ERIC-PCR and Protein Patterns of *Staphylococcus aureus* isolated from Nigerian HIV-Positive Patients Admitted in different Hospitals in Imo State Nigeria

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Abstract: Opportunistic infections due to endogenous microorganisms have been a global problem in immune compromised Human Immunodeficiency Virus (HIV) positive patients. *Staphylococcus aureus* is a significant opportunistic pathogen among HIV patients in both nosocomial and community settings. There is scarce information on the relatedness of *S. aureus* strains isolated from HIV patients in Nigeria. The goal of the present study was to determine the genetic relatedness of *S. aureus* isolated from HIV-positive patients from three different hospitals in Imo State, Nigeria. Fifty nine *S.. aureus* strains obtained from HIV.positive patients attending Heajt to Heart centres at Owerri General Hospital, Okigwe General Hospital and Awo-Omama General Hospital all in Imo State, Nigeria, and presenting symptoms of pneumonia, oral thrush, skin and urinary tract infections, were subjected to enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis and protein profiling to assess their genetic variability. Distinct 12 clusters with a clone circulating in two different hospitals were obtained. The strains in few clusters represent a clone because they are 100% related and therefore could be said to be of the same origin The protein profile showed different band sizes ranging from 26.5 to 300 kDa of which few strains from the three different hospitals were related. Inherent variations among *S. aureus* were observed and it could be due to the selective pressure produced by antibiotics used as part the treatment regime for these patients. The methods used afforded a valuable contribution in defining inherent variations among strains which would be useful in understanding the epidemiology and in designing prevention and control measures against the infections caused by this pathogen in HIV patients.

Keywords: ERIC-PCR, protein profiles, HIV, *Staphylococcus aureus.*

Introduction

Staphylococcus aureus is a bacterium that belongs to the family *Staphylococcaceae*. This microorganism causes infection in almost every tissue of human body (Lowy, 2003; Sabouni *et al*., 2013). *Staphylococcus aureus* belong to the resident microbiota of skin, intestine, upper respiratory tract and vagina (Lowy, 1998; Shadyab and Crum-Cianflone, 2012). They become pathogenic when factors such as pH, temperature and nutrient availability are altered supporting favourable conditions for overgrowth (Mims *et al*., 2004; Emeka-Nwabunnia *et al*., 2015). *S. aureus* adapts rapidly to environmental changes by deploying those factors required for growth and survival within a particular environment (Pelaez-Lorenzo *et al*., 2013). Such bacterial opportunistic infections are distinct clinical entity frequently described in HIV infected patients (Hidron *et al*., 2010; Emeka-Nwabunnia *et al*., 2015). Different strains of *S.aureus* are associated with several infections in HIV positive patients (Shadyab and Crum-Cianflone, 2012; Schaumberg *et al*., 2014) and are a major pathogen found in blood stream infections, skin and soft tissue infections, nose and throat infections and urinary tract infections (Olalekan *et al*., 2012). There is need to define the genetic pattern of these strains in order to promote disease surveillance and epidemiological studies. Several polymerase chain reactions (PCR)-based DNA fingerprinting methods have been used for bacterial typing. Enterobacterial repetitive intergenic consensus (ERIC) is a PCR-based technique, using primer pairs designed along the intergenic repeat units characterised by conserved palindromic structures (Hulton *et al*., 1991) to detect changes in the DNA sequence at sites in the genome. DNA fragment patterns produced are specific for different strains of pathogenic bacteria, such as *S. aureus* (Del Vecchio *et al*., 1995; Weinstein, 1996; Weiser and Busse, 2000). This approach is used for molecular epidemiological typing as it is relatively fast and easy (Williams *et al*., 1990). The polymorphism within the set of DNA fragments generated has been used in discriminating microorganisms both at the interspecies and intra species level (Welsh and McClelland, 1993) including *Staphylococcus aureus* (Van Belkum *et al*., 1993; Mamishi *et al*., 2012). Another molecular technique that has proved to be useful in typing bacterial strains is sodium dodecyl sulphate polyacrylamide gel

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electrophoresis (SDS-PAGE) of whole cell bacterial proteins, wherein differences seen in protein bands have been successfully used to group bacteria (Krech *et al.*, 1988; Huey and Hall, 1989). *S. aureus* strains are reported to express different proteins in different circumstances (Ganjian *et al.*, 2012) due to genetic response to environmental conditions (Pelaez-Lorenzo *et al.*, 2013). Opportunistic bacteria associated with human immunodeficiency virus (HIV) such as *Staphylococcus aureus* have not been well documented in Nigeria. Establishing the genetic pattern of this opportunistic bacterium associated with human immunodeficiency virus (HIV) in Nigeria could help understand the epidemiology, track outbreaks and spread of this organism among HIV-infected patients in a hospital setting. This study was aimed at understanding the genetic diversity of *S. aureus* prevalent among HIV Patients of Imo State, Nigeria using ERIC-PCR and protein profile typing methods.

