

## Clonality of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Hospital and Community Samples in Nsukka, Nigeria.

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### Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a human pathogen that has emerged as a serious public health problem both in hospital and community settings. This study was aimed to determine the clonal variations of MRSA from healthcare and community environments. *S. aureus* was isolated and identified from hospital and community samples using standard laboratory procedures and MRSA strains were identified using Kirby Bauer disk diffusion method. Out of 505 *S. aureus* isolates recovered in this study, 295 (58.4%) were oxacillin resistant and were therefore considered to be methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The prevalence of MRSA in the hospital environment was 34.8% and 28.7% in the community environment. Out of 21 selected MRSA isolates examined for *mecA* gene by PCR, only six (53, 69, 74, 84, 88 and 89) showed positive amplification for *mecA*. Of these six, one (53) was a hospital isolate, while the other five were community isolates. Polymerase chain reaction (PCR) was used to detect both the *Staphylococcus* specific 16s rRNA and *mecA* genes with amplicon sizes of 750 bp and 310 bp, respectively. All oxacillin resistant isolates possessed the *S. aureus* specific sequence gene, but not all possessed the *mecA* gene. Genetic relatedness of the isolates was determined using protein profile from SDS-PAGE and restriction patterns from restriction fragment length polymorphism (RFLP). The protein profiles and restriction patterns revealed heterogeneity among the isolates. Phylogenetic analyses were done on the protein profiles and restriction patterns using bioinformatics software (UPGMA). The results revealed four different patterns suggesting that there are about four MRSA clones among the samples as well as genetic relatedness between hospital and community isolates.

**Key words:** Genotyping, Phylogenetic analyses, Polymerase chain reaction (PCR), Restriction fragment length polymorphism (RFLP), SDS-PAGE.

### I. Introduction

*Staphylococcus aureus* is an established cause of skin and soft tissue infections (SSTI) and invasive life-threatening infections <sup>1,2</sup>. It is also the leading cause of hospital-associated infections <sup>3,2</sup>. Antibiotic resistance in *S. aureus*

continues to be a public health concern worldwide, particularly since the emergence of methicillin-resistant strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of morbidity and mortality worldwide<sup>4,5,6</sup>. MRSA was first reported in hospitalized patients in a British hospital in the 1960s<sup>5</sup> and was subsequently considered to be primarily a nosocomial pathogen<sup>4,5,7,8</sup>. However, recent reports have shown a changing epidemiology for MRSA. Two major phenotypic forms of MRSA are currently recognized; Hospital-acquired (HA) and Community-acquired (CA) MRSA. Several methods have been used to classify MRSA as either HA-MRSA or CA-MRSA, including genotypic testing, based on pulsed-field gel electrophoresis (PFGE), phenotypic testing, based on antimicrobial susceptibility and epidemiological analysis, based on time of admission in a hospital to a positive culture<sup>7</sup>. The major difference, however, is that while HA-MRSA is resistant to many classes of antibiotics, CA-MRSA is susceptible to multiple classes of antibiotics<sup>7,8</sup>. Reports have shown that there is increasing occurrence of CA-MRSA and it has even been speculated that CA strains may eventually replace HA strains in the hospital<sup>8</sup>. In the light of this increased occurrence, there has been speculation about whether the community strains arise through transmission by discharged patients and health care workers or whether they arise by some other mechanism<sup>5,8</sup>. It has also been reported that one of the cardinal features of the rapid emergence of MRSA in many parts of the world is the dissemination of specific clones, which has contributed to the accelerated increases in the incidence of MRSA<sup>9</sup>. It was also reported that MRSA infection was of clonal nature, with few dominant lineages<sup>10</sup>. Several clonal complexes have been reported from different regions of the globe<sup>11</sup>. The continuous spread of staphylococcal infection in several communities is now becoming a threat to the populace and mostly the children<sup>12</sup>. Consequently, surveillance for this emerging pathogen and investigation of the genetic relatedness of strains isolated from different environments is of great importance.

Reports have been made about circulation of MRSA clones and subtypes in some developed countries<sup>13,4,7,8</sup>, but there is a paucity of information from the developing countries, in general and Nigeria, in particular, about both HA- and CA-MRSA. Some studies in Nigeria have shown MRSA prevalence of between 22 and 35% in samples collected from hospitals<sup>14,15,16</sup>, but there are no data available about the situation in the community.

In this study, samples were collected from both hospital and community environments and examined for presence of MRSA. Sub-typing of the isolates was also done using molecular methods.

## II. Materials and Methods

### Sampling

A total of 600 samples were collected from four different hospitals in Nsukka, while 300 samples were collected from the community (outside the hospital). The hospital samples comprised urine (n=200), sputum (n=50), wound swabs (n=80), swabs from hospital formites (n=20) and nasal swabs from hospital personnel (n=250, while the community samples comprised 300 nasal swabs from persons living in the community who were not admitted in the hospital. Swab samples were collected using sterile cotton swabs moistened with peptone water. The samples were promptly transported to the laboratory for analysis.

