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Prevalence Of Methicillin Resistant *Staphylococcus Aureus* From Clinical Specimens In Ibadan, Nigeria

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----------ABSTRACT----------

This study was carried out to investigate the prevalence of methicillin-resistant Staphylococcus aureus and antibiotic sensitivity pattern in clinical isolates in Ibadan. Clinical samples (150) were collected from various health centers in Ibadan over a period of 7 months. The samples were cultured on bacteriological media for the isolation of Staphylococcus aureus using standard methods of isolation and identification of bacteria. The Staphylococcus aureus were tested for methicillin susceptibility using 5µg oxacillin disc while the identified MRSA were tested for susceptibility to other antibiotics. Out of 23 Staphylococcus aureus isolated, 52.2% were recovered from urine specimens, 30.4% wounds swab, 13.0% ear swab and 4.3% from nasal swab. 7(30.4%) out of the Staphylococcus aureus were MRSA as indicated by their resistance to oxacillin . The yield of MRSA was highest from urine (71.4%) and least from wound swab (28.6%). The MRSA were highly resistant to Amoxicillin (100%), Augumentin (85.7%), Cotrimozaxole (71.4%), Tetracycline (57.1%), Gentamycin (42.9%), Ofloxacin (28.9%) and Ciprofloxacin (28.5%). The MRSA 6(85.7%) isolated showed multi resistance to at least 4 or more of the antibiotic tested, five out of the seven MRSA harbor single plasmid of molecular weight 25.0kbp.The prevalence of MRSA is high; the MRSA showed a very high degree of resistance to β-lactam antibiotics indicating the infectiveness of these antibiotics and need to reassess the policies on antibiotic usage in hospital environment.

KEY WORDS: *prevalence, methicillin-resistant Staphylococcus aureus, antibiotic sensitivity pattern, Clinical samples, bacteriological media.*

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I. INTRODUCTION

Staphylococus aureus (including MRSA strains) are cluster-forming facultatively aerobic, Gram-positive cocci with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media. They also ferment mannitol turning Mannitol salt Agar (MSA) Yellow (Bannerman, Murray, Baron, Jorgensen, pFaller and Yolken, 2003). Staphylococcus aureus (S. aureus) is the leading cause of gram positive bacterial infections and linked with a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. Although Staphylococcus aureus infections were historically treatable with common antibiotics, emergence of drug- resistant organisms is now a major concern. MRSA is a strain of Staphylococcus aureus that is resistant to methicillin or β-lactamase resistant to penicillin. Usually these strains of Staphylococcus aureus are resistant to more than one antibiotic hence, the infections due to this strain of MRSA are very difficult to treat. The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins, often called enzymes (clumping factor) used for their identification. MRSA has been severally shown to cause variety of diseases ranging from mild, superficial dermatological diseases to severe and potentially fatal systemic debilitiations (Chambers, 1997; Moran, 2005).In spite of the availability of considerable number of effective antimicrobial chemotherapeutic agents, MRSA still remains an important and increasing cause of post-surgical wound infections (Gorttileb, Fowler, and Kong, 2000; Graffunder and Venezia,2002). Some invasive infections such as nosocomial bacteremia and septicemia (sepsis) (Myelotte and Tayara, 2000), acute endocarditis and osteomyelitis, pneumonia and other soft tissue infections (STIs) (Graffunder and Venezia, 2002; Rello and Diaz, 2003), are also traceable to them.The increasing prevalence of MRSA multiple – drug resistant strains which limits the therapeutic options available for the management of MRSA associated infections has become a worrisome issue worldwide (Frazee, Lynn, Charlebois, Lambert, Lowery, and Perdreau Remington, 2005) and has posed a serious therapeutic challenge leaving glycopeptides as the drug of choice.

Furthermore, methicillin – resistant Staphylococcus aureus (MRSA) has been known to be an important bacterial pathogen causing nosocomial and community – onset infection which has shown increasing endemic and epidemic spread in the last four decades (Kuehnert , Hill, and Kupronis, 2005; Frazee *et al.*, 2005) while its control has become a serious concern burden in terms of medical and socio-economic costs and cause significant morbidity and mortality (Maranan, Moreira,, Boyle Vavra, and Daum, 1997; Carbon, 1999; Bratu, Erarno, Kopec, Coughlin, Ghitan, Yost, Chanprnick, Landman, and Wuale, 2005).MRSA prevalence varies greatly with geographical location, type of hospital and studied population. High prevalence has been recorded in tertiary hospitals in US, Southern European countries, Asia and South America (Diekema, Pfaller, Schmitz, Smayevsky, and Bell, 2001). In Africa, MRSA prevalence varies with different countries, high in some and low in others (Bell, Turnidge and Sentry Apac participants, 2002). In Nigeria, studies on methicillin-resistant *Staph aureus* have been conducted, particularly in southwestern zone (Adesida, Boelens, Kehinde, Babajide, and Snijders, 2005; Shittu and Lin, 2006; Ghebremedhin, Olugbosi, Raj, Layer , Bakare, and Konig, 2009; Adeleke and Asani, 2009; Onipede, Onayade, Elusiyan, Obiajuwon, Ogundare, Olaniran, Adeyemi, and Oyelami, 2009). Despite this epidemiological data on MRSA in Nigeria, available data are still relatively limited when compared to information from developed countries which may be attributable to high level of awareness of MRSA infections and its clinical and societal consequences.In view of the above, the aim of this study is to determine the prevalence of methicillin-resistant *Staphylococcus aureus* from clinical specimen in Ibadan, Nigeria.

