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Full Length Research Paper

Characterization of *Staphylococcus aureus* isolates obtained from health care institutions in Ekiti and Ondo States, South-Western Nigeria

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***Staphylococcus aureus* is one of the major causative agents of infection in all age groups following surgical wounds, skin abscesses, osteomyelitis and septicaemia. In Nigeria, it is one of the most important pathogen and a frequent micro-organism obtained from clinical samples in the microbiology laboratory. Data on clonal identities and diversity, surveillance and new approaches in the molecular epidemiology of this pathogen in Nigeria are limited. This study was conducted for a better understanding on the epidemiology of *S. aureus* and to enhance therapy and management of patients in Nigeria. A total of 54 *S. aureus* isolates identified by phenotypic methods and obtained from clinical samples and nasal samples of healthy medical personnel in Ondo and Ekiti States, South-Western Nigeria were analysed. Typing was based on antibiotic susceptibility pattern (antibiotyping), polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs) of the coagulase gene and pulsed field gel electrophoresis (PFGE). Of the 54 isolates, 50 were confirmed as *S. aureus* by PCR detection of the *nuc* gene. The antibiotic susceptibility testing showed that all the isolates were susceptible to fusidic acid and clindamycin, but 100% resistant to penicillin, 70% to tetracycline, 22% to erythromycin, 6% to gentamicin and 4% to ciprofloxacin. Only one isolate was confirmed to be a methicillin-resistant *S. aureus* (MRSA). The study established the importance of confirming phenotypic identification of *S. aureus* and MRSA by molecular techniques. There was a high level of agreement between the three methods in the typing of methicillin-susceptible *S. aureus* (MSSA). Furthermore, the recognition of a predominant MSSA clone from clinical and nasal samples indicates the possible cross-infection from medical personnel to patients. A total of nine of the 50 isolates were Panton-Valentine leukocidin (PVL) positive suggesting that they are community-associated *S. aureus* isolates. The study provided baseline information on the need for effective infection control measures in health-care institutions in South-Western Nigeria.**

Key words: *Staphylococcus aureus*, phenotypic and molecular typing, methicillin-susceptible *S. aureus* clone

INTRODUCTION

Staphylococcus aureus is one of the important pathogens in many countries causing infection in hospitals and the community. It causes a variety of diseases, ranging in severity from boils and furuncles to more serious diseases such as septicaemia, pneumonia and endocarditis Lowy, 1998). Since the emergence of *S. aureus* strains with resistance to methicillin in 1961 (Jevons, 1961), it has become a well-known aetiological agent for a wide variety of infections. Methicillin-resistant *S. aureus* (MRSA) infections have become a common problem in hospital and community-acquired infections, and has been associated with prolonged hospital stay and increased costs (Kopp et al., 2004; Lodise and McKinnon, 2005; Nixon et al., 2006).

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A number of recent investigations have indicated that *S. aureus* is the main etiological agent of many infections in Nigeria (Anah et al., 2008; Odetoyin et al., 2008; Adeleke et al., 2009; Bekibele et al., 2009; Onipede et al., 2009). However, in many studies, identification and antibiotic susceptibility testing of *S. aureus* isolates have been based on phenotypic methods and few data exists on the characterization of *S. aureus* isolates using molecular methods (Adesida et al., 2005; Shittu et al., 2006; Ghebremedhin et al., 2009; Okon et al., 2009). In order to have adequate information for treatment of staphylococcal infection and the formulation of effective infection control measures, data on susceptibility patterns and characterization of *S. aureus* are of great importance. This study characterized *S. aureus* isolates obtained from two neighbouring states in South-Western Nigeria (Ekiti and Ondo States), using phenotypic and molecular techniques. Ekiti State was created from Ondo State by the Military Government of the Federal Republic of Nigeria on October 1, 1996.

MATERIALS AND METHODS

Microbiological analysis and identification

In this study, a set of 54 *S. aureus* isolates (identified by phenotypic methods) obtained between December 2005 to October 2006 from clinical samples (41) and nasal (13) samples of healthy medical personnel in the neighbouring states were analysed. A total of 23 isolates were obtained from the State Specialist Hospital, Akure and the Federal Medical Centre, Owo (Ondo State) while 31 isolates were obtained from the State Specialist Hospital, the Joe Jane Medical Centre, and the University of Ado-Ekiti Health Centre, Ekiti State. Phenotypic Identification of *S. aureus* isolates was based on growth and fermentation on mannitol salt agar, and colonial morphology on nutrient agar. Other tests included Gram stain (Gram positive cocci in clusters) and positive results for catalase, coagulase and DNase tests. The phenotypic characterization of the isolates was carried out at the Microbiology Laboratory, University of Ado-Ekiti, while the molecular study was conducted at the Department of Microbiology, University of KwaZulu-Natal, Republic of South Africa.

