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Full Length Research Paper

Comparative protein profiling of antibiotic resistant *S. aureus* strains from hospital and community- associated infections

***[1]Torimiro N, [1]Ako-Nai AK and [2]Omitogun OG**

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria
[2]Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Nigeria

ABSTRACT

The study compared antibiotic resistance pattern of community and hospital associated *Staphylococcus aureus* strains in order to evaluate the relationship between the strains. The strains were identified from a total of 150 specimens collected from human subjects using standard bacteriological methods. The antibiotic susceptibility pattern was determined using the disk diffusion plating method. The whole cell protein profile was determined using 10% sodium dodecylsulphate-polyacrylamide gel electrophoresis. A total of 107(71.3%) *S. aureus* isolates were identified. In all, 67 (62.6%) of the isolates were cultured from the hospital while 40 (37.4%) of the isolates were cultured from the community. The incidence of antibiotics resistance was high in both settings. But 9 of the strains were resistant to methicillin/oxacillin. The protein profiling of the methicillin resistant strains, showed that 2 strains from the community clustered in the hospital phenon. Dissemination of resistant strains appears to be based mainly on clonal spread; indiscriminate use of anti-infective agents is secondary. Hence, effective infection control measures are important.

Keywords: *Staphylococcus aureus,* antibiotic, methicillin, resistance, protein, electrophoresis.

INTRODUCTION

Staphylococcus aureus is a pathogen that is specifically responsible for many human diseases ranging from soft tissue to bloodstream infections (Ako-Nai et al., 1991; Diekema et al., 2001; Torimiro et al., 2005). The organism is an important cause of nosocomial infections. It has been reported that hospitalized patients with *S. aureus* infection have a high risk of mortality as compared with hospitalized patients without the infection (Noskin et al., 2005). *S. aureus* has been reported to be increasingly difficult to treat because of their multiple resistances to anti-infective agents (Archer and Climo, 1994; Chopra, 2003; Diekema et al., 2001; Lowy, 2003; Deurenberg and Stobberingh, 2008). Worthy of note is that resistance to methicillin is often linked to resistance to several other unrelated groups of antibiotics thereby limiting choice for treatment (Lowy, 2003; Woodford and Livermore, 2009). Methicillin resistant *S. aureus* (MRSA) strain is primarily a nosocomial pathogen whereas most out-patients are infected with methicillin susceptible *S. aureus* strains (Woodford and Livermore, 2009).

The epidemiology of Methicillin-resistance *S. aureus* (MRSA) has changed with the apparent emergence of community-associated MRSA with no risk factors for hospital- associated methicillin resistant *S. aureus* (HAMRSA)(Lowy, 2003; Shukla 2005). It has also been reported that there is an increasing emergence of health care associated infections caused by *S. aureus* with molecular characteristics of community-associated methicillin resistant *S. aureus* (CAMRSA) (Klein et al., 2007; Maree et al., 2007) which may suggest a transmission of CAMRSA in the hospital (Gonzalez, 2006). The replacement of the traditional HAMRSA strains with CAMRSA has been observed to be supported by the characteristics of CAMRSA that

*Corresponding Author's Email: ntorimiro@yahoo.com; ntorimiro@oauife.edu.ng

includes a rapid growth rate which provide it with a competitive advantage over HAMRSA (Baba et al., 2002). Reports have shown that the compromised hospital infection control practices in many hospitals in developing countries often result in dissemination of nosocomial pathogens disseminated to the community (Meers, 1988; Okello et al., 1997). Okeke et al. (1999) have suggested the susceptibility pattern of the community strains can be influenced by these practices, apart from the indiscriminate use of antibiotics.

There are reports on antibiotic resistance and epidemiology of *S. aureus* in Ile-Ife. However, there is a paucity of information on the comparison of hospital-associated and community-associated antibiotic resistant *S. aureus* strains using protein profiling technique. This present study aims to determine the possible relationship between the hospital and community-associated antibiotic resistant *S. aureus* strains isolated from various specimens using antibiotyping and the sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) of the whole cell protein.