Material and Methods

Patients

Patients were recruited from three hospitals in Imo State, Nigeria namely Owerri General Hospital, Okigwe General Hospital and Awo-Omama General Hospital, after consultation with physicians between June, 2011 and December, 2012. Inclusion criteria includes all HIV-infected patients presenting with pneumonia, oral thrush, skin and urinary tract infections and no change in antiretroviral regimen in the previous six months. Excluded patients include those who had been on admission or had an invasive procedure the previous one year as well as those with metabolic or orthopaedic condition treated with steroids or hormones. For confidentiality the patients were given codes. Patients' age, sex and type of collected samples were taken and recorded accordingly. Ethical approval was obtained from ethics committee of the three study hospitals (Ref Number: HMB/AD/872/11/132) in accordance with the ethical standards. Each subject signed a statement of informed consent. Also oral informed consents of the leaders of the HIV support groups of the three hospitals were also obtained before sample collection.

Cultural Identification

Staphylococcus aureus isolates investigated in this study originated from sputum, oral, vagina and skin swabs collected from participants mentioned above. Samples were suspended in 15 mL sterile peptone water broth, and incubated at 37 °C for 24 h. After incubation, ten micro litres was transferred to sterile blood agar and incubated at 37 °C for 24 hours. The colony morphology of isolates that developed on blood agar was observed and pure colonies obtained by subculturing onto nutrient agar plates and incubation at 37 °C for 24 h. The organisms were identified by Gram staining reaction, catalase and coagulase test and growth on Mannitol Salt agar, after incubation at 37 °C for 24 hours. All strains that were gram positive, catalase and coagulase positive and were able to grow on Mannitol salt agar were presumptively identified as *S. aureus* species. The identity at species level was further confirmed by conventional polymerase chain reaction (PCR).

Molecular Identification

A single colony was subcultured in 5 mL of Mueller Hinton broth and was incubated at 37 °C for 24 h prior to DNA harvesting. Whole genomic DNA was extracted by boiling according to the method of Zhang *et al.* (2004). Molecular identity of the cultures was tested based on PCR targeting the 16S rRNA according to McClure *et al.* (2006). Crude bacterial lysates of all cultures were prepared targeting a 756-bp internal fragment of the gene with primer concentrations of 100 µM as follows; forward: 5'- AAC TCTGTT ATT AGG GAA GAA CA -3', and reverse 5'- CCA CCT TCC TCC GGT TTG TCA CC -3' (Inqaba Biotechnical company Pty, South Africa). PCR reaction mixture was optimized by adding to each 25 µL eppendorf tubes, 16.4 µL of sterile distilled deionised water, 5 µL of PCR buffer, 1.5 µLof 1x $MgCl_2$, 0.5 µl of dNTP, 0.25 µLeach of the forward and reverse primers and 0.1 µL of *Taq* DNA polymerase (Promega, USA). To completely make it a 25 µL reaction mixture, 1µL of DNA from the processed clinical isolates was added. PCR was carried out in a thermal cycler (Eppendoff Vapoprotect, Germany) with the reaction cycles consisting of an initial denaturation of 94 °C for 5 min; 34 cycles of 55 °C for 30 seconds, 52 °C for 1min and 72 °C for 1 min. A final extension step at 72 °C was continued for another 10 min. The PCR products were resolved on 2% agarose gels containing 0.5 µg/mL ethidium bromide and visualised on UV transilluminator using a photo documentation system (Clinix Science, China).

ERIC-PCR Assay

The primers ERIC-1R and ERIC-2 (5' - ATG TAA GCT CCT GGG GAT TCA C - 3' and 5' - AAG TAA CTG ACT GGG GTG AGC G - 3') with primer concentrations of 100 µM were used as described by Rafiee *et al.* (2000). PCR components consisted of 12.5 µL of master mix (Promega), 1 µL of $MgCl_2$, 0.25 µL each of the primers and 9 µL of nuclease free water to complete it 25 µL. The PCR amplification was performed using a programmable thermal cycler (Eppendoff Vapoprotect, Germany) according to the following cycling conditions. Initial activation step at 94 °C for 5 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and extension at 72 °C for 2 min followed by final extension step at 72 °C for 10 min and the products were resolved in 2% agarose gel, stained with ethidium bromide and the results photograph captured by Gel Documentation system (Clinix Science, China).

Statistical analysis

Gel data was documented for measurement of gel migration distances of PCR products. All specific distances (band Positions) were then scored for presence or absence of bands at that position in the fingerprint from a given isolate in comparison to a corresponding band size on standard DNA ladder. Each DNA fragment generated was treated as a separate character and scored as a discrete variable using 1 to indicate presence, and 0 for absence. A rectangular binary data matrix was obtained and statistical analysis was performed using the Numerical taxonomy and multivariate analysis system NTSYS-pc (Rohlf, 1993) statistical package. A pair wise similarity matrix was generated by means of simple matching coefficient and unweighted pair-group method using arithmetic average. Cluster analysis was performed to develop a dendogram and a batch mode of NYSYS-pc was used to show the inter-relationship and genetic similarity matrix among the strains. Resultant dendogramme were generated by Unweighted Pair – Group Method with Arithmetic mean (UPGMA) method (Saitou and Nei, 1987).