Antibiotic susceptibility testing

The susceptibility testing of isolates to ten antibiotics was carried out by the disk diffusion method according to the National Committee for Clinical Laboratory Standards (now Clinical Laboratory Standards Institute) guidelines (NCCLS, 2003). The antibiotics (Mast Diagnostics, UK) included penicillin (10 U), oxacillin (1 µg), cefoxitin (30 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin, (2 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5µg) and fusidic acid (10 µg). *S. aureus* ATCC 25923 was the control strain in every test run. Detection of inducible resistance of clindamycin by erythromycin (D-test) was performed on the *S. aureus* isolates as previously described (Fiebelkorn et al., 2003). Multi-resistance was defined asresistance to at least three classes of antibiotics.

Molecular detection of the *nuc* and *mecA* genes by PCR

DNA isolation was carried out according to the method previously reported (Udo et al., 1999). All the isolates were confirmed as *S. aureus* and MRSA by PCR detection of the *nuc* and the *mecA* genes respectively based on the conditions described previously (Shittu and Lin, 2006).

PCR-RFLP of the coagulase gene

PCR amplification of the coagulase gene was performed as described previously (Goh et al., 1999). *S. aureus* ATCC 25923 served as the positive control in the PCR reaction. Restriction fragment length polymorphisms (RFLPs) of the amplicons were determined by digestion with *Alu*I (Fermentas, UK) based on modification of the protocol previously described (Annemuller et al., 1992). The sizes of the PCR products and of the restriction DNA digests (RFLPs with respect to the overall number of 81-bp tandem repeats) were estimated by comparison with a 100 bp molecular size standard marker and visual inspection. The strains were grouped as described previously (Shittu and Lin, 2006).

PFGE typing

PFGE typing of *Sma*I (Fermentas, UK)-digested DNA was carried out by a modification of a protocol described previously by Bannerman et al. (1995). Electrophoresis was performed in 0.5X TBE buffer by a contour-clamped homogeneous electric field method using a CHEF MAPPER system (Bio-Rad) as described previously. The gels were stained with ethidium bromide for 1 h, visualized under UV light and photographed using a SynGene Bioimaging System. The banding patterns were interpreted visually and the relatedness of the strains was determined according to the recommendation of Tenover et al. (1995). Strains showing the same PFGE pattern were grouped as a pulsotype and assigned alphabetically (A, B, C, etc).

Detection of panton-valentine leukocidin (PVL) genes

The PVL genes (*lukS-PV* and *lukF-PV*) were detected by PCR as described by Lina et al. (1999).

RESULTS AND DISCUSSION

The recent epidemiological analysis of *S. aureus* isolates using molecular techniques have provided important baseline information on the emergence of antibiotic-resistant *S. aureus*, and the diversity of hospital and community-acquired MRSA in Nigeria (Adesida et al., 2005; Shittu et al., 2006; Ghebremedhin et al., 2009; Okon et al., 2009). However, there is no report on the characterization of *S. aureus* isolates in Ondo and Ekiti States in South-Western Nigeria. Of the 54 isolates analysed, 50 (37 - clinical; 13 - nasal) isolates were confirmed as *S. aureus* by PCR detection of the *nuc* gene. They comprised 21 (13 - clinical; 8 - nasal) and 29 (24 - clinical; 5 - nasal) isolates from Ondo and Ekiti States respectively. Misidentification of bacterial pathogens has dire consequences on the patient along with increased medical cost and this observation has established the importance of confirming phenotypic identification of *S. aureus* and MRSA by molecular techniques.

The knowledge of the local antimicrobial resistance patterns of bacterial pathogens is essential to guide em-

Table 1. Relationship between antibiotyping, PCR-RFLP of the coagulase gene, detection of the PVL gene and PFGE in MSSA and MRSA isolates from Nigeria.