II. MATERIALS AND METHODS

Samples and Samples Collection

The materials made use of in the course of analysis of samples in this study include equipment, bacterial culture, media and chemicals. These materials with their manufacturers are listed in Appendix . A total of 150 clinical specimens consisting of urine, nasal swab, ear swab and wound swab were collected from the Department of Medical Microbiology, University College Hospital Ibadan, Oluyoro Catholic Hospital, Hebron Medical Centre Odo –Ona, Amazing Grace Hospital Orita, College and Cedar Hospital Molete, all in Ibadan for the period of 7 months. The swab samples were collected using commercially prepared sterile swab sticks (Oxoid U.K.).The urine samples are clean-catch midstream urine collected into sterile disposable universal bottles. They were kept refrigerated at 4^oC until delivery, to the laboratory. All the samples where cultured immediately on appropriate media, within 12 hours of collection.

Isolation and Identification : The sterile culture media plates of Mannitol Salt Agar (MSA), Blood agar (BA) and MacConkey Agar (MCA) were dried to remove water of condensation in the plates as well as on the surface of the culture media, by slightly exposing them inside the 37^oC incubation before use. The swab sample was rubbed over one quarter of each of the different agar plates (i.e. MSA, BA and MCA), the rest part of the plates were streaked with a sterile wire loop to obtain discrete colonies. The inoculated culture media were incubated at 37^oC in an incubator for 24-48hrs. Suspected discrete colonies of *Staphylococcus aureus* were sub-cultured on Nutrient Agar plates to obtain pure culture and for further analysis.Each organisms was identified according to Cowan and Steel (2003) method of bacteria identification , by their colonial appearance such as size, shape, consistency, colour, elevation and its differential characteristics such as pigmentation, lactose fermentation on MacConkey and Gram Staining were done to further identify the isolates.

Biochemical Identification

Gram staining Technique : A smear of the suspected colony from the culture plate was made on clean, grease – free slide. The smear was heat-fixed on slide by passing the slide over Bunsen burner flame briefly. The slide was then covered with Crystal violet stain and allowed to stain for 1 minute. The stain was decanted, rinsed with tap water and stained with Lugol's iodine for 1 minute the stain was decanted and the film (smear) decolorized with acetone for few seconds. The slide was quickly washed with distilled water and counter stained with Safranin for 1 minute. The slide was finally washed with water dried and examined under the microscope using the oil-immersion objective. Suspected *Staphylococcus aureus* isolates were Gram-positive cocci (appearing purples) and were arranged in clusters.

Catalase test : A drop of 3% hydrogen peroxide solution was placed on a clean, grease-free glass slide; the edge of another clean slide was used to pick the test organisms and was dipped into the hydrogen peroxide. Observed bubble formation was regarded positive.

Coagulase Test : Part of the colony was emulsified on a clean grease free glass slide. 10µl of citrated human plasma was added and was observed for the presence of agglutination which indicates a positive reaction.

Tube Coagulase test : Isolates to be tested for Coagulase production were incubated in Mueller Hinton broth at 37^{o}C for 24hrs. 0.2ml of the overnight broth culture was added to 0.5ml citrated human plasma in a sterile glass test tube with gentle mixing, the test tubes were incubated at 37^{o}C and observed for coagulation at the 1hr of 2hrs, 4hrs and 24hrs. Formation of a firm opaque clot which remains in the place when the tube is tilted on its side was considered positive.

Mannitol fermentation : Plates of Mannitol salt agar were prepared according to the manufacturer's directions allowed to cool at 50^{o}C and poured in a sterile disposable plate to set. The plates were labeled against the samples to be tested. The organism to be identified was inoculated on the set dried cooled plate with the aid of a sterile wire loop (streaking), Incubated at 37^{o}C for 18hrs and was checked for evidence of growth on the agar surface and as well for colour change from red to golden yellow.Mannitol salt agar enables us to determine the different characteristics. The first is the organism ability to tolerate a high salt concentration environment which was indicated by the evidence of growth on the mannitol salt plate. Secondly, fermentation of the sugar mannitol by producing an acids an end product which changes the red pH indicator in the media to yellow. Change from red to yellow colour was considered positive.

DNase test : The test is used to identify *Staphylococcus aureus* which produces deoxyribonuclease (DNase) enzyme. It was done by preparing 100ml of DNase agar and adding 1.35ml of methyl green as indicator. Clear zone on an inoculated plate of *S. aureus* indicates positive.

Antimicrobial Testing : Pure isolates of identified *Staphylococcus aureus* were subjected to antimicrobial susceptibility testing using the disk diffusion method as recommended by KirbyBauer method according to the National Committee for Clinical and Laboratory Standards criteria (NCCLS 2005) with the following antibiotics: Gentamicin (10ug), Augmentin (30µg), Ofloxacin (5µg), Tetracycline (10µg), Cotrimoxazole (25µg), Cloxacillin (5µg), Ciprofloxacin(5µg), Erythromycin (5µg), Amoxycillin (10µg), Oxacillin (5µg). Were inoculated with *Staphylococcus aureus* containing 0.5 Macfarland standard bacteria on Mueller Hinton agar plates. The plates were allowed to dry for 5mins. The antibiotics disc were placed on the centre of the agar plates with the aid of sterile pointed tip forceps and incubated at 37^{o} C for 24hrs. The presence of a clear zone around the antibiotic disc is measured with meter rule.