Protein Profile Assay

Each bacteria in 5 ml Mueller Hinton broth and incubated at 37 °C was pelleted by centrifugation at 3000 rpm for 5 min. Phosphate buffered saline (PBS, 100 µL), 10 µL of beta-mercaptoethanol and 100 µL of sodium dodecyl sulphate (SDS) lysis buffer (1X) [20% SDS, 15 mM Tris base and 2.5% glycerol] were added for extraction of whole cell protein together with bromophenol blue. The mixture was incubated in a dry bath at 100 °C for 15 min. Approximately 40 µL of the protein sample was taken and subjected to SDS-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) containing 5% stacking and 10% of resolving gels and separated based on Laemeli discontinuous buffer system (Laemmli, 1970). After electrophoresis on a vertical slab under a constant voltage of initially at 60V in the stacking gel and increased to 80V when the dye entered the resolving gel until the dye almost ran out of the gel, the gels were stained with coomassie brilliant blue. A protein marker (Thermo Scientific Spectra Multicolour Ladder) was used as a standard to estimate the molecular weight of protein bands. Gel was placed in an adequately labelled container containing coomassie brilliant blue (staining solution) for 1 hour. After destaining, the gel was photographed with the help of digital camera for permanent recording of results.

Results

A total of 2748 HIV positive patients were examined. From them, 405 samples of those presenting symptoms of interest were collected. Table 1Characteristics of participants with respect to hospitals of sample collection and the 59 cultures positive for *S. aureus* for those presenting symptoms for pneumonia, oral thrush, skin and urinary tract infections shown in Table 2. All 59 strains amplified the expected 756 bp of the 16S rRNA gene confirming the identification of *S. aureus*.

Table 1: Characteristics of participants according to the hospital where samples were collected

	Owerri General/Specialist Hospital	Okigwe General Hospital	Awo-Omama General Hospital
No of Patients examined	1065	952	731
No of patients enrolled	172	130	103
No of individuals with *S.aureus* Positive Growth	25	18	16
% *S.aureus* Positive Growth	42.4	30.5	27.1
Mean age (years) (Those with *S.aureus* Positive Growth	38.5 ± 10	35.2 ± 13	39.6 ± 12
Sex (male / female)	10 / 15	2 / 16	4 / 12
Duration of HIV (months)	120 ± 17	102 ± 11	109 ± 20
Duration of HAART (months)	52 ± 23	77 ± 10	48 ± 9
CD4 count (Cells/mm^3)	536 ± 72	327 ± 88	637 ± 97
CD8 count (Cells/mm^3)	1089 ± 424	1068 ± 428	1124 ± 358

Continuous variables expressed as mean ± standard deviation; HAART, highly active antiretroviral therapy

Table 2: Prevalence of infections associated with *Staphylococcus aureus* isolates

Diseases/Infections	Prevalence (%)
Lung infection (Pneumonia)	21 (35.59)
Skin/ Soft Tissue Infection/ Rashes	14 (23.73)
Urinary Tract Infection	16 (27.11)
Oral infection	8 (13.56)

ERIC-PCR -fingerprints

In this study ERIC-PCR fingerprints were generated for 42 strains using the primers ERIC-1R and ERIC-2 as dimers while 17 of the isolates were untypeable with this fingerprinting technique. Fragments with band sizes ranging from 0.1Kb to 2Kb were observed. Cluster analysis generated ERIC profiles showed similarity values of 91.9% with confidence interval (CI) of 77.5 – 84.9%. In this study, clusters with strains that showed 100% similarity were considered to be of the same clone. The primers yielded patterns which clustered the isolates into 12 ERIC types (A, B, C, D, E, F, G, H, I, J, K and L). A dendogram based on the combined similarity matrix of Dice coefficient with the two ERIC primers is presented in Figure 1.