### Bacteriological methods

One loopful of each urine and sputum sample as well as swabs were inoculated onto mannitol salt agar (MSA) plates. The plates were incubated aerobically at 37°C for 24h. The characteristic colonies were aseptically isolated and characterized using established microbiological methods including colonial morphology, Gram's staining reaction, haemolytic reaction, catalase and coagulase tests. Identified *Staphylococcus aureus* colonies were stored on nutrient agar slants at 4°C for further use.

### PCR amplification of *Staphylococcus* specific sequence gene and *mecA* gene

DNA was isolated from selected MRSA isolates by boiling method according to the method of<sup>17</sup>. The extracted DNA was amplified with primers targeting the *Staphylococcus* 16S rRNA gene and the methicillin-resistant (*mecA*) gene, with amplicon sizes of 750 and 310 bp, respectively. The primer for the *Staphylococcus* specific gene had the sequence: 756-bp forward, 5'-AAC TCT GTT ATT AGG GAA GAA CA-3' and 750- bp reverse, 5'- CCA CCT TCC TCC GGT TTG TCA CC 3'. On the other hand, the primers for detecting methicillin-resistance had the sequence:

*mecA1* (5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3') and *mec2* (5'- CCA ATT CCA CAT TGT TTC GGT CTA A-3'). All primers were synthesized by Inqaba Biotechnical Company, South Africa.

PCR reaction mixture was prepared in a 25µl reaction volume containing: 12.5 µl sterile distilled water, 0.25 µl each of the forward and backward primers and 2 µl of DNA. PCR was carried out in a thermal cycler (peGlab Biotechnology GmbH, England) with the reaction cycles consisting of an initial denaturation at 94°C for 5 min, 34 cycles at 55°C for 30 seconds, denaturation at 55°C for 1 min and annealing at 72°C for 1 min. A final extension step at 72°C was continued for another 10 min. The PCR products were resolved on 1.5% agarose gels containing 0.5 µl/ml ethidium bromide and visualized on a UV transilluminator using a photo documentation system (Climix science, Japan). The sizes of the amplification products were estimated by comparison with a 100 bp molecular size standard ladder.

### Protein profiling by SDS PAGE

Protein profiles of all the Methicillin-resistant *Staphylococcus aureus* strain (MRSA) were determined by running SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to laemeli discontinuous buffer system<sup>18</sup>.

The isolates were grown in nutrient broth for 72 h at 37°C. A 1 ml aliquot of each culture was placed in a microcentrifuge tube. The cells were pelleted by centrifuging at 7,000 rpm for 5 min. The pellets were washed twice in 1 ml of phosphate buffered saline (PBS). The supernants were gently decanted and the pellets resuspended in 100 µl SDS lysis buffer (2X). Loading dye (60 µl) was added to each sample. The samples were incubated at 100°C for 10 min in a water bath and then immediately cooled to about 20°C. A 10 µl volume of each protein sample was taken and subjected to SDS-PAGE. The proteins were separated in a 15% resolving gel and 5% stacking gel. Electrophoresis was carried out at 80 volts in the stacking gel for 30 min and 100 volts in the resolving gel for about 2 hours in a 1X SDS-PAGE running buffer using a Bio-Rad Mini- Protein II Dual Slab Cell. The protein bands were visualized by Coomassie blue staining and the molecular weights of the proteins were calculated using Sigma SDS-PAGE molecular weight standards.

### Restriction enzyme digestion and pulsed field electrophoresis (PFGE)

Genotyping of the MRSA isolates was performed using *AluI* enzyme digestion. Restriction fragments were separated by PFGE in a contour-clamped homogenous electric field apparatus (CHEF DRIII, Bio-Rad Hercules, CA, USA).

### Phylogenetic Analysis

Phylogenetic analyses and plotting of neighbor joining tree of the MRSA isolates were performed on band patterns obtained from both SDS-PAGE and RFLP, using Mega version 5.2.2 bioinformatics software.

## III. Results

Out of 505 *S. aureus* isolates recovered in this study, 295 (58.4%) were oxacillin resistant and were therefore considered to be methicillin-resistant *Staphylococcus aureus* (MRSA) strains. There was a higher prevalence of MRSA in the hospital environment (34.8%) than in the community environment (28.7%). Out of 21 selected MRSA isolates examined for *mecA* gene by PCR, only six (53, 69, 74, 84, 88 and 89) showed positive amplification for *mecA* (Figures 1 and 2). Of these six, one (53) was a hospital isolate, while the other five were community isolates. Relatedness of the hospital and community strains was evaluated by studies of SDS-PAGE patterns of their proteins and RFLP patterns. SDS-PAGE proteins profiles of the isolates revealed at least four clusters. Isolates 69 and 74 were found to be the same strain and shared a close relationship with the 44 and 61 cluster. The next cluster was the 42 and 81 cluster, while isolate 57, which was *mecA*, had less than 1% relatedness with any of the other clusters (Figure 3).

Several different patterns were generated from RFLP of the isolates and analysis of these patterns also revealed the presence of four restriction patterns (Figure 4). Isolates 88 and 89 were the same strain and were different but related to the other clusters. Isolates 88 and 89 were the same strain and were different but related to the other clusters. Isolate 57 was again found to have little or no relationship with other isolates. 53, the hospital isolate shared about 35% relatedness to 69 and 74 which were isolated from the community and about 10% relatedness to 84, 88 and 89, which are also community isolates.