Oxacillin Dics Diffusion : Oxacillin disc diffusion (5µg) was used to test for oxacillin resistance. Muellar Hinton Agar (MHA) plates containing 4% NaCl were inoculated with 10µL of 0.5 McFarlad (10^{6}CFU/ml) suspension of the isolate by streaking and incubated at 35^{o}C for 16-18hrs, a zone diameter of $\leq$ 10mm was considered resistant for oxacillin according to the National Committee for Clinical Laboratory Standard.

Plasmid Extraction and Profiling : Plasmid extraction was carried at FUNAAB with slight modification has described by Birnboim and Doly (1979). Pure isolates were inoculated on MRSA broth and incubated. The grown cells were harvested and suspended in 200µl of solution A (100 mM glucose – 50 mMTris hydrochloride (pH 8)-10 mM EDTA) containing 10 mg of lysozyme per ml and 10µl/ml mutanolysin and incubated for 30 minutes at 37^{o}C in an incubator. 400µl of freshly prepared 1% sodium dodecyl sulfate in 0.2 N NaOH was added and the samples were mixed by inverting tubes ,300µlof a 30% potassium acetate solution (pH 4.8) was added and the samples were mixed by vortexing. After incubating on ice for 5 minutes, the debris was removed by a 5-minute centrifugation in a centrifuge (model 5415R; Eppendorf). The supernatant was removed and extracted once with a phenol-chloroform mixture (1:1) and precipitated with an equal volume of isopropanol. The plasmid DNA was then dissolved in 100µl of TE buffer.

Gel electrophoresis : Electrophoresis of the DNA was carried out on a 0.8% agarose gel in a 0.5X concentration of Tris-Borate-EDTA (TBE) buffer. Agarose gel was prepared by boiling 0.8g of agarose powder in 100mls of 0.5X TBE buffer. After boiling, the solution was allowed to cool and 10µl of ethidium bromide was added to the cooled agarose solution. This was poured into a casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30 minutes and the comb was removed. 20µl of bromophenol blue. A DNA molecular weight marker was also loaded into one of the wells. The gel was thereafter electrophoresised in a horizontal tank at a constant voltage of 60V for about 1 hour 30 minutes. After electrophoresis, plasmid DNA bands were viewed by fluorescence of bound ethidium bromide under a short wave ultraviolet light transilluminator and the photograph were taken using a digital camera. Molecular weight of the DNA bands was calculated by comparing with Lambda DNA Hind III digest (Bangloregenei) as standard marker.

III. RESULTS

Out of the 150 bacteria isolated from clinical specimens, 66 (44%) were identified to be S*taphylococcus species* (Table 1). Only 23 (34.8%) were identified biochemically to be S*taphylococcus aureus*, out of this, 52.2% were recovered from urine specimens, 30.4% wounds swab, 13.0% ear swab and 4.3% from nasal swab. (Figure.1) *Staphylococcus aureus* is the leading cause of gram positive bacterial infections and produces a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. A prevalence rate of 30.4% was recorded for MRSA. Although this prevalence was higher than 20.3% reported by Ghebremedhin *et al*,(2009) in Ibadan and 12.5% reported by Okon *et al*., (2011) in Maiduguri, it was however lower than that reported in Ilorin (34.7%) and Jos (43%) both in Nigeria (Ikeh, 2003;Taiwo *et al.*, 2004).Of the 23 *Staphylococcus aureus*, 7(*30.4%*) were MRSA as indicated by their resistance to oxacillin and 16(69.6%) were MSSA as indicated by their sensitivity to oxacillin (Table 2). 71.4% of the MRSA were recovered from urine and 28.6% from wound swab (Figure 2). The different prevalence results obtained from several studies indicated that the prevalence of MRSA varies from one area to another. It is clear that MRSA has become a global nosocomial pathogen with attendant therapeutic problems and warrant urgent infection awareness, considering the common practice of unregulated sale of antimicrobial agent and movement of people which may be agent necessary for rapid dissemination (Okon, Basset, Uba, Oyawoye, Yusuf, Shittu and Blanc, 2011).The result of antibiotic susceptibility of methicillin resistant isolates to other antibiotics is shown in (Table 3). The results show high resistance to Amoxicillin (100%), Augumentin (85.7%), Cotrimoxazole (71.4%) and less resistance rate was observe against Gentamycin (15.1%), Tetracycline (57.1%), Erythromycin (42.9%) ofloxacin (42.9%) and Ciprofloxacin (28.5%). The prevalence of methicillin resistance associated with urinary tract infections was 71.4% and 28.6% among skin and soft tissue infections. These difference might be due to prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stays, resulting in enhanced selection pressure. This reflects the fact that critically ill patients have a greater chance of becoming colonized or infected. (Rubeena, Chughtai and Aslam, 2004)Table 4 shows the multidrug resistance pattern of isolates. Multidrug resistance is determined by the resistance of an isolates to 4 or more antibiotic. The highest resistance was found in isolates 3 (42.9%) with resistant to 4 antibiotics.