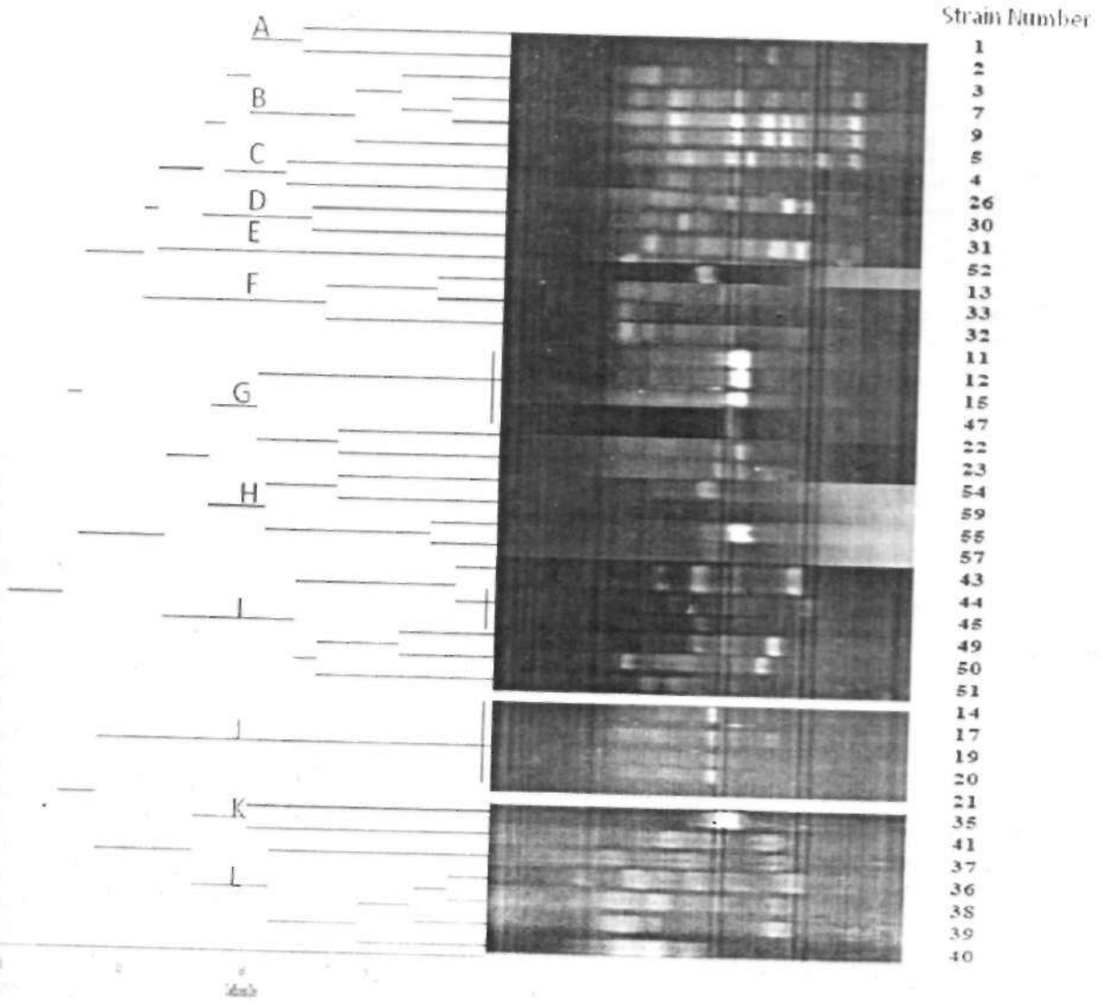
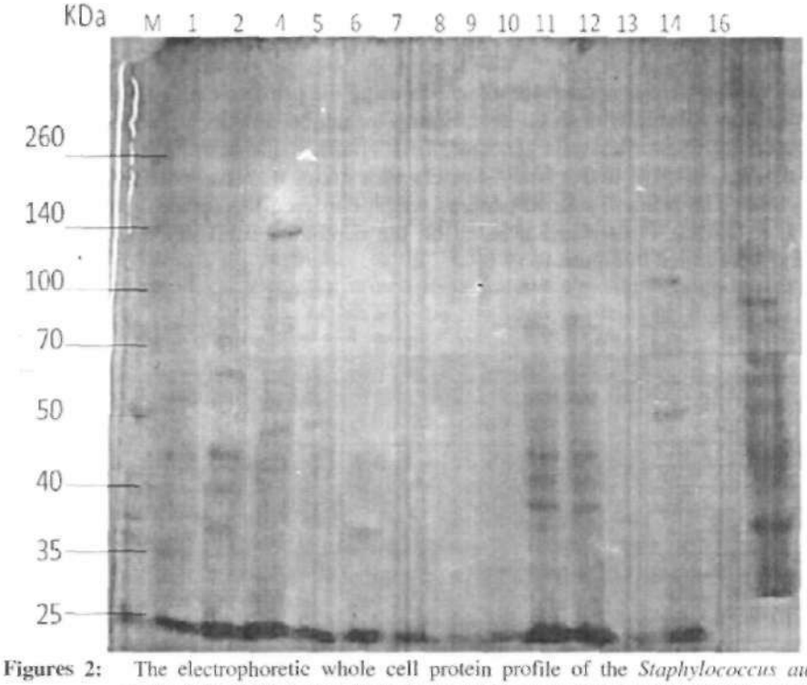


Figure 1:Dendogram of *S. aureus* clonality isolated from patients

Protein profile

The protein profile of 56 *S. aureus* strains were obtained and of them, 33 *S. aureus* yielded 1 to 13 clear and distinct polypeptide bands with molecular weights ranging from 26.5 to 300 KDa. The respective patterns were shown in Figure 2. The bovine serum albumin standard curve was generated by plotting the log of the molecular weight of low standards versus the relative mobility (Rf).