#### IV. Discussion

In the past, MRSA strains were believed to be restricted almost exclusively to the hospital environments, where they have been associated with a variety of nosocomial infections, but more recent reports have shown the emergence of community-associated strains. This has led to the grouping of the organism into hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) <sup>1,19,20</sup>. The two types are thought to be genotypically dissimilar, targeting different but overlapping populations and causing different diseases <sup>19</sup>. The two types are also believed to differ in terms of their antibiotic-resistance profiles. Methicillin- or oxacillin-resistant phenotype is encoded by the *mecA* that is contained within a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). Therefore, *S. aureus* strains that are methicillin resistant by disk-diffusion tests are expected to be *mecA*<sup>+</sup> genotypically. In this study, high prevalence of MRSA were recorded in both hospital and community samples examined by disk-diffusion tests. However, only about 28.6% of the oxacillin-resistant isolates demonstrated expression of the *mecA* gene by PCR. This result is consistent with other studies that have shown that some *S. aureus* isolates may show resistance to methicillin without the expression of *mecA* <sup>1,6</sup>. This further buttresses the importance of molecular methods in identification of MRSA although the disparity in results has been explained, by some authors, to be due to the fact that resistance in *S. aureus* involves two different mechanisms: the expression of  $\beta$ -lactamase and *mecA* gene <sup>6</sup>.

Attempts were made, in this study, to evaluate the genetic relatedness of the MRSA isolates from both the hospital and community environments. Analysis of protein profiles generated by SDS-PAGE and patterns generated by RFLP showed that there are at least four different MRSA clones circulating in Nsukka. The identification of up to four clonal varieties in the small geographical area is unlike the situation reported for Trinidad and Tobago, where a single MRSA clone was reported in 2007 <sup>5</sup>, but similar to the case of Finland where multiple genotypes were identified <sup>4</sup>. This finding is a cause for concern because it indicates a rapid evolution of MRSA strains in the region, which in turn could represent a serious health problem. It has been reported that one of the cardinal features of the rapid emergence of MRSA in many parts of the world is the dissemination of specific clones, which has contributed to the accelerated increases in the incidence of MRSA <sup>9</sup>.

There was genetic relatedness between community and hospital isolates in this study, suggesting that there is no clear delineation of HA- and CA-MRSA in the area. This calls to mind the question previously raised by some authors regarding whether the community strains resulted from transmission by discharged patients and healthcare workers or as a result of horizontal transfer of *mecA* gene from resistant types to previously susceptible *S. aureus* <sup>4</sup>. Considering the situation in Nigeria, where there is free movement of both patients and personnel between the hospital and community, the two explanations are entirely plausible, especially the latter, in view of the multiple genotypes observed in this study.

#### V. Conclusion

This study has shown that several different clones of MRSA are in circulation, there is no clear genetic distinction between the hospital and community isolates and that *S. aureus* infections in the region should be taken seriously, bearing in mind the high mortality rates often associated with MRSA infections.

#### Conflict of interest statement

Authors declare that they have no conflict of interest.