MATERIALS AND METHODS

Specimens collection and Identification of the isolates

Permission for participation was voluntarily obtained from patients according to the Institution's ethics committee guideline of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife, Nigeria. Purulent specimens from eyes, ears, wounds and other soft tissue infections were collected from 150 patients using sterile cotton-tipped applicators (Evepon, Nigeria). The specimens were immediately transported to the laboratory after collection, aseptically introduced into culture bottles containing freshly prepared nutrient broth (Oxoid, UK) and incubated at 35°C for 18 h. Using established bacteriological protocols, isolates that were Gram positive cocci arranged in clusters, catalase positive and tube coagulase positive were identified as *S. aureus* (Cheesebrough, 1991). Based on questionnaire responses, the *S. aureus* isolates were classified as either hospital and community associated when period of hospitalization is greater than 48 h or less than 48 h respectively (Wylie and Norwicki 2005).

Antibiotic susceptibility typing of the isolates

The antibiotics susceptibility disk diffusion testing was carried out as described by Kirby and Bauer (1966). Methicillin resistance was determined using a 1µg oxacillin. Susceptibility to amoxicillin (25µg), chloramphenicol (30µg), cotrimoxazole (25µg), gentamicin (10µg), erythromycin (5µg), tetracycline (10µg), ofloxacin (5µg), streptomycin (10µg) ceftriaxone (30µg), pefloxacin (5µg) and ciprofloxacin (10µg) were also determined. Typed culture *S. aureus* ATCC 25923 and ATCC 4330 were included as control. The inoculums of the test and reference strains were adjusted to 0.5 McFarland standard (10^7 CFU/ml). Multi-resistance was defined as resistance to more than two classes of antibiotics.

SDS-PAGE ANALYSIS

Preparation of inoculum

The representative strains of *S. aureus* isolates representing multiple antibiotic resistance pattern and the MRSA strains were streaked on nutrient agar plates and incubated at 37°C for 24 h. Four colonies from these plates were inoculated into 20ml of nutrient broth and incubated at 37°C for 18 h.

Extraction of whole cell protein from *S. aureus* isolates

A modified method of Saçilik et al. (2000) was employed. The cells were harvested by spinning the culture broth for 3 min at 12100rpm (Beckman, USA). The pellets were washed three times with sterile distilled water. Sodium dodecyl sulphate (SDS) sample buffer containing 0.06M Tris, 2.5% glycerol, 0.5% SDS, 1.25% 2-mercaptoethanol and bromophenol blue 0.001% (w/v) were added to the pellets and stirred. Protein samples were prepared for PAGE at a proportion of one part sample to three parts of sample buffer. Thereafter, the proteins were denatured at 95°C for 5 min in a water bath. The solutions were centrifuged at 12100rpm for 3 min and the supernatants were collected in eppendorf tubes and stored at –4°C until the electrophoresis process was carried out.

Estimation of protein concentration

The protein concentration was estimated by the method of Schacterk and Pollack (1973). 0.5ml of alkaline copper reagent comprising 0.5N NaOH, 10% $NaCO_3$, 0.1% Sodium/Potassium (Na/K) tartarate and 0.05% $CuSO_4.5H_2O$ were mixed with 0.5 ml of the sample in a test tube and allowed to stand for 10 min. Thereafter, 2ml of Folins reagent was added rapidly and forcefully. The test tubes were placed in a water bath at 55°C for 5 min and allowed to cool rapidly in water. The absorbance was read at 650nm using a spectrophotometer (Cintral, Australia). Bovine serum albumin (BSA) stock at 0.25mg/ml stock was used as the standard. The protein concentration was adjusted using distilled water to give a final concentration of 0.12mg/ml for all the samples.

Table 1. Antibiotic resistance patterns of *S. aureus* isolates from hospital and community sources in Ile-Ife, Nigeria

Antibiotics	Number (%) of resistant isolates from		Total (%) (n = 107)
	Community n= 40 (%)	Hospital n = 67 (%)	
Amoxicillin	29 (72.5)	49 (73.1)	78 (72.9)
Ceftriaxone	30 (75)	52 (77.6)	82 (76.6)
Chloramphenicol	10 (25)	8 (11.9)	18 (16.8)
Ciprofloxacin	0 (0)	5 (7.5)	5 (4.7)
Cotrimoxazole	15 (37.5)	29 (43.3)	44 (41.1)
Erythromycin	14 (35)	22 (32.8)	36 (33.7)
Gentamicin	7 (17.5)	31 (46.1)	38 (35.5)
Ofloxacin	1 (2.5)	4 (6.0)	5 (4.7)
Oxacillin	5 (12.5)	4 (5.97)	9 (8.4)
Pefloxacin	2 (5.0)	4 (6.0)	6 (5.6)
Streptomycin	26 (65.0)	37 (52.2)	63 (58.9)
Tetracycline	24 (60)	46 (66.7)	70 (65.4)