Figures 2: The electrophoretic whole cell protein profile of the *Staphylococcus aureus* isolates. Lane M represents the molecular weight marker while lanes 1-16 is a representive of the 56 *S. aureus* strains: a representative figure.

Discussion

The result of this study indicated that ERIC-PCR could differentiate *S. aureus* strains by obtaining distinguishable band patterns for each *S. aureus* strain typed. Genetic fingerprinting and phylogenetic diversity (genetic variability of a species) between different *S. aureus* strains in this study were determined by converting ERIC data into a Dice similarity matrix and analyzed by UPGMA to produce a phylogenetic tree. The DNA band pattern obtained is similar to a bar code, allowing the identification of each individual. *S. aureus* strains produced DNA fingerprints with clear bands after ERIC amplification which grouped the strains into 12 clusters (Figure 1). Cluster A (with 2 strains), B (4 strains) and C (2 strains) are all from Owerri General Hospital, cluster D (2 strains) and F (3 strains) are from Okigwe General Hospital. Cluster E (1 strain) is from Owerri General Hospital, cluster G (6 strains) has 4 strains from Owerri General Hospital and 2 strains from Okigwe General Hospital. The 4 strains from Owerri General Hospital showed 100% similarity (the same clone) and therefore could be said to be from the same origin with similar resistance patterns (data not shown). Cluster H (4 strains) has two strains each from Okigwe General Hospital and Awo-Omama General Hospital. Cluster I (6 strains) has 4 strains from Owerri General Hospital and 2 strains from Okigwe General Hospital. Cluster J (5 strains) has 3 strains from Awo-Omama General Hospital and 2 strains from Okigwe General Hospitals. The strains in this cluster represent a clone therefore could be said to be of the same origin and they also showed the same resistance pattern (data not shown). This could be possible because of inter-referral of HIV patients among these hospitals. Previous studies have suggested that patient transfer or patient referral pattern (Donker *at al.*, 2010) could affect the prevalence of *S. aureus* in hospitals (Smith *et al.*, 2005; Kho *et al.*, 2008). Hospitals that share patients also tend to share genetically similar population of *S. aureus*. The 100% similarity in cluster J from this study provides the genetic evidence that confirms the importance of patient sharing among these three hospitals. Cluster K (2 strains) and cluster L (5 strains) are from Awo-Omama General Hospital. The low genetic diversity of isolates from the general hospitals that is, 100% similarity of 4 strains from Owerri General Hospital, the 5 strains in cluster J from Awo-Omama and Okigwe General Hospitals, would enhance the treatment of these strains using similar antibiotics since they showed the same resistance pattern (data not shown). There have been reports of treatment failure due to resistant *S. aureus* in which there was high Minimum Inhibitory

Concentration (MIC) and reduced susceptibility to Vancomycin (Emeka-Nwabunnia *et al.*, 2015). Drug – drug interactions limit the co-administration of most antibiotics with several antiretroviral agents including protease inhibitors and non-nucleoside reverse transcriptase inhibitors.

ERIC markers revealed relationship between hospital origin, mutation and genetic variation among *S. aureus* strains in this study (Table 3), and this demonstrated its fingerprinting and diagnostic potential. The genotyping to determine the relatedness of the *S. aureus* strains was done using enterobacterial repetitive intergenic consensus (ERIC) polymerase chain reaction (PCR) typing method. The ERIC-PCR was used because it has been found to be reliable, reproducible, rapid, inexpensive and comparatively highly discriminatory (Healy *et al.*, 2005). ERIC element is a small repetitive units of 126 bp containing a conserved central inverted repeat of 40 bp. There is some evidence that these sequence elements amplified by the ERIC primers exist in the genome of Gram-positive bacteria based on the data collected by Weiser and Busse, (2000). Van Belkum *et al.* (1993), used ERIC primers and showed reliable differentiations of strains of *S. aureus*. The high quality and discriminatory ability of enterobacterial repetitive intergenic consensus (ERIC) PCR profiling encouraged the use of this method for analyses of *S. aureus* diversity, transmission, persistence, and virulence. The robustness of ERIC fingerprinting may reflect the length of the primers used (22 bp), which allows high-efficiency priming of further amplification from products of any ERIC amplification cycle. The use of the ERIC protocol allows significant labour and financial savings without a loss in sensitivity or reliability when many samples are to be analyzed. Application of ERIC-PCR fingerprinting for genotyping of *S. aureus* strains has been reported in Iran by Mamishi *et al.*, (2012) in a study conducted among health workers and hospital patients where they also established genetic variance among the strains of the isolates.