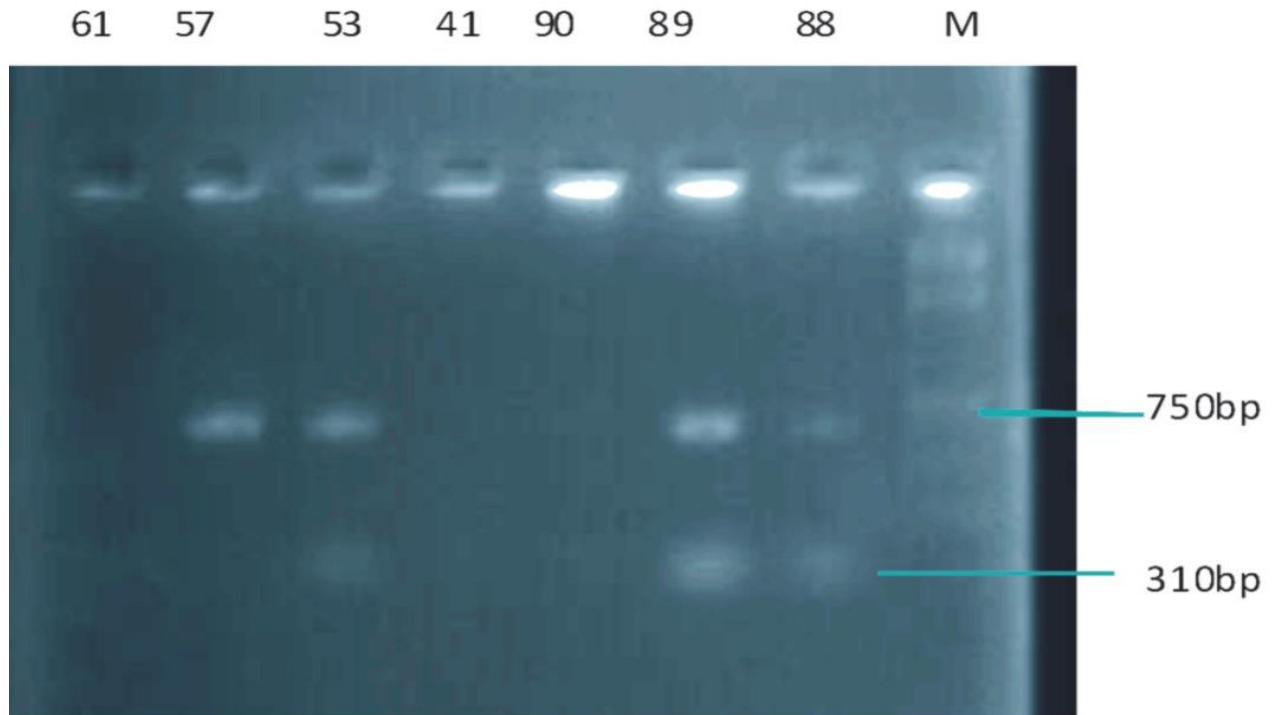


Fig 1: Agarose gel electrophoresis of PCR-amplified products of MRSA isolates. Lane M shows 1Kb DNA Ladder; Lanes 61 to88 are MRSA isolates showing positive amplifications for the 750 bp *Staphylococcus* specific sequence gene while lanes 53, 89 and 88 showed positive amplifications of the 310 bp *mecA* gene.

**+C      -C2      -C      84      74      69      M**

750 bp

310 bp

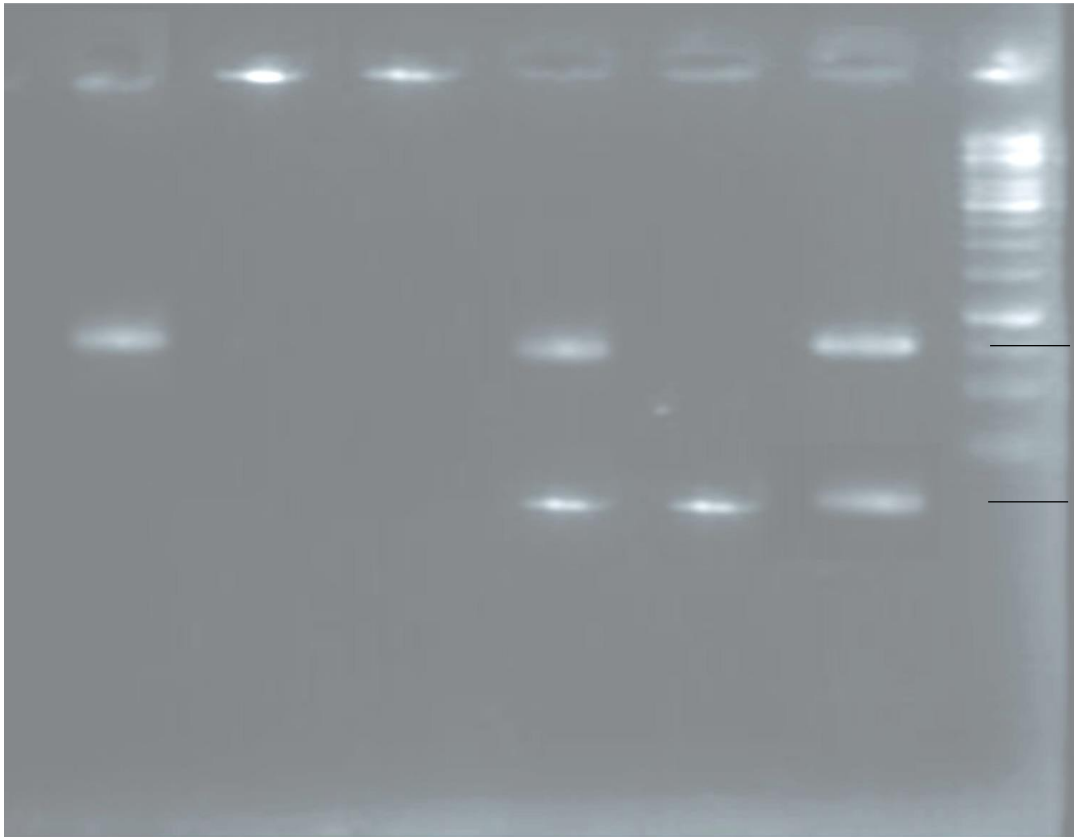


Fig 2: Agarose gel electrophoresis of PCR-amplified products of MRSA isolates. Lane M shows 1 Kb DNA Ladder; Lane +C is a positive control for the *Staphylococcus* sequence gene (ATCC 25923); Lanes -C2 and -C are negative controls; Lanes 69, 74 and 84 are MRSA isolates showing positive amplification of *mecA* gene.