Electrophoretic procedure

The protein profile of all the *S. aureus* isolates was observed using the SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) discontinous buffer system of Laemmli (1970). Electrophoresis was performed with a discontinuous buffer system. A dual vertical slab (Bio-Rad Mini-Protean –3) with the specification- 16 cm (Length) by 12 cm (Width) by 18 cm (Height) was cast to allow for 10mm of stacking gel, to give a final polyacylamide content of 10% of the separating gel. The stacking gel was prepared from the stock solution that was used for the separation gel to give a polyacylamide content of 4%. The gel was allowed to polymerise for 1hr and the gel surface was rinsed completely with distilled water. A volume of 10µl low range molecular weight marker (BioRad, USA) was loaded in the first lane of the gel and the protein extracts on the other lanes. The electrophoresis was carried out for 1hr at a constant current of 35mA and 100V until the bromophenol blue dye migrated to the bottom.

Photography of protein band patterns

After the process of electrophoresis, the gels were removed carefully and stained overnight in a solution containing 0.1% Coomassie blue R250, 40% methanol and 10% glacial acetic acid in water. The visualisation of the protein bands required the removal of excess dye from the gels and this was done by destaining the gels in 10% glacial acetic acid, 40% distilled water and 40% methanol until the background was clear. Afterwards, the gels were scanned using a Hewlett Packard scanner.

Computation of similarity

The positions of unequivocally scorable protein bands were transformed into binary character matrix (“1” for the presence and “0” for the absence of a band at a particular position). The similarity of the scorable protein bands were expressed as the Dice coefficient correlation, which was converted for convenience to a percentage value. Strains were clustered by the unweighted pair-group method arithmetic average (UPGMA), which was compiled by the PAST Data Software Package (Hammer *et al.,* 2001).

RESULTS

Identification and antibiotic resistant pattern of the *S. aureus* isolates

A total of 67 (62.6%) from the hospital and 40 (37.4%) from the community *S. aureus* isolates were identified. The antibiotic resistance pattern of *S. aureus* isolates cultured from hospital and community sources is shown in Table1.

The electrophoretic protein profiling of the *S. aureus* isolates

The whole cell proteins of all the MRSA isolates obtained were analysed. In the case of the methicillin sensitive strains from both sources, one representative strain that exhibited multi resistance from 3 to 7 classes of antibiotics were used to carry out the electrophoretic

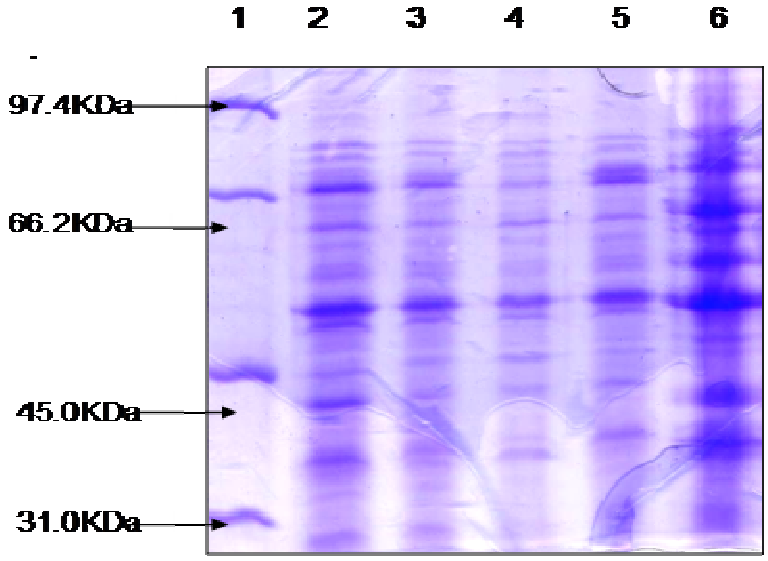

Figure1a. Electrophoretic whole cell protein profiles of community associated methicillin resistant *Staphylococcus aureus*. Lane 1: Molecular size marker; Lane 2: CAMRSA1; Lane 3 CAMRSA2; Lane 4: CAMRSA3; Lane 5: CAMRSA4; Lane 6: CAMRSA5.