SDS-PAGE of whole cell profiling is another method of classifying or typing *S. aureus*. On the 10% SDS-PAGE electrophoresis, the typed 33 *S. aureus* strain analysis yielded approximately 1 to 13 clear and distinct polypeptide bands with molecular weights ranging from 26.5 to 300 KDa. *Staphylococcus aureus* are able to adapt to changing environments by showing a number of genetic response which include production of proteins. Out of the 33, 5 stains showed bands size of 38.5 KDa which has been previously determined as extracellular matrix protein-binding protein (Emp). The Emp protein has been shown by other studies to be a member of the group of secreted *S. aureus* molecules that interact with the extended spectrum of host ligand thereby contributing to *S. aureus* pathogenicity (Hussain *et al.*, 2001a). Extracellular adherence protein (Eap) as well as its analogues with molecular weight ranging from 60 -72 KDa has been confirmed to enhance adherence of *S. aureus* to host components (Palma *et al.*, 1999; Hussain *et al.*, 2001b) and these are bands showed by 28 (84.8%) out the 33 strains in this study. A major protein target structure for Eap on the *S. aureus* surface is the 30-32 KDa protein which has phosphatase activity and binds to human IgG. Out the 28 strains, 20 (71.4%) with these bands were noticed in this study. The affinity between the Eap and 30-32 protein structure has been shown by studies to affect cell signalling and phagocytosis (Flock and Flock, 2001). Whole cell protein typing produced bands in 8 *S. aureus* strains that could not be typed by ERIC – PCR. This difference in protein profile may be due to their genetic variation. The minor differences in the protein pattern may be due to environmental factors or antibiotic resistance of these strains isolated from patients using different antibiotics. These results show that each species yielded a different electrophoretic pattern. Furthermore, the slight dissimilarities may be due to the difference in the origin of the strains. Torimiro *et al.*, (2013) also used protein profiling to classify antibiotic resistant *S.aureus* strains from hospitals among non HIV patients in which they determined that the resistant *S.aureus* strains they analysed were from two main sources.

Nucleic acid-based approaches for investigating microbial communities rely invariably on the coordinated functions of protein molecules that serve as enzymes for recognizing, replicating, and amplifying specific nucleic acid sequences. In addition to this fundamental linkage between protein function and the assessment of genetic diversity, protein molecules represent the final result of genetic expression, and through their functions as physiological catalysts, structural components, signal transducers, and mediators of intercellular communication, they control key reactions in ecological processes performed by microorganisms (Ogunseitan, 1996; Ogunseitan, 2000). *Staphylococcus aureus* are able to adapt to changing environments by showing a number of genetic response which include production of proteins. □-lactams resistant organisms produce □-lactamase with penicillin binding protein in effecting resistance by destroying the □-lactam rings in the antibiotics and reduces them to inactive penicilloic acid (Lowy, 2003). The protein banding pattern was observed to be unidentical for most of the isolates.

A high discriminatory power is an important characteristic of a typing technique defined as the probability that isolates with related and identical phenotypic and genotypic profiles are clonal and part of the same transmission. The discriminatory index showed that ERIC PCR had a better discriminatory index than whole cell protein fingerprinting at typing this *S. aureus* strains from HIV patients used in this study. It was observed that both molecular methods could not type only 8 strains. The non-typeable strains may pose an important challenge in clinical settings because it could be due to mutation in *S. aureus* strains

as previously observed by Votintseva *et al.*, (2014). Deletion E (174bp) has also been previously described by Baum *et al.*, (2009) in clinical *S. aureus* strains where strains with this deletion could not be amplified therefore was not typed along with other strains. The non-typeable samples that did not amplify with the standard set of primers may have deletions affecting the binding site for the original primers.

The incidence of *Staphylococcus aureus* infection in the hospitals and community continues to rise globally. Genotyping by ERIC-PCR typing method showed 12 clusters which revealed that some of the *S. aureus* strains from different hospitals were clonally related. The protein analysis revealed the presence of some strains with bands that has similar characteristics to important adhesive and pathogenicity enhancing proteins such as Emp and Eap. Molecular methods evaluated in this study proved to be useful rapid tools that can be implemented for screening and monitoring *S. aureus* strains among HIV patients to ensure that outbreak is prevented by correct infection control principles as one clonal type was found circulating in two hospitals.

Conflict of Interest:
The authors wish to declare that there is no conflict of interest.

Acknowledgement. The partial financial support (Grant with REF NUMBER: FUTO/REG/RC/GEN. 67) towards this study from the Education Trust Fund, Government of Nigeria is gratefully acknowledged.

References

Baum, C., Haslinger-Loffler,B., Westh, H., Boye, K., Peters, G., Neumann, C. and Kahl, B. C. (2009). Non-spa-typeable clinical *Staphylococcus aureus* strains are naturally occurring protein A mutants. *Journal of Clinical Microbiology*. **47**(11): 3624–3629.