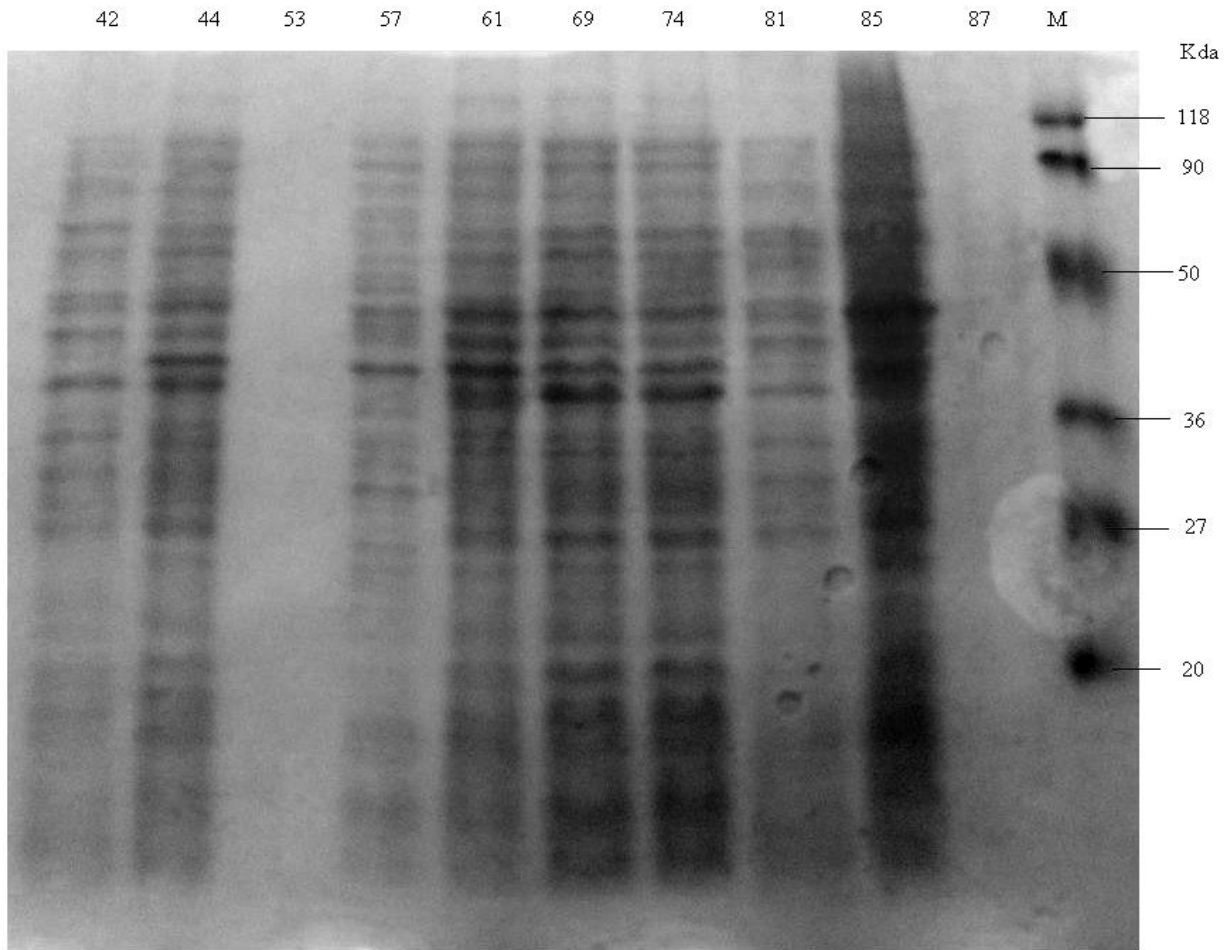


Fig 3: Protein profiles of MRSA isolates from hospital and community environments in Nsukka; Lane M shows molecular weight Ladder; Lanes 87,85,81,74, 69, 61, 57, 53, 44 and 42 are the MRSA strains.