In Table 5 it was shown that out of the 7 MRSA isolates only 5 harbor single plasmid of molecular weight 25.0 kbp. The plasmid profile of MRSA isolates is as shown in Plate 1. MRSA isolated show high resistance to Amoxicillin and Augumentin, which support the findings, that MRSA strains are equally resistant to all β-lactam antibiotics (Weems, 2001; Gross – Schulman, Dassey, Mascola and Anaya, 1998) which may be due to the presence of chromosomal mecA gene that specifies the production of an abnormal penicillin binding protein (PBP) which has low affinity for binding β-lactam antibiotics. There is a high susceptibility to Ofloxacin and ciprofloxacin in this study. This may be due to the absence of resistance conferring genes in these MRSA strains as reported by (Polyzou *et al*., 2001). The observed high MRSA susceptibility to Ofloxacin and Ciprofloxacin in this study support some previous reports (Fridkin, Hagema, Morrison, Sanza, Como-Sabetti, Jernigan, Harriman, Harrison, Lynfield and Farley, 2005; Nordmann and Nass,2005).Thus, the existence of MRSA susceptible to these non β-lactam antibiotics may provide an opportunity for the recommendation of these drugs for empirical treatment.

Table 1: Distribution of isolates obtained from clinical specimen

Isolates	**Number of isolates (n=150)**	**Percentage (%)**
Proteus spp	8	5.3
Pseudomonas spp	4	2.7
E. coli	48	32
Klebsiella spp	24	16
Staphylococcus aureus	23	15.3
Other Staphlococcus spp	43	28.7
Total	**150**	**100.0**

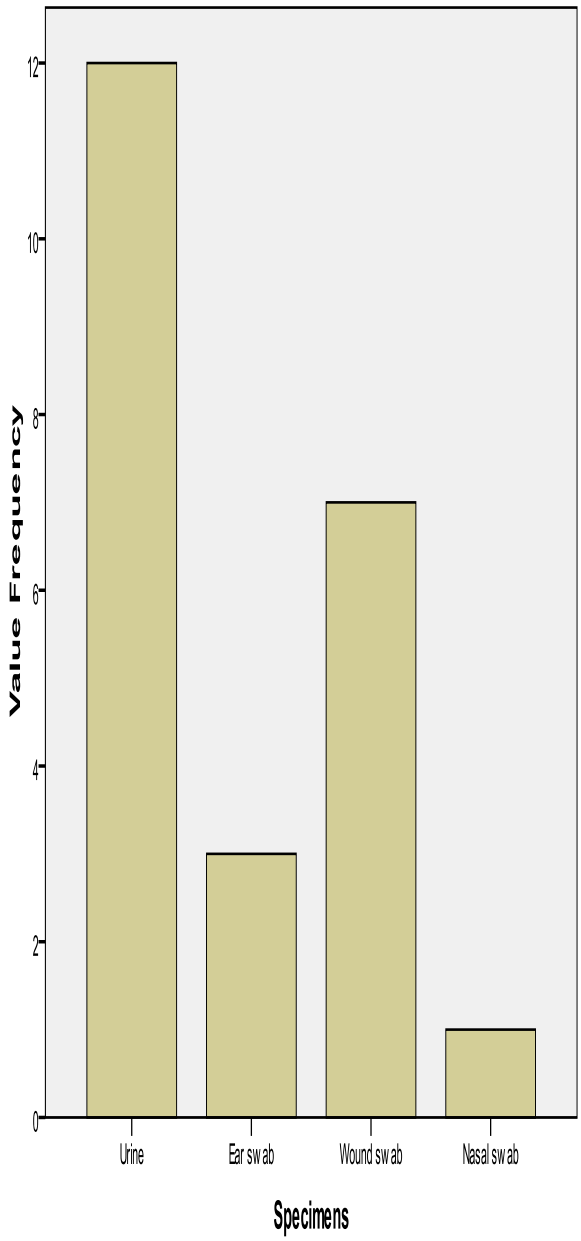

Figure 1: Bar graph showing specimens distribution of *Staphylococcus aureus* as obtained from the different clinical specimens.

Table 2: Prevalence of MRSA and MSSA

	Resistant pattern to oxacilin	
Bacterial isolates	**No resistance**	**% resistance**
MRSA	7	30.4
MSSA	16	69.6%
Total	23	100.0%

