigeria, wounds, when inflicted on affected persons, are frequently left untended to until they get infected and sometimes, become purulent. This could explain why wound swabs have the highest rate of resistance to oxacillin. Amplification of PVL genes (lukF-PV and lukS-PV) in the present study showed that a total of 4 different isolates were found to be positive among 25 selected isolates (16%) (GEY142, GER127, AAS67, and AER 05). A positive result depicts the presence of CA-MRSA. In a region or a population with a high prevalence of CA-MRSA, e.g., where >10% of clinical *S. aureus* isolates are MRSA isolates, β-lactam antibiotics are no longer reliable for empiric therapy (Hankin and Everett, 2007). This study showed that for PCR amplification of msrA genes, a total of 7 isolates were positive among 25 selected isolates (28%). The isolates include AAS67, GER127, AW27, AW11, GP194, GEY148, and AW327 respectively. HA-MRSA is always resistant to the macrolides (Harbarth *et al*., 1999) while many CA-MRSA are susceptible, although resistance can range up to 25%. This explains why the some of the isolates positive for PVL genes (AAS67 and GER127) were also positive for msrA genes. The higher incidence of 28% recorded in this study as compared to the 16% of PVL-positive isolates indicates that HA-MRSA was more prevalent than CA-MRSA. This is not surprising, as health care policies and general hospital management guidelines are poorly enacted in the average Nigerian hospitals.

5. Conclusion

This study established an alarmingly high prevalence of SSTIs in Zaria. There is however, absence of complicated SSTIs. The present studies went further to bridge the information gap and expand the knowledge of *S. aureus* skin and soft tissue infections, in the process, elucidating its local antimicrobial resistance pattern. A higher prevalence of HA-MRSA was established using msrA gene markers. The combination of susceptibility testing and

molecular assay provided useful information on the antibiotic resistance and molecular diversity (variation) of *S. aureus* in Zaria.

References

Besier, S., Ludwig, A., Zander, J., Brade, V., Wichelhaus, T.A., 2008. Linezolid Resistance in *Staphylococcus aureus*: Gene Dosage Effect, Stability, Fitness Costs, and Cross-Resistances. *Antimicrobial Agents and Chemotherapy*; 52, 1570-1572.

Centre for Disease Control and Prevention 2003. Guidelines for Environmental Infection Control in Health-Care Facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). *Morbidity and Mortality Weekly Report*; 52(RR10), 1-42.

Clinical Laboratory Standards and Institute 2009. Performance Standards for Antimicrobial Susceptibility Testing, 16th informational supplement. M100-S16 Wayne, Pa.

Fayomi, O.D., Oyediran, E.I.O., Adeyemi, A.T., Oyekale, O.T., 2011. Prevalence And Antibiotic Resistance Pattern of Methicillin-Resistance *Staphylococcus Aureus* among In- Patients at a Tertiary Health Facility in Ido-Ekiti, Nigeria. *The Internet Journal of Laboratory Medicine*, 4(2)

Ghebremedhin, B., Olugbosi, M.O., Raji, A.M., Layer, F., Bakare, R.A., Konig, B., Konig, W., 2009. Emergence of a Community-Associated Methicillin-Resistant *Staphylococcus aureus* with Unique Resistance Profile in Southwest of Nigeria. *Journal of Clinical Microbiology*. 47, 2975-2980.

Harbarth, S., Dharan, S., Liassine, N., 1999. Randomized, Placebo-Controlled, Double- Blind Trial to Evaluate the Efficacy of Mupirocin for Eradicating Carriage of Methicillin-Resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*; 43, 1412–1416.

Ikeh, E.I., 2003. Methicillin-Resistant Staphylococcus aureus at Jos University Teaching Hospital. *African Journal of Clinical and Experimental Microbiology*; 4(1), 52-55.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M.O., Gauduchon, V., Vandenesch, F., andEtienne, J., 1999. Involvement of Panton-Valentine Leukocidin-Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. *Clinical Infectious Diseases*; 29, 1128-1132.

Lowy, F.D., 1998. *Staphylococcus aureus* Infection. *New England Journal of Medicine.* 339(8), 520-532.