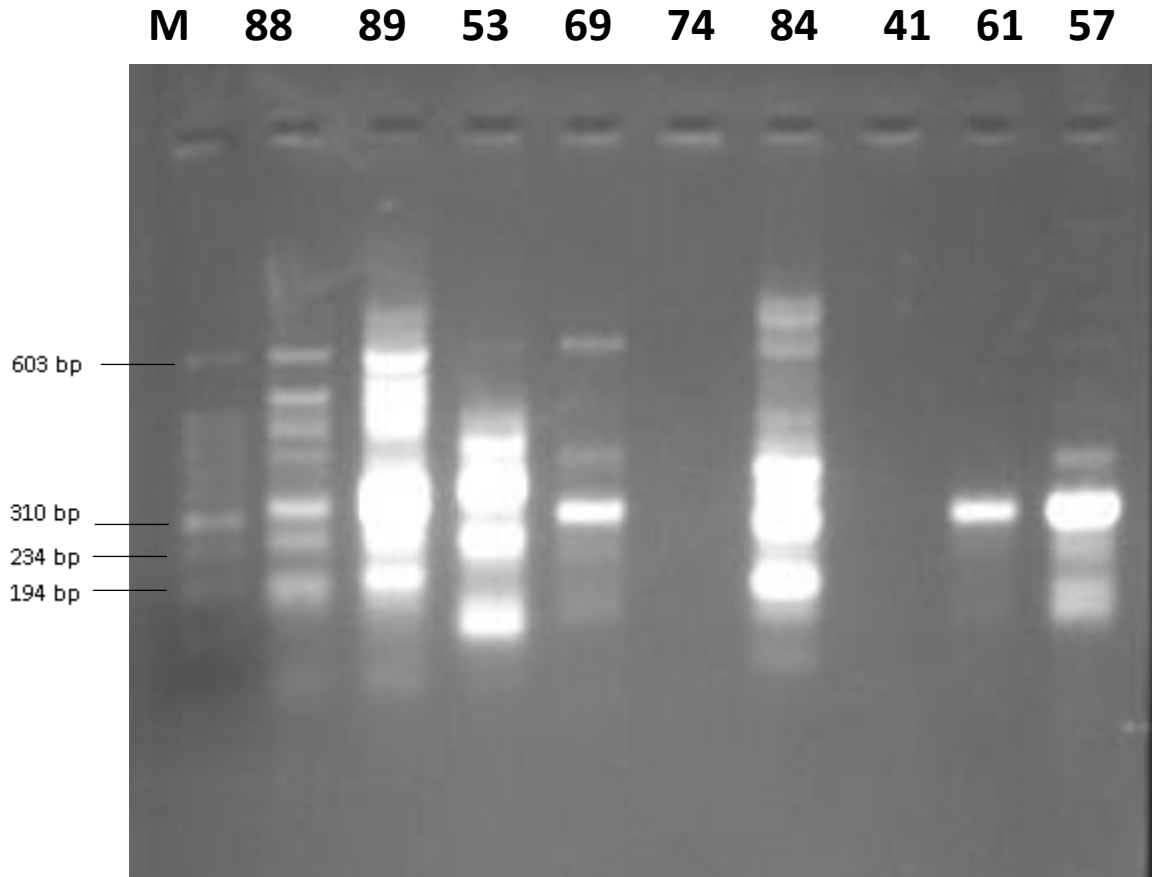


Fig 4: Alu I Restriction fragment length polymorphism (RFLP) pattern of MRSA isolates from hospital community environments in Nsukka; Lane M is a 100 bp molecular weight ladder while Lanes 88, 89, 53, 69, 74, 84, 41, 61 and 57 are MRSA isolates.

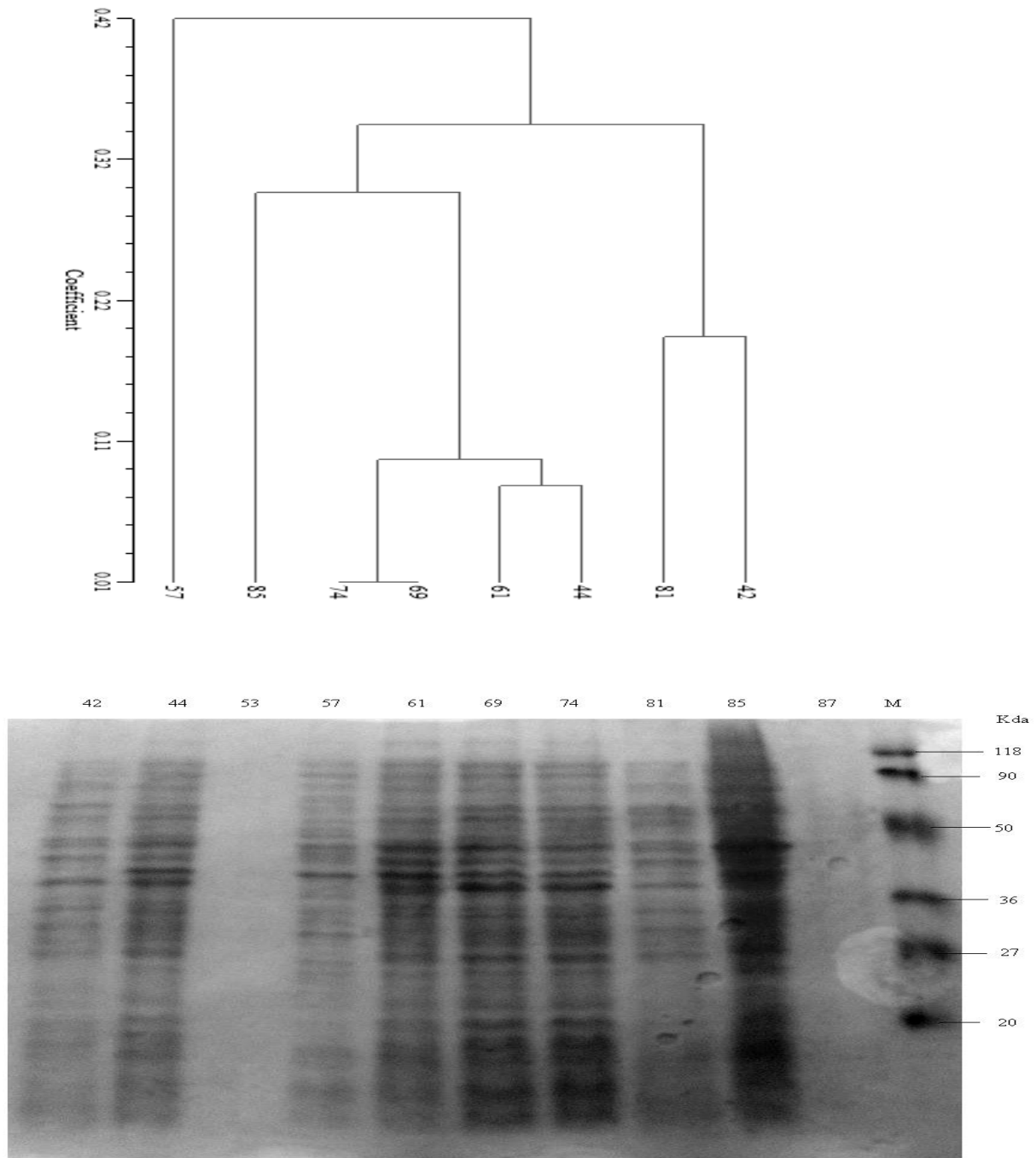
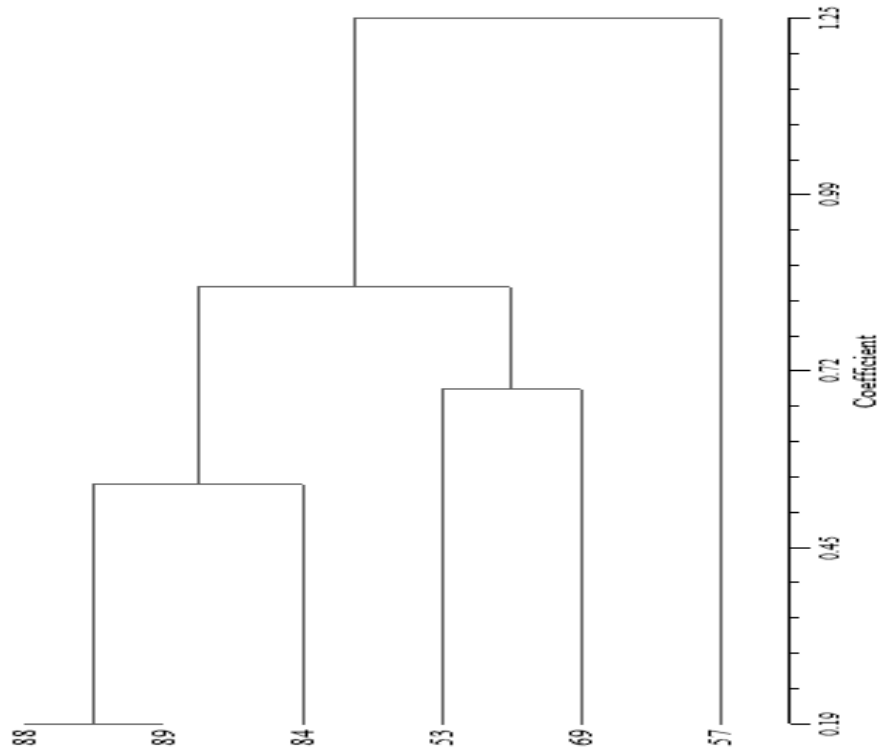