Del Vecchio, V.T., Petroziello, J.M., Gress, M.J., McCleskey, F.K., Melcher, G.P., Crouch, H.K. and Lupski, J.R. (1995). Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore - enhanced repetitive-sequence PCR. *Journal of Clinical Microbiology*. **33**(8): 2141–2144.

Donker, T., Wallinga, J. and Grundmann, M. (2010). Patient referral patterns and the spread of hospital acquired infections through national health care networks. *Plos Computational Biology*. **6**: 10007 – 10015.

Emeka-Nwabunnia, I., Chiegboka, N. A., Udensi, U. J. and Nwaokorie, F. O. (2015). Vancomycin resistant *Staphylococcus aureus* Isolates from HIV-Positive Patients in Imo State, Nigeria. *Science Journal of Pubulic Health*. **3:** 1 – 7.

Flock, M. and Flock, J. I. (2001). Rebinding of extracellular adherence protein Eap to *Staphylococcus aureus* can occur through a surface-bound neural phosphatase. *Journal Bacteriology*. **183**(13): 3999 – 4003.

Ganjian, H., Nikokar, I., Tieshayar, A., Mostafaei, A., Amirmozafari, N. and Kiani, S. (2012). Effects of salt stress on the antimicrobial drug resistance and protein profile of *Staphylococcus aureus*. *Jundishapur Journal of Microbiology*. 5(1): 328–331.

Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., Raza, S., Schrock, R., Manry, J., Renwick, A., Nieto, R., Woods, C., Versalovic, J. and Lupski, J. R. (2005). Microbial DNA typing by automated repetitive sequence based PCR. *Journal ofClinicalMicrobiology*.43 (1): 199–207.

Hidron, A. I., Kempker, R., Moanna, A. and Rimland, B. N. (2010). Methicillin-resistant *Staphylococcus aureus* in HIV-infected Patients. *Infectious Drug Resistance*. 3: 73 – 86.

Huey, B. and Hall, J. (1989). Hypervariable DNA fingerprinting in *Escherichia coli*: mini satellite probe from bacteriophage M13. *Journal ofBacteriology*.171: 2528.

Hulton, C. J. S., Higgins, C. F. and Sharp, P. M. (1991). ERIC sequences: a novel family of repetitive elements in the genomes of *Escherichia coli*, *Salmonella typhimurium* and other enterobacteria. *Molecular Microbiology*. 5(4): 825–834.

Hussani, M., Becker, K., von Eiff, C., Schrenzel, J., Peters, G. and Herrman, M. (2001a). Identification and characterization of a novel 38.5 kilodalton cell surface protein of *Staphylococcus aureus* with extended spectrum binding activity for extracellular matrix and plasma proteins. *Journal of Bacteriology*. 183(23): 6778 – 6786.

Hussani, M., Becker, K., von Eiff, C., Schrenzel, J., Peters, G. and Herrman, M. (2001b). Analogs of Eap proteins are conserved and prevalent in clinical *Staphylococcus aureus* isolates. *Clinical and Diagnostic Laboratory Immunology*. 8(6): 1271 – 1276.

Kho, A. N., Lemmon, L., Commiskey, M., Wilson, S. J. and McDonald, C. J. (2008). Use of regional health information exchange to detect

crossover of patients with MRSA between urban hospitals. *Journal of American Medical and Information Association.* 15: 212 – 216.

Krech, T., de Chastonay, J. and Falsen, E. (1988). Epidemiology of diphtheria: polypeptide and restriction enzyme analysis in comparison with conventional phage typing. *European Journal of Clinical Microbiology and Infectious Diseases.* **7:** 232–237.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature.* 227: 680–685.

Lowy, F. D. (1998). *Staphylococcus aureus* infections. *New Engl and Journal of Medicine.* **339**: 520–532.

Lowy, F. D. (2003). Antimicrobial resistance: The example of *Staphylococcus aureus. Journal of Clinincal Investigation.***111**: 1265–1273.

Mamishi, S., Mahmoudi, S., Sadeghi, R. H., Movahed, Z., Hadipour, R. and Pourakbari, B. (2012). Genotyping of *Staphylococcus aureus* strains among healthcare workers and patients in the tertiary referral children's medical hospital in Tehran, Iran. *British Journal of Biomedical Science.***69**(4): 1 – 5.

McClure, J., Conly, J. M., Lau, V., Elsayed, S., Louie, T., Hutchins, W. and Zhang, K. (2006). Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine Leukocidin genes and simultaneous discrimination of methicillin susceptible from resistant staphylococci. *Journal of Clinical Microbiology.* **44**(3): 1141–1144.

Mims, C., Dockrell, H. M. and Georing, R. V. (2004). *Medical Microbiology.* 3rd ed. United Kingdom, Elservier Mosby, Edinburgh.

Ogunseitan, O. A. (1996). Protein profile variation in cultivated and native freshwater microorganisms exposed to chemical environmental pollutants. Microbial *Ecology.* **31**: 291–304.