Micek, S.T., 2007. Alternatives to Vancomycin for the Treatment of Methicillin Resistant *Staphylococcus aureus* Infections. *Clinical Infectious Diseases*; 45, S184-190.

Nwankwo, E.O., Nasiru, M.S., 2011. Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. *The Pan African Medical Journal.*8, 4. ISSN 1937-8688. http://www.panafrican-med journal.com/content/article/8/4/full

Pallin, D.J., Egan, D.J., Pelletier, A.J., Espinola, J.A., Hooper, D.C., Camargo, C.A. Jr 2008. Increased US emergency department visits for skin and soft tissue infections, and changes in antibiotic choices, during the emergence of community-associated methicillin- resistant *Staphylococcus aureus*. *Annual Emergency Medicine;*51,291-298.

Popovich, K.J., Weinstein, R.A., Hota, B., 2008. Are Community-Associated Methicillin- Resistant *Staphylococcus aureus* (MRSA) Strains Replacing Traditional Nosocomial MRSA Strains? *Clinical Infectious Diseases*; 46, 787–794.

Singh, V.K., Moskovitz, J., Wilkinson, B.J., Jayaswal, R.K., 2001. Molecular Characterization of Chromosomal Locus in *Staphylococcus aureus* that Contribute to Oxidative Defence and is Highly Induced by the Cell-Wall-Active Antibiotic Oxacillin. *Microbiology;* 147(11), 3037-3045.

Shittu, A.O., Lin, J., 2006a. Antimicrobial Susceptibility Patterns and Characterization of Clinical Isolates of *Staphylococcus aureus* in Kwazulu-Natal Province, South Africa. *BMC Infectious Disease*. 6(1), 125

Shittu, A.O., Okon, K., Adesida, S., Oyedara, O., Witte, W., Stro mmenger, B., Layer, F., Nube, U., 2011. Antibiotic Resistance and Molecular Epidemiology of *Staphylococcus aureus* inNigeria. *BMC Microbiology*; 11, 92 doi:10.1186/1471-2180-11-92. http://www.biomedcentral.com/1471-2180/11/92

Skiest, D.J., Brown, K., Cooper, T.W., 2007. Prospective Comparison of Methicillin- Susceptible and Methicillin-Resistant Community-Associated *Staphylococcus aureus* Infections in Hospitalized Patients. *Journal of Infections*; 54, 427–434.

Stevens, D.L., Bisno, A.L., Chambers, H.F., Everett, E.D., Dellinger, P., Goldstein, E.J.C., 2005. Infectious Diseases Society ofAmerica. Practice Guidelines for the Diagnosis and Management of Skin and Soft-Tissue Infections

[published errata appear in Clin Infect Dis 2005;41:1830 and 2006;42:1219]. *Clinical Infectious Disease*; 41, 1373–1406.

Templer, S.J., Brito, M.O., 2009. Bacterial Skin and Soft Tissue Infections. p p . 9 –16, 26. *Hospital Physician*. www.turner-white.com

Truong, H., Shah, S.S., Ludmir, J., Tawanana, E.O., Bafana, M., Wood, S.M., Moffat, H., teenhoff, A.P., 2011. *Staphylococcus aureus* skin and soft-tissue infections at a tertiary hospital in Botswana. *South African Medical Journal*; 101(6), 413-416.

Turnidge, J.D., Rao, N., Chang, F., Fowler, V.G. Jr, Kellie, S.M., Arnold, S., Lee, B.Y., Tristan, A.(2008). *Staphylococcus aureus.* Updated December, 2008.

Udo, E.E., Al-Sweih, N., Noronha, B., 2006. Characterisation of Non-Multiresistant Methicillin-Resistant *Staphylococcusaureus* (including EMRSA- 15) in Kuwait Hospitals. *Clinical Microbiology Infections*; 12, 262-269.

Winstead, Y., Emmerich, H., Manning, M.L., Winstead, D., Nelson-Bachmann, P., Kornecki, Z., 2010. Clinical Management of Skin and Soft Tissue Infections in the Emergency Department of a Suburban Hospital. *Advanced Emergency Nursing Journal*; 32(2), 155-167.