Fig 5: Protein profiles and dendrogram of MRSA isolates from hospital and community environments. Lane M is molecular weight ladder, while lanes 42 to 87 are MRSA isolates.



**M 88 89 53 69 74 84 41 61 57**

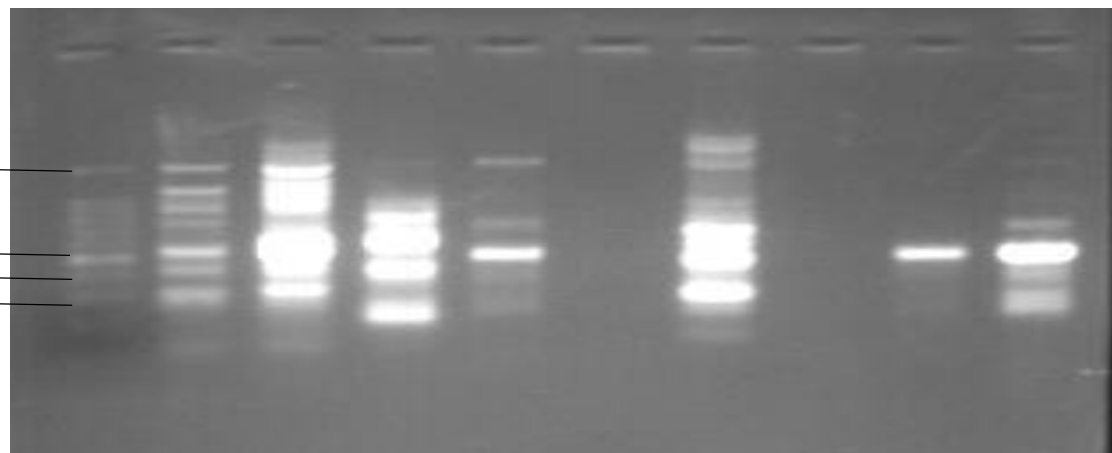


Fig 6: RFLP profiles and dendrogram of MRSA isolates from hospital and community environments. Lane M is a 100 bp molecular weight ladder, while lanes 88 to 57 are MRSA isolates.