Ogunseitan, O. A. (2000). Microbial proteins as biomarkers of ecosystem health. In: Scow, K., Fogg, G. E., Hinton, D. and Johnson, M. L. (Eds.). *Integrated Assessment of Ecosystem Health.* Boca Raton CRC Press.

Olalekan, A. O. Schaumberg, F., Nurjadi, D., Dike, A. E. Ojurongbe, O., Kolawole, D. O., Kun, J. F. and Zanger, P. (2012). Clonal expansion accounts for an excess of antimicrobial resistance in *Staphylococcus aureus* colonizing HIV-positive individuals in Lagos, Nigeria. *International Journal of Antimicrobial Agents.* **40**(3): 268 – 272.

Palma, M., Haggar, A. and Flock, J-I. (1999). Adherence of *Staphylococcus aureus* is enhanced by an endogenous secreted protein with broad binding activity. *Journal of Bacteriology.* **181**(9): 2840 – 2845.

Pelaez – Lorenzo, C., Veledo, M. T., Gonzalez, R., de Frutos, M. and Diez – Masa, C. (2013). Protein fingerprinting of *Staphylococcus aureus* by capillary electrophoresis with an induced fluorescence detection. *Methods in Molecular Biology.* **984**: 237–251.

Rafiee, M., Bara, M., Stephens, C. P. and Blackwell, P. J. (2000). Application of ERIC PCR for the comparison of isolates of *Haemophilus parasuis. Austrian Vetenary Journal.***78**: 846–849.

Rohlf, F. J. (1993). Numerical taxonomy and multivariant analysis system version 2.0. State University of New Yolk. Stony Brooth, NY.

Sabouni, F., Ranjbani, R., Pourakbari, B., Mahmoudi, S., Teymuni, M., Taghi, H., Ashtiaul, M., Movahedi, Z. and Mamishi, S. (2013). *Staphylococcus aureus* infections in children in an Iranian referral paediatric hospital. *Journal of Prevention and Medical Hygiene.* **54**: 205–207.

Saitou, N. and Nei, M. (1987). The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution.***4**(4): 406 – 425.

Schaumberg, F., Alabi, A. S., Peters, G. and Becker, K. (2014). New epidemiology of *Staphylococcus aureus* infections in Africa. *Clinical Microbiology and infections.* **20**(7): 589 – 596.

Shadyab, A. H. and Crum-Cianflone, N. F. (2012). Methicillin-resistant *Staphylococcus aureus* (MRSA) infections among HIV-infected persons in the era of highly active antiretroviral therapy: a review of the literature. *HIV Medicine.* **13**(6): 319–32.

Smith, D. L., Levin, S. A. and Laxminarayan, R. (2005). Strategic interactions in multi-institutional epidemics of antibiotics resistance. *Proceedings of National Academy of Science, USA.* **102**: 3153 – 3158.

Torimiro, N., Ako-Nai, A. K. and Omitogun, O. G. (2013). Comparative protein profile of antibiotic resistant *S. aureus* strains from

hospital and community associated infections. *International Research Journal of Microbiology.* 4(2): 64 – 71.

Van Belkum, A., Bax, R., Peerbooms, P., Goessens, W. H. F., Van Leeuwen, N. and Quint, W. G. V. (1993). Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for discrimination of methicillin-resistant *Staphylococcus aureus* strains. *Journal of Clinical Microbiology.***31**: 798–803.

Votintseva, A. A., Fung, R., Miller, R. R., Knox, K., Godwin, H., Wyllie, D. H., Bowden, R., Crook, D. W. and Walker, S. A. (2014). Prevalence of *Staphylococcus aureus* protein A (spa) mutants in community and hospitals in Oxford shire. *BMC Microbiology.* **14**(63): DOI: 10.1186/1471-2180-14-63.

Weinstein, M. P. (1996). Current blood culture methods and systems: clinical concepts technology and interpretation of results. *Clinical Infectious Diseases.* 23: 40 – 46.

Welsh, J. and McClelland, M. (1993). The characterization of pathogenic micro-organisms by genomic fingerprinting using arbitrarily primed polymerase chain reaction. (AP-PCR). In *Diagnostic molecular microbiology* (Ed. D. H. Persing, T. F. Smith, F. C. Tenover, T. J. White), Washington DC, USA, ASM Press.

Wieser, M. and Busse H. J. (2000). Rapid identification of *Staphylococcus epidermidis*. *International Journal of Systems and Evolutionary Microbiology.* 50: 1087–1093.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, L. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic marker. *Nucleic Acids Research.* 18: 6531–6535.

Zhang, K., Sparling, J., Chow, B. L, Elsayed, S., Hussain, Z., Church, D. L., Gregson, D. B, Louie, T. and Conly, J. M. (2004). New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. *Journal of Clinical Microbiology.*42: 4947–4955.