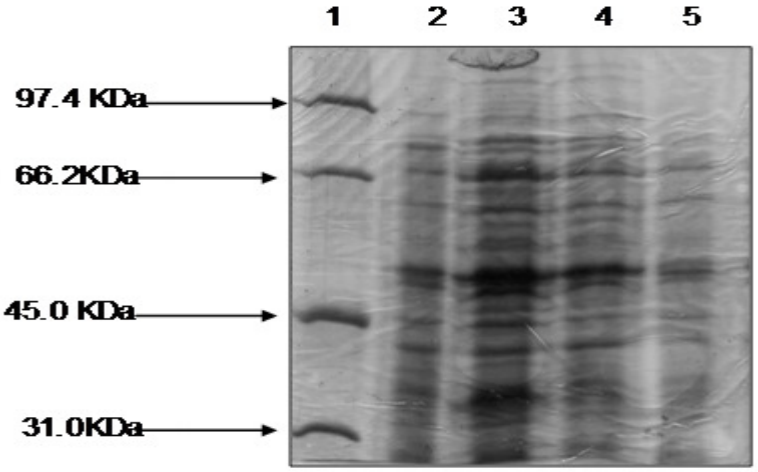

Figure 1b. Electrophoretic whole cell protein profiles of hospital associated methicillin resistant *Staphylococcus aureus*. Lane 1: Molecular size marker; Lane 2: HAMRSA1; Lane 3: HAMRSA2; Lane 4: HAMRSA3; Lane 5: HAMRSA4.

analysis of the whole cell proteins. The number of bands observed in the four phenons showed protein patterns that contained 22-30 discrete bands. The isolates and their corresponding patterns are shown in Figures 1a, 1b, 2a and 2b. The bovine serum albumin standard curve was generated by plotting the log of the molecular weight of low range standards versus the relative mobility (Rf).

The cluster analysis of the protein bands

The cluster analysis of the protein markers data as shown (Figure 3) placed the isolates into four major phenons which included CAMRSA, HAMRSA, hospital associated methicillin sensitive *S. aureus* (HAMSSA) and community associated methicillin *S. aureus* (CAMSSA).

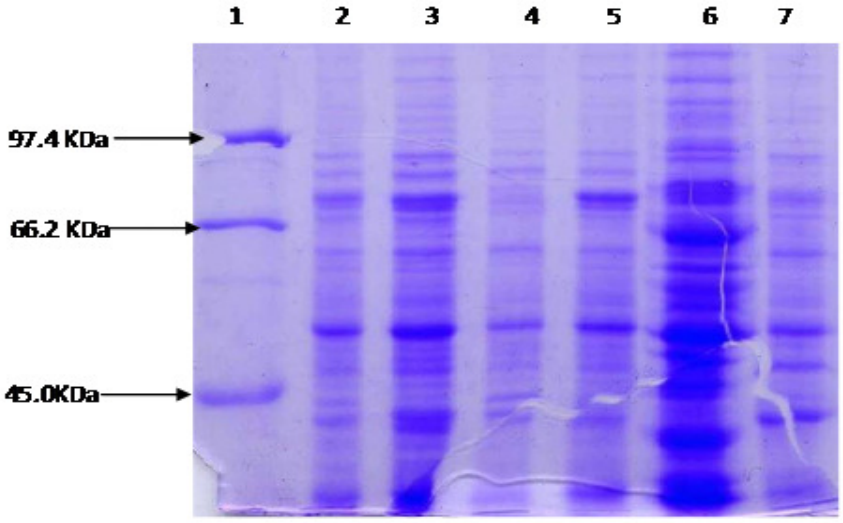

Figure 2a. Electrophoretic whole cell protein profiles of community associated methicillin sensitive *Staphylococcus aureus*. Lane 1: Molecular size marker; Lane 2: CAMSSA1; Lane 3: CAMSSA2; Lane 4: CAMSSA3; Lane 5: CAMSSA4; Lane 6: CAMSSA5; Lane 7: CAMSSA6