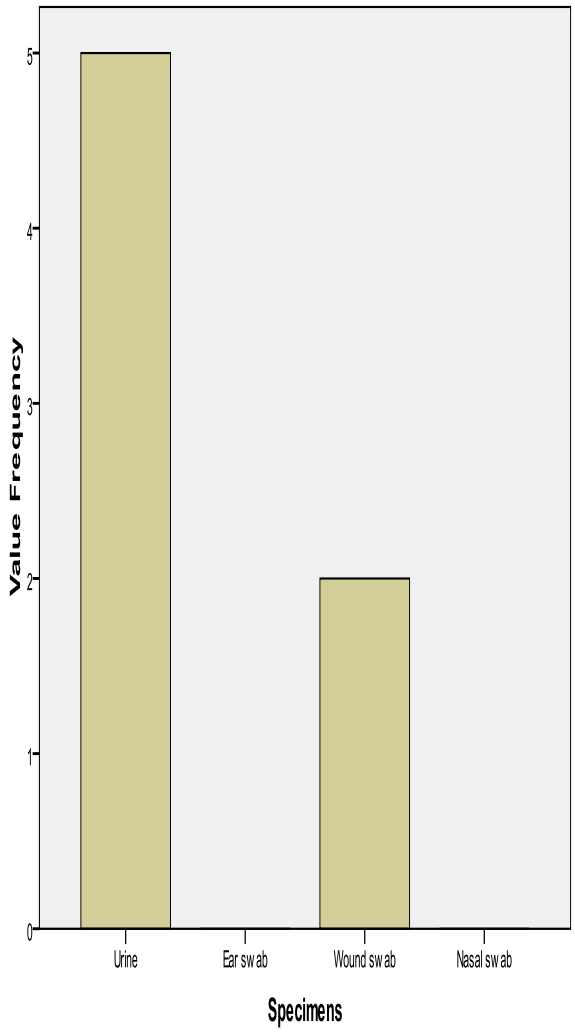

Figure 2: Bar graph showing specimens distribution of MRSA isolates

Table 3:Antimicrobial resistance pattern of MRSA obtained from clinical specimen

Antibiotics	**Antibiotics resistance pattern of bacterial isolates**	
	Resistance No (%)	**Resistance (%)**
Amoxicillin (AMX)	7	(100%)
Cotrimoxazole (COT)		
Gentamycin (GEN)	5	(71.4%)
Augumentin (AGU)		
Tetracycline (TET)	3	(42.9%)
Ciprofloxacin (CIP)		
Erythromycin (ERY)	6	(85.7%)
Ofloxacin (OFL)		
	4	(57.1%)
Oxacillin(OX)		
	2	(28.5%)
	4	(57.1%)
	3	(28.9%)
	7	(100%)

Table 4: Multi-drug resistance profile of Isolates from clinical isolates

Antimicrobial agent	Frequency of multi-drug resistance number of Resistance isolates (n) = 7	Percentage Resistance (%)
Resistant to 1 agent	0	0
Resistant to 2 agents	0	0
Resistant to 3 agents	1	14.3
Resistant to 4 agents	3	42.9
Resistant to 5 agents	1	14.3
Resistant to 6 agents	2	28.6
Resistant to 7 agents	0	0
Resistant to 8 agents	0	0

Table 5: Plasmid profile of MRSA

Isolates ID	Plasmid
13	Present
98	Present
105	Present
86	Present
37	Present

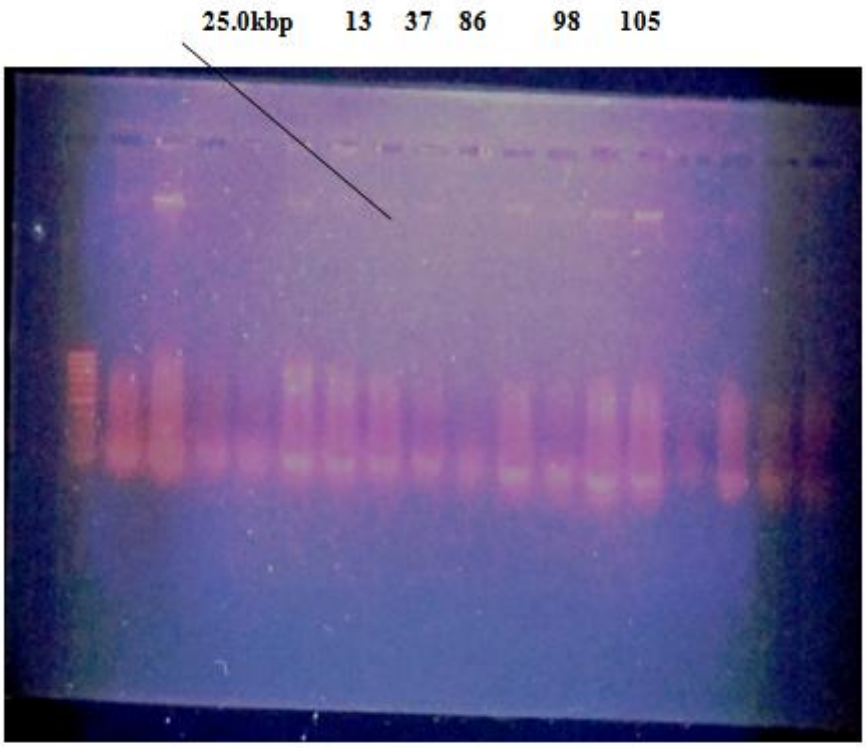

Plate 1: showing plasmid profile of MRSA isolates

IV. CONCLUSION AND RECOMMENDATION

In this study, the prevalence of MRSA may be considered to be high to warrant urgent infection awareness. The high resistance to the β-lactam used in this study shows that these agents are becoming ineffective in the treatment of isolates from clinical specimen, while the high susceptibility to some non β – lactam antibiotics in this study may provide an opportunity for the recommendation of these drugs for empirical treatment. Also, results from the study show the need to reassess policies on antibiotic use within the hospital environment. More research and monitoring of trend of nosocomial infection with MRSA required. Empirical treatment of MRSA infection should be based on prior determination of local resistance patterns. Provision and adequate implementation of educational programs on hospital community and personal hygiene.

REFERENCES

[1] Anah M.U., Udo J.J., Ochigbo S.O., Abia-Bassey L.N. (2008). Neonatal septicemia in Calabar, *Nigeria. Trop. Doct*.38: 126-128.

[2] Adesida S.H., Boelens B., Kehinde A., Babajide B., and Snijders S. (2005). Major epidemic clones of *Staphylococcus aureus* in Nigeria. *Microb. Drug Resist*, 11: 115-121.