## References

- [1]. Chatterjee SS, Ray P, Aggarwal A, Das A, Sharma M. A community-based study on nasal carriage of *Staphylococcus aureus*. *Indian Journal of Medical Research*. 2009; 130: 742-748.
- [2]. Prospero M, Veras N, Azarian T, Rathore M., Nolan D, Rand K, Cook RL, Johnson J, Morris JG, Salemi M. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in the genomic era: a cross-sectional study. *Scientific Reports*. 2013.
- [3]. Jarvis WR, Schlosser J, Chinn RY, Tweeten S, Jackson M. National prevalence of methicillin-resistant *Staphylococcus aureus* in inpatients at US health care facilities. *American Journal of Infection Control*. 2007; 35: 631-637.
- [4]. Salmenlinna S, Lyytikäinen O, Vuopio-Varkila J. Community-acquired methicillin-resistant *Staphylococcus aureus*, Finland. *Emerging Infectious Diseases*. 2002; 8: 602-607.
- [5]. Akpaka PE, Kisooson S, Rutherford C, Swanston WH, Jayaratne P. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates from regional hospitals in Trinidad and Tobago. *International Journal of Infectious Diseases*. 2007; 11: 544 – 548.
- [6]. Al-Ruaily MA, Khahl OM. Detection of (*mecA*) gene in methicillin resistance *Staphylococcus aureus* (MRSA) at prince A/Rhman Sidery hospital, Al-Jouf, Saudi Arabia. *Journal of Medical Genetics and Genomics*. 2011; 3(3): 41 – 45.
- [7]. Popovich K, Hota B, Rice T, Aroutcheva A, Weinstein RA. Phenotypic prediction rule for community-associated methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*. 2007; 45: 2293-2295.
- [8]. Kouyos R, Klein E, Grenfell, B. Hospital-community interactions foster coexistence between methicillin-resistant strains of *Staphylococcus aureus*. *PLoS Pathogen*. 2013; 9: e1003134.
- [9]. Ko KS, Lee JY, Suh JY, Oh WS, Peck KR, Lee NY, Song JH. Distribution of major genotypes among methicillin-resistant in *Staphylococcus aureus* clones in Asian countries. *Journal of Clinical Microbiology*. 2005; 43: 421-426.
- [10]. Ilcyszyn WM, Sabat AJ, Akkerboom V, Szkariat A, Klepacka J, Sowa-Sierant I, Wasik, B, Kosecka-Strojek M, Buda A, Miedzobrodzki J, Friedrich AW. Clonal structure and characterization of *Staphylococcus aureus* strains from invasive infections in paediatric patients from South Poland: Association between Age, spa types, clonal complexes and genetic markers. *PLoS ONE*. 2016; 11(3): e0151937.
- [11]. Goudarzi M, Seyedjavadi SS, Nasiri MJ, Goudarzi H, Nia RS, Dabiri H. Molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from patients with bacteremia based on MLST, SCCmec, spa and agr locus types analysis. *Microbiology and Pathogen*. 2017; 104: 328-335.
- [12]. Akinduti AP, Osiyemi J A, Banjot T, Ejilude O, El-Ashker M, Adeyemi AG, Obafemi Y D, Isibor P O. Clonal diversity and spatial dissemination of multi-antibiotics resistant *Staphylococcus aureus* pathotypes in SouthWest, Nigeria. *PLoS ONE*. 2021; 16(2): e0247013.
- [13]. Leski T, Oliveira D, Trzcinski K, Santos Sanchez I, Aires de Sousa M, Hryniewicz W, de Lencastre H. Clonal distribution of methicillin-resistant *Staphylococcus aureus* in Poland. *Journal of Clinical Microbiology*. 1998; 36:3532-3539.
- [14]. Taiwo SS, Onile BA, Akanbi AA. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Ilorin, Nigeria. *African Journal of Clinical and Experimental Microbiology*. 2004; 5: 189-197.
- [15]. Shittu A, Lin J, Kolawole, D. Antimicrobial susceptibility patterns of *Staphylococcus aureus* and characterization of MRSA in Southwestern Nigeria. *BMC Infectious Diseases*. 2006; 6:125.
- [16]. Alli Terry OA, Ogbolu DA, Akorede E, Onemu OM, Okanlawon BM. Distribution of *mecA* gene amongst *Staphylococcus aureus* isolates from Southwestern Nigeria. *African Journal of Biomedical Research*. 2011; 14:9-16.

- [17]. Zhang K, Sparling J, Chow B L, Elsayed S, Hussain Z, Church D L, Gregson DB, Louie T, Conly J. M. 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*. 2004; 42:4947-4955.
- [18]. Harlow ED, Lane D. Antibodies: Laboratory manual .CSHL press. 1988; Pp 484 – 493.
- [19]. Liu GY. Molecular pathogenesis of *Staphylococcus aureus* infection. *Pediatric Research*. 2009; 65:71R-77R.
- [20]. Fagade OE, Ezeamagu CO, Oyelade AA, Ogunjobi AA. Comparative study of antibiotic resistance of *Staphylococcus species* isolated from clinical and environmental samples. *Assumption University Journal of Technology*. 2010; 13: 165-169.

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