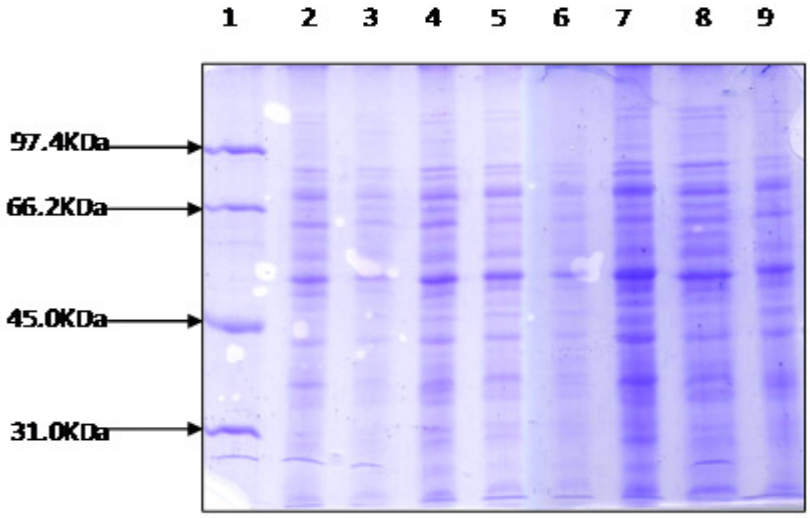

Figure 2b. Electrophoretic whole cell protein profiles of hospital associated methicillin sensitive *Staphylococcus aureus*. Lane 1: Molecular size marker; Lane 2: HAMSSA1; Lane 3: HAMSSA2; Lane 4: HAMSSA3; Lane 5: HAMSSA4; Lane 6: HAMSSA5; Lane 7: HAMSSA6; Lane 8: HAMSSA7; Lane 9: HAMSSA8.

However, CAMRSA2 and CAMRSA5 clustered in the HAMRSA phenon. A similarity value of 53% was observed in the HAMRSA and CAMRSA, while HAMSSA and CAMSSA were 48% related. Moreover, HAMRSA and HAMSSA strains and CAMSSA and CAMRSA were observed to be over 60% related. The clustering of the molecular band which was derived based on the calculation of the molecular weights of the protein bands observed in the four phenons, showed a close relationship between the CAMRSA and HAMRSA isolates, while the HAMSSA and CAMSSA isolates clustered together. The correlation coefficient of the molecular weight analysis was 0.9814 i.e approximately 98.1% when converted to per cent values.