[3] Adeleke S.I., Asani M.O. (2009). Urinary tract infection in children with nephritic syndrome in Kano, Nigeria. *Ann. Afr. Med*.8:38-41.

[4] Anupurba S. Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM (2003). Prevelance of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern uettar Pradesh *Indian. J. Med. Microbiol* 21: 49-51.

[5] Ayliffe, A. G., (1997). The progressive intercontinental spread of methicillin resistant *Staphylococcus auerus. Clin infect. Dis* 24: 574-579.

[6] Azees, O. A., utsalo, S. J., Epoke, J. (2008). Distributions and antibiotic susceptibility pattern of methicillin – resistant *Staphylococcus aureus* isolates in a University Teaching Hospital in Nigeria. *Sahel Med. J*. 11(4): 142-147.

[7] Bannerman, T. I; P. R. Murray, E. I. Baron, I. H. Jorgensen, M. A. pFaller and R. H. Yolken (2003). *Staphylococcus, Micrococcus* and other catalase – positive cocci that grow aerobically in: *Manual of Clinical Microbiology*. Yolkeneds 8th edition, *ASM Press Washington DC* pp. 384-404.

[8] Becker, K., Friedrich, A. W., Lubritz, G., Weilert, M., Peters, G., Von Eiff, (2003). C. pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J. Clin microbial*. 41 (4): 1434-1439.Matinez, J. I., F. (2000). Mutation frequencies in antibiotic resistance *Antimicrob, Agent Chemother* 44: 1771-1777.

[9] Bell J. M., J. D. Turnidge and Sentry Apac participants, (2002). High prevalence of oxacillin – resistant staphylococcus aureus isolates from hospitalized patients in Asia – pacific and South Africa Results from SENTRY. Antimicrobial surveillance program, 1998-1999. *Antimicrob. Agents Chemother*., 46: 897-881.

[10] Birnboim H.C., and Doly J. (1979) 'A rapid alkaline extraction procedure for screening recombinant plasmid DNA'. *Nucleic Acids Research* 7:1513-1523.

[11] Bekibele C.O., Kehinde A.O., Ajayi B.G (2009).Upper lid skin bacterial count of surgical eye patients in Ibadan, Nigeria. *Afr. J. Med. Med. Sci*.37:273-277.

[12] Bratu. S., Erarno, A. Kopec, R., Coughlin, E., Ghitan, M., Yost R., Chanprnick, E. K., Landman, D., Wuale, J. (2005) Community associated methicillin – resistant *Staphylococcus aureus* in hospital nursery and maternity units. *Emerg. Dis*. 11 (6): 808-813.

[13] Carbon, C; (1999).Costs of treating infections caused by methicillin resistant staphylococci and vancomycin – resistant enterococci *J. Antimicrob, Chemother*. 44: 31-36.

[14] Cowan, ST and Steel, KJ (1993). Enterobacteriacea, In .G. I. Barrow, R.K.A. Felthan, (Eds) *Manual of for the identification of Medical Bacteria* (3rd Edition), Cambridge University Press, United Kingdom, 213-218.

[15] Chamber, HF (1979). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implication. *Clin. Microbial*. Rev. 10: 781-791.

[16] Chambers, H. E. (1997). Methicillin resistant in Staphylococci molecular and biochemical basis and clinical implications *Clin Microbial. Rev*. 10: 781-791.

[17] Cheesbrough Monica (2000). *District Laboratory Practice in Tropical Countries*. Part II. 2nd Edition. Cambridge University press 157-158.

[18] Chambers, H. F., (2001). The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect. Dis* 7: 178-182.

[19] Diekema, D. J., M, Pfaller,M A Schmitz, F.J, Smayevsky J and Bell J (2001). Survey of infections due to staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western pacific region for the SENTRY antimicrobial surveillance program, 1997-1999. *Clin. Infect. Dis*., 32: S114-S132.

[20] Duguid I. P. Staphylococcus cluster – forming Gram – positive cocci In: Mackie and Macarthney Practical Medical Microbiology J. G. Collee, J. P. Duguid, A. G. Fraser B. P. Marnioneds 13th the edition (1989). Church: II living stone London Pp 303-316.

[21] Frazee, B. W., Lynn, J, Charlebois, E. D., Lambert, L, Lowery, D; Perdreau Remington, F. (2005).High prevalence of methicillin - resistant *staphylococcus aureus* in emergency department skin and soft tissue infection. *Ann. Emerg. Med*. 45 (3); 311-320.

[22] Fridkin SK, Hageman JC, Morrison, M, Sanza LT, Como-Sabetti, K, Jernigan JA, Harriman K, Harrison LH, Lynfield R, Farley MM (2005). Methicillin-resistant *Staphylococcus aureus* disease in three communities. *N. Engl. J. Med*. 352: 1436-1444.

[23] Gottilebs, G. S; Fowler, V. G. Jr., Kong, I. K. (2000).*Staphylococcus auerus* bacteremia in the surgical patients a prospective analysis of 73 post opernative patients who developed *staphylococcus aureus* bactermia at a tertiary care facility. *J. Ann. Collsurg* 190: 50-57.

[24] Grafunder E M., Veriezia, R. A.; (2002). Risk factors associated with nosocomial methicillin – resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobial. *J. Antimicrob chemother* 49: 999-1005.