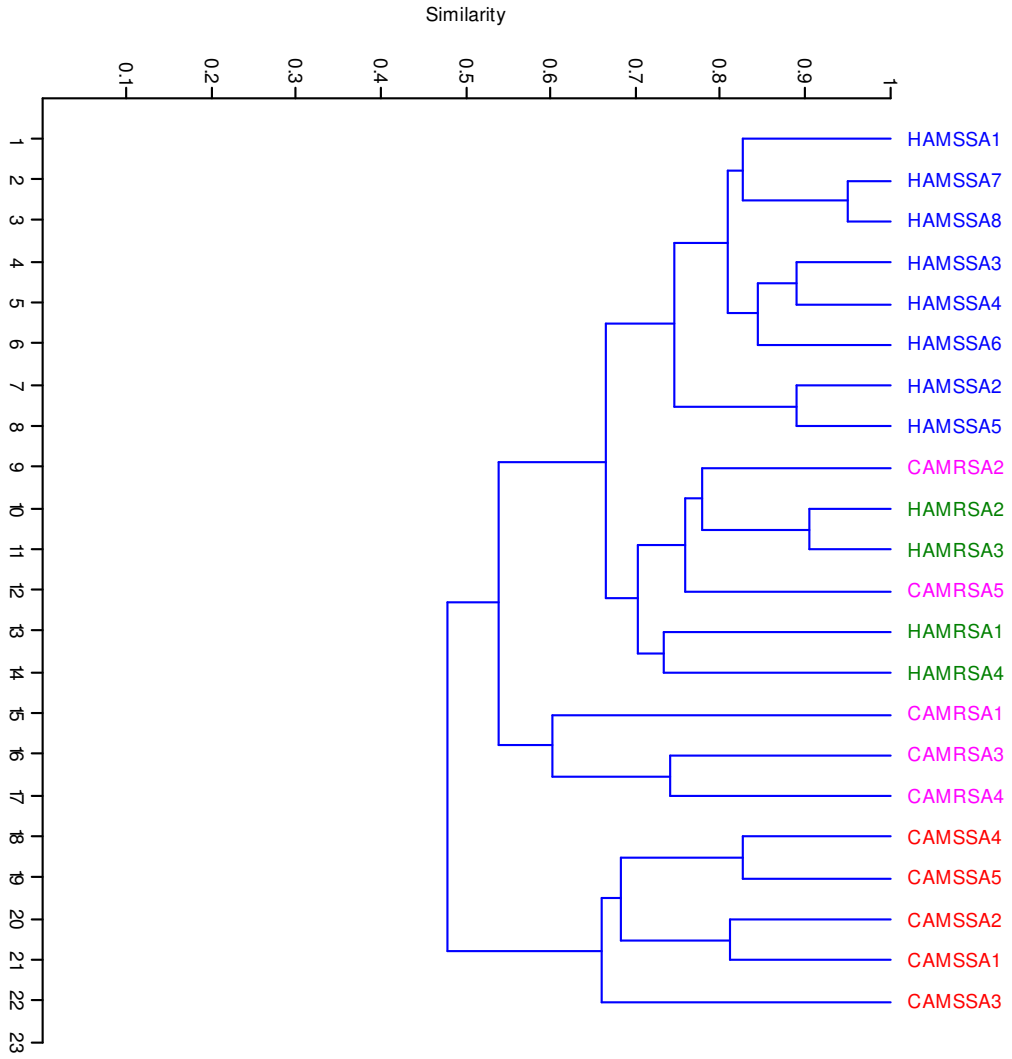

Figure 3. Dendrogram showing the relationship of *S. aureus* strains from hospital and community sources using the Dice similarity coefficient.

DISCUSSION

The study considered the antibiotyping of *Staphylococcus aureus* strains obtained from the hospital and the community sources, and ascertained the relationship of these strains using SDS-PAGE protein profiling technique.

β-lactams resistant organisms produce β-lactamase with penicillin binding protein in effecting resistance by destroying the β-lactam rings in the antibiotics and reduces them to inactive penicilloic acid (Medeiros, 1984; Lowy, 2003). The *S. aureus* isolates cultured from the two settings showed that resistance to the β-lactam antibiotics was high and appeared to be similar. These antibiotype similarities may suggest β-lactams could be ineffective for the treatment of infections/diseases caused by these strains in the hospital and community settings. Other studies have reported high rates of *S. aureus* resistance to β-lactams in Ile-Ife (Lamikanra and Olusanya, 1988; Ako-Nai et al., 1991; Torimiro et al., 2005). The β-lactam antibiotics are relatively available, accessible and cheap and therefore, prone to abuse (Agom et al., 1990; Okeke and Lamikanra, 1995) and this phenomenon have been observed to be major drivers in the emergence of drug resistance.

A total of 65% of the *S. aureus* isolates obtained from the community were resistant to streptomycin compared with 52.2% *S. aureus* isolates from the hospital. But, 46.1% of *S. aureus* isolates from hospital setting were resistant to gentamicin compared with 17.5% from

community setting. The injectable mode of administration of gentamicin makes it less attractive to use and misuse (Umolu et al., 2002). This could be a reason for the observed low incidence of resistance among the community isolates as compared to the hospital isolates in this study. Overall, less than 6% of the *S. aureus* isolates obtained from the two settings were resistant to the quinolones which is similar to reports by other investigators (Onanuga et al., 2005; Shittu et al., 2006) suggesting that these drugs would be effective in treating infections caused by this pathogen.

Methicillin-resistant *S. aureus* (MRSA) continues to be a major problem in the hospital and community settings (Diekema et al., 2001; Appelbaum, 2007, Maree et al., 2007). In this study, nine of the *S. aureus* isolates from the hospital and community settings were phenotypically identified as MRSA indicating a low prevalence of MRSA in the study centre. High level of MRSA had been reported in developing countries of Georgia and India (Revazishvili et al., 2006; Jeshina and Surekha, 2009), which may suggest that the prevalence of MRSA may vary from locality to locality. Deurenberg et al. (2007) have reported prevalence rate of MRSA ranges from 0.6% in the Netherlands to 66.8% in Japan.

The protein patterns contained 22-30 discrete bands. The cluster analysis of the protein marker data placed the isolates into two phenon as predefined. The CAMRSA and HAMRSA were grouped into two phenons at 53% similarity level. The first cluster contained 3 CAMRSA strains at 63% similarity. The second cluster presented a 70% similarity of HAMRSA phenon which presented a low degree of heterogeneity. However, it was observed that in the second cluster, there was an overlap of individual strains from both groups with similarity. The overlapping strains CAMRSA2 and CAMRSA5 in the HAMRSA cluster, could suggest a possible relationship or transmission of strains from the hospital to the community. Recent studies have suggested that MRSA infection rates in the community are possibly correlated with *S. aureus* infection rates in the hospital (Jones et al., 2002). Though, Salgado and associates (2003) reported previous hospitalisation of patients has been associated with CAMRSA.

Conversely, the similarities observed between CAMRSA and HAMRSA had raised the possibility that some CAMRSA strains originated in hospitals (Aires de Sousa and de Lencastre, 2003) and it has been shown that a CAMRSA and a HAMRSA clone have a common ancestor (Deurenberg and Stobberingh, 2008).

Although, this study did not apply the molecular tool often used to characterize MRSA strains but the pool of hospital MRSA strains observed in the CAMRSA phenon, using the protein profile method suggest a possible relationship between the source, strains and genetic variability among the *S. aureus* isolates. This underscores the potential usefulness of the method for fingerprinting and diagnosis.

In conclusion, it was observed there is no significant difference in the resistant pattern of *S. aureus* from the community and hospital setting. The clustering of CAMRSA strains in the HAMRSA phenon may suggest a possible transmission from either the community to the hospital or vice versa. The findings suggest the control of indiscriminate use of anti-infective agents and strict infection control measures to prevent the spread of resistant strains from the hospital to the community and vice versa.

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REFERENCES

Agom JK, Akanni AO, Dawodu TO (1990). Quality of ampicillin/cloxacillin preparations on the Nigerian market. J. Pharm. 21:36-38.

Aires de Sousa M, De Lencastre H (2003). Evolution of sporadic isolates of methicillin resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community acquired MRSA. J. Clin. Microbiol. 41:3806-3815.

Ako-Nai KA, Ogunniyi AD, Lamikanra A, Torimiro SEA (1991). The characterisation of clinical isolates of *Staphylococcus aureus* in Ile-Ife, Nigeria. J. Med. Microbiol. 34: 109-112.

Appelbaum PC (2007). Microbiology of Antibiotic Resistance in *Staphylococcus aureus.* Clin. Infect. Dis. 45:S165-170

Archer GL, Climo MW (1994). Antimicrobial susceptibility of coagulase negative staphylococci. Antimicrob. Agents and Chemother. 38:2231-2237.

Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, Nagai Y, Iwawa N, Asano K, Naimi T, Kuroda H, Cui L, Yamamoto K, Hiramatsu K (2002). Genome and virulence determinants of high virulence community-acquired MRSA. Lancet 359: 1819-1827.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 45:493-496.

Cheesebrough M (1991). Medical Laboratory Manual for Tropical Countries Vol II: Microbiology.Educational low-priced Books Scheme, Great Britain; 60-63.

Chopra I (2003). Antibiotic resistance in *Staphylococcus aureus*: concerns, causes and cures. Expert Rev. Anti-Infective Ther. 1(1):45-55.

Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus.* Clin. Microbiol. Infection 13:222-235.

Deurenberg RH, Stobberingh EE (2008). The evolution of *Staphylococcus aureus*. Infection, Gen. Evol. 8: 747-763.

Diekema DJ, Pfaller MA, Schmitz FJ, Schmitz J, Smayevsky J, Bell R, Jones N, Beach M, the Sentry Participants Group (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. 32:114–132.

Gonzalez BE, Rueda AM, Shelburne SM, Musher DM, Hamill RJ, Hulten KG (2006). Community-associated strains of methicillin-resistant *Staphylococcus aureus* as the cause of healthcare-associated infection. Infection Cont. Hosp. Epidemiol. 27: 1051-1056.

Hammer O, Harper DAT, Ryan PB (2001). PAST: Paleontological statistics software package for Education and data analysis. http//palaeo-electronica.org.

Jeshina J, Surekha K (2009). Molecular characterization of methicillin resistant *Staphylococcus aureus* strains isolated in Kerala, South India. Curr. Res.Bacteriol. 2 (1):1-6.

Jones ME, Mayfield DC, Thornsberry C, Karlowsky JA, Sahm DF, Peterson D (2002). Prevalence of oxacillin resistance in *Staphylococcus aureus* among inpatients and outpatients in the USA during 2000. Antimicrob. Agents Chemother. 46:3104-3105.