[25] Gross – Schulman S., Dassey D, Mascola L. Anaya C. (1998). Community-acquired methicillin resistance *Staphylococcus aureus. JAMA* 280: 421-422.

[26] 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. *J. Clin. Microbiol*. 47: 4740-4744.

[27] Hackbarth, C. J, Kacagoz, T., Kacogoz, S., Chambers, H. F. (1995). Point mutations in *Staphylococcus aureus* PBP 2 gene affect penicillin binding kinetics and are associated with resistance. *Antimicrob agents.Chemother*, 30: 103-106.

[28] Holloway, K. (2000). Antimicrobial resistance: the facts. Essential Drug Monitor WHO 28 & 29:7-8.

[29] Hedin G., Fang, H. (2003). Rapid screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time PCR assay. *J. Clin. Microbiol* 41(7): 2894-2899.

[30] Hart, C.A and Kariuki, S (1998). Antimicrobial resistance in developing countries. *BMJ* 317:647-650.

[31] Ikeh, E. I. (2003). Methicillin – Resistant *Staphylococcus aureus* (MRSA) at Jos teaching hospital *Afr, J. Clin. Exptl. Microbial* 4(1): 52-55.

[32] Jevons, M. P., (1961). "Celbarin" – resistant Staphylococci *S. Med J* 1: 124-125.

[33] Kesah, C., Rdjeb, B., Odugbemi, T. (2003). Prevalence of methicillin – resistant *Staphylococcus aureus* in eight African hospitals and *malta Clin Microbial, Infect*, 9(2) 153-156.

[34] Kopp, BJ, Mix, DE, Armstrong, EP (2004).Clinical and economic analysis of methicillin – susceptible and resistant *Staphylococcus aureus* infections. *Ann. Pharmacother*. 38: 1377-1382.

[35] Kuehnert, M. J., Hill, H. A., Kupronis, B. A. (2005). Methicillin resistant *Staphylococcus aureus* hospitalization, United States, *Emerg. Infect. Dis.* 11(6): 868-872.

[36] Lelievre, H.,, Lina G, Jones, ME, Olive,, C. Forey F, Roussel – Delvallez, M, Nicholas Chanoine, M, Bebear CM, Jarlier V, Andremont A, Vandensech F, Etienne J. (1999). Emergence and spread in French hospitals of methicillin – resistant staphylococcus aureus with increasing susceptibility to gentamicin and other antibiotics. *Journal Clin. Microbiol*. 37: 3452-3457.

[37] Lodise, TP, McKinnon, P S (2005). Clinical and economic impact of methicillin resistance in patients with *Staphyloccus aureus* bactermia. Diagnosis. *Microbial, Infect. Dis*. 52: 113-122.

[38] Lowy, FD (1998), *Staphylococcus aureus* infection. *N. Engl. J. med*. 339 520-532.

[39] Lyon, B., Skurray, R. (1978). Antimicrobial resistance of *Staphylococcus aureus*: genetic basis Microbial 51:88-134.

[40] Mangeney, N., Bakkauch, A, Pons, J. L., Dupeyon, C., Niel, P., Lehan, G. (1995). Methicillin – resistant *Staphylococcus aureus* subtyping: interest of combined antibiotyping and esterase electrophoretic typing *J, Appl. Bacteriol*. 79: 347-351.

[41] Maranan, M.C., Moreira, B., Boyle Vavra, S., Daum R. S. (1997). Antimicrobial resistance in Staphylococcus epidemiology, molecular mechanisms and clinical relevance. *Inf. Dis. Clin*. 11: 813-849.

[42] McDougal, L. K., Steward, C. D., Killgore, C. F (2003). Pulse field gel electrophoresis typing of oxacillin – resistant *Staphylococcus aureus* isolates from the United State establishing a national database. *J. Clin. Microbial*, 41: 5113-5120.

[43] Moran, G. J., Amii, R. N., Abrahaman, F. M.., Talan, D. A. (2005). Methicillin – resistant *Staphylococcus aureus* in community acquired skin infections – *Emerg infect. Dis* 11 (11): 928-930.

[44] Matinez, J.I. (2000). Mutation Frequencies in antibiotic resistance. *Antimicrob Agents Chemother*. 44:1771-1777.

[45] Mulvey, M. R., MacDouglall, I., Cholin, B (2005) community associated methicillin resistant *Staphylococcus aureus*, Canada *Emerg, Infect. Dis*, 11(6): 844-850.

[46] Musa, H. A., Shears, P. Khagali, A. (1999). First report of methicillin resistant *Staphylococcus aureus* from hospitalized patients in Sudan. *J. Hosp. Infect.* 42(1): 74-75.

[47] Mylotte, J. M., Tayara, A. (2000). *Staphylococcus aureus* bacteremia predictors of 30-day mortality in a large cohort *Clin Infect. Dis* 31: 1170-1174.

[48] Nixon M, Jackson B, Varghese P, Jenkins D, Taylor G. (2006), methicillin – resistant *Staphylococcus aureus* on orthopedics wards: incidence, spread, mortality, cost and control. *J. Bone Joint surg. Br*. 88: 812-817.

[49] National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing(2005). 12th informational supplement.M100-S12.Wayne, PA: National Committee for Clinical Laboratory Standard.

[50] Nwankwo, EoK; Abdulhadi, S.; Magagi, A and Ihesiulor, G. (2010) Methicillin resistant *S. aureus* (MRSA) and their antibiotic sensitivity pattern in Kano, Nigeria. *Africa Journal of Clinical and Experimental Microbiology* 11(i): 129-136.