Klein E, Smith DL, Laxminarayan R (2007). Hospitalisations and deaths caused by methicillin resistant *Staphylococcus aureus*, in the United States 1999-2005. Emerg. Infect. Dis. 13 (12):1840-1846.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Lamikanra A, Olusanya OI (1988). A long-term study of nasal carriage of *Staphylococcus aureus* in healthy Nigerian Students. Trans. Roy. Soc. Trop. Med. and Hyg., 82: 500-502.

Lowy FD (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. J. Clin. Invest. 3 (9):1265–1273.

Maree CL, Daum R, Boyle-Vavra S, Matayoshi K, Miller L (2007). Community associated MRSA isolates causing healthcare associated infections. Emerg. Infect. Dis. 13:236-242.

Medeiros AA (1984). β-lactamase. Br. Med. Bull. 40(1):18-27.

Meers PD (1988). Infection control in developing countries. J. Hosp. Infections, 11: Suppl A, 406-410.

Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Smulders M, Lapetina E, Gemmen E. (2005). The burden of *Staphylococcus aureus* infections on hospitals in the United States: An analysis of the 2000 and 2001 nationwide inpatient sample database. Arch. Internal Med. 165: 1756-1761.

Onanuga A, Oyi AR, Olayinka BO, Onaolapo JA (2005). Prevalence of community associated multi-resistant *Staphylococcus aureus* among healthy women in Abuja, Nigeria. Afr. J. Biotechnol. 4 (9):942-945.

Okello D, Konde-Lule J, Lubanga R, Arube-Wani J (1997). Waste disposal in private medical clinics in Kampala, Uganda. J. Clin. Epidemiol. 50(l):45S.

Okeke IN, Lamikanra A (1995). Quality and bioavailability of tetracycline capsules in a Nigerian semi-urban community. International J. Antimicrob. Agents 5:245-250.

Okeke IN, Lamikanra A, Edelman R (1999). Socioeconomic and Behavioral Factors Leading to Acquired Bacterial Resistance to Antibiotics in Developing Countries. Emerg. Infect. Dis. 5 (1):18-27.

Revazishvili T, Bakanidze L, Gomelauri T, Zhgenti E, Chanturia G, Kekelidze M, Rajanna C, Kreger A, Sulakvelidze A (2006). Genetic background and antibiotic resistance of *Staphylococcus aureus* strains isolated in the Republic of Georgia. J. Clin. Microbiol. 44(10):3477-3483.

Saçilik SC, Osmanagaoglu O, Basustaoglu OK, Cokmus C (2000). Protein profiles and prevalence of methicillin resistant *Staphylococcus aureus* MRSA) in Gulhane military academy hospital in Turkey. Turkish J. Biol. 24:809-816.

Salgado CD, Farr BM, Calfee DP (2003). Community acquired methicillin resistant *Staphylococcus aureus:* A meta-analysis of prevalence and risk factors. Clin. Infect. Dis. 36:131-139.

Schacterk GL, Pollack RL (1973). A simplified method for the quantitative assay of small amounts of protein in biologic materials. Analytical Biochem. 51:654-656.

Shittu A, Lin J, Kolawole DO (2006). Antimicrobial susceptibility patterns of *Staphylococus aureus* and Characterization of MRSA in South Western Nigeria. WOUNDS. 18 (4):77-84.

Shukla SK (2005). Community associated methicillin resistant *Staphylococcus aureus* and its emerging virulence. Clin. Med. Res. 3(2):57-60.

Torimiro N, Torimiro SEA, Fadahunsi OD (2005). Antibiotic resistance of clinical strains of *Staphylococcus aureus* among different age groups in Ile-Ife. Sci.Focus 10 (3):1-4.

Umolu PI, Okoli EN, Zomoh IM (2002). Antibiogram and β-lactamase production of *S. aureus* isolates from different human clinical specimens in Edo State, Nigeria. West Afr. J. Med. 12:124-127.

Woodford N, Livermore DM ((2009). Infection caused by Gram-positive bacteria: A review of the global challenge. J. Infection 59(S1):S4-S16.

Wylie JL, Nowicki DL (2005). Molecular epidemiology of community and health-care associated methicillin resistant *Staphylococcus aureus* in Manitoba, Canada. J. Clin. Microbiol. 43 (6):2830-2836.