[51] Nordmann P., Naas T. (2005). Transmission of methicillin-resistance *Staphylococcus aureus* in a university hospital. *Afri. Clin. Exper. Microbiol* 6:46-52.

[52] Okon, K.O., Basset, A., Uba, P., Oyawoye, O.M., Yusuf, Z.I., Shittu, A.O., and Blanc, J. (2011). Epidemiology and characteristic pattern of Methicillin-Resistance *Staphylococcus aureus* Recovered from Tertiary Hospital in Northeastern, Nigeria. *International Journal of Tropical Medicine* 6(5): 106-112.

[53] Onipede, A.O., Onayade, A.A., Elusiyan, J.B., Obiajuwon, P.O., Ogundare, E.O., Olaniran, O.O., Adeyemi, L.A., Oyelami, O.O. (2009).Invasive bacterial isolates from children with severe infections in a Nigeria hospital. *J. Infect. Dev. Ctries*. 2:429-436.

[54] Odedoyin W.B., Aboderin A.O., Ikem R.T., Kolawole B.A., Oyelese A.O. (2008). Asymptomatic bacteriuria in patients with diabetes mellitus in Ile-Ife, South-West, Nigeria. *East Afr. Med.J*.85:18-23.

[55] Peacock, J.E., Jr. Marsik, F.I, Wenzel, R.P. (1980). Methicillin – resistant *Staphylococcus aureus* introduction and spread within a hospital *AunInterm Med*. 93: 526-532.

[56] Polyzou A, Slavakis A, Pournaras S (2001). Predominance of a methicillin resistant *Staphylococcus aureus* clone susceptible to erythromycin and several other non-β-lactan antibiotics in a Greek hospital. *J. Antimicrob Chemother* 48: 231-234

[57] Pournaras S, Slavakis A, Polyzou A, ManitisAnl, Sofianou D, Tsakris A. (2001). Nosocomial spread of an unusual methicillin – resistance *Staphylococcus aureus* clone that is sensitive to non-β-lactam antibiotics, including tobramycin. *J. Cclin. Microbiol*. 39: 779-781.

[58] Rasheed, J.K.; Tenover, F.E. P.R. Murray., E.J. Yolken (2003). Detection and characterization of antimicrobial resistance genes in bacteria In: *Manual of Clinical Microbiology*. Eds, 8thedtn.ASM press, Washington DC pp. 1197-1212.

[59] Rello, J., Diaz, E. (2003). Pneumonia in the intensive care unit. *Crt. Care. Med*. 37: 2544-2551.

[60] Rice, L. B., Sahm, D., Bonomo, R. A., P. R. Murray, E. J. Baron, J. H. Jorgenesen, M. A. PFaller, R. H. Yolken (2003). Mechanisms of resistance to antimicrobial agent In. *Manual of Clinical Microbiology* 8thEdtn.Asm press, Washington DC. Pp 1674-1101.

[61] Rubeena Hafeez, Chughtai A.S and Aslam M. (2004). Prevalence and antimicrobial susceptibility of methicillin resistance *Staphylococcus aureus* (MRSA). *International Journal of Pathology*; 2(1):10-15

[62] Standing medical advisory committee (1998). Sub group on Antimicrobial Resistance. The path of least resistance. London: Department of Health.

[63] Stevens D. D. (2004). Optimizing outcomes in methicillin resistant *Staphylococcus aureus* infections focus on nosocomial pneumonia and SSTI, highlights from a satellite symposium at the 1th Annual International Congress on Infectious Disease (ICID), Cancun, Mexico, pp1-8.

[64] Said-Salim B., Mathema B., Kreiwirth B.M. (2003). Community acquired methicillin-resistant *Staphylococcus aureus* an emerging pathogen .Infection *Contr. Hosp. Epidemiol* 24(6):451-455.

[65] Shittu, A.O., Lin, J. (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. *BMC Infect. Dis*. 6(1):125.

[66] Taiwo, S.S., Onile B.A., Akanbi, A. A., (2004). Methicillin -resistant *Staphylococcus aureus* (MRSA) isolates in Nigeria. *Afr. J. Clin. Exptl. Microbiol*. 5(2): 189-197.
[67] Voss, A and Doebbeling, D.N, (1985).The worldwide prevalence of methicillin resistant *S. aureus*. *Int. J. Antimicrob. Agents* 5: 101-106.
[68] Weems, J. J. (2001).The many faces of *Staphylococcus aureus* infections. *Postgraduate Med*. 110(4): 24-36.

Appendix
Interpretative Chart of Zone Size Values

Antimicrobial Agent	Disk potency	Suscep - tibility	Interme -diate	Resis- tance
Cefotaxime	30µg	≥23	15-22	≤14
Cetazidime	30µg	≥18	15-17	≤14
Ceftriazone	30µg	≥35	-	-
Ofloxacin	5µg	≥21	16-20	≤15
Gentamicin	10µg	≥15	13-14	≤12
Tetracycline	30µg	≥17	15-18	≤14
Trimethroprim + sulfamethoxazole	12.5µg+ 23.75µg	≥16	11-15	≤10
Amoxicilin	25µg			
Ciprofloxacin	5µg	≥21	16-20	≤15
Perfloxacin	5µg	≥21	16-20	≤15
Augumentin	30µg			