Type	Coagulase gene Molecular weight (±20 bp)	PCR-RFLP Coagulase gene	PFGE type	PVL Gene (number of strains)	Antibiotype - resistance pattern (number of strains)	*mecA*-positive	*mecA*-negative	Total number of strains
1	470	486	ND	-	PEN (1)	0	1	1
2	550	567	ND	-	PEN (1)	0	1	1
3a	720	243, 405	E	-	PEN, TET, GN, CIP (2)	0	2	2
3b		243, 486	C	-	PEN, CHL, TET (2)	0	2	2
3c		81, 243, 405	E1	-	PEN, CHL, TET, GN (1)	0	1	1
4	800	81, 243, 486	A	+ (1)	PEN (2) PEN, CHL, TET, ERY (10) PEN, CHL, TET (9)	0	21	21
5a	400, 800	405, 810	F	-	PEN (9)	0	9	9
5b		81, 324, 405, 810	ND	+ (1)	PEN (1)	0	1	1
6	850	81, 162, 567	B	-	PEN, OX, TET	1	0	1
7	980	162, 324, 486	D	-	PEN (1) PEN, TET (2) PEN, CHL, TET, ERY (1)	0	4	4
8	400, 980	162, 324, 405, 486	ND	+ (7)	PEN (2) PEN, TET (5)	0	7	7

Key: ND: Not determined, PEN - Penicillin, OX - Oxacillin, GN - Gentamicin, ERY - Erythromycin, CHL - Chloramphenicol, TET - Tetracycline, CIP – Ciprofloxacin.

pirical and pathogen specific therapy. The antibiotic susceptibility testing showed that all the isolates were susceptible to fusidic acid but they were 100% resistant to penicillin, 35 (70%) to tetracycline, 11 (22%) to erythromycin, 3 (6%) to gentamicin and 2 (4%) to ciprofloxacin. The susceptibility of *S. aureus* to fusidic acid observed in this study is in agreement with a previous study conducted in South-Western and North-Eastern Nigeria (Shittu et al., 2006; Okon et al., 2009), indicating that fusidic acid is an excellent and effective agent for the treatment of *S. aureus* infections in Nigeria. However, in order to minimize the emergence of fusidic-acid resistant strains, Howden et al. (2006) advised that monotherapy with fusidic acid should be discouraged and a combination with another anti-staphylococcal agent is recommended. A total of 25 of the 49 MSSA isolates (51%) were multi-resistant while the predominant antibiotypes were resistance to penicillin only (17 isolates), resistance to penicillin, chloramphenicol and tetracycline (11 isolates), resistance to penicillin, chloramphenicol, tetracycline, and erythromycin (11 isolates), and resistance to penicillin and tetracycline (7 isolates). All the isolates did not exhibit inducible or constitutive resistance to clindamycin by the D-test method and only one isolate from Ekiti State was confirmed to be MRSA.

Phenotypic and genotypic methods have been used to a great advantage in classifying epidemiologically related strains. Characterization based on antibiotic susceptibility testing has been regarded as a timely and inexpensive tool for MRSA phenotyping and for identifying specific clones (Amorim et al., 2007; Nimmo et al., 2008). PCR-RFLP of the coagulase gene has been widely used in the clinical laboratory because of its ease and speed, and has been widely used in genotyping clinical and non-clinical *S. aureus* strains (Montesinos et al., 2002; Mitani et al., 2005; Shittu and Lin, 2006; Ishino et al., 2007; Moon et al., 2007; Saei et al., 2009). Moreover, macro-restriction followed by pulsed-field gel electrophoresis (PFGE) is considered the gold standard method for epidemiological typing of a variety of bacterial species including *S. aureus* since it is a highly discriminatory, stable and reproducible method (Faria et al., 2008). Typing data of the isolates based on antibiotyping, PCR-RFLP of the coagulase gene and PFGE are illustrated in Table 1. A single amplicon which ranged from 470 - 980 bp was observed in 32 MSSA isolates while two PCR products of 400, 800 and 400, 980 bp were identified in ten and seven isolates respectively. A total of seven groups were observed among the MSSA isolates and no restriction fragment was detected in types 1, 2 and 5a. The RFLPs of the *S. aureus* isolates are illustrated in Figure 1. Type 4 (800; 81, 243, 486bp) was the predominant type followed by types 5a and 8. The MRSA isolate was grouped in type 6. Data on antibiotyping and PCR-RFLP of the coagulase gene showed that the predominant antibiotype (resistance to penicillin) was observed in types 1, 2, 4, 5, 7 and 8. Moreover, no restriction fragment was detected in the MSSA isolates that were resistant only to penicillin in type 5a. Furthermore, three antibiotypes were identified in the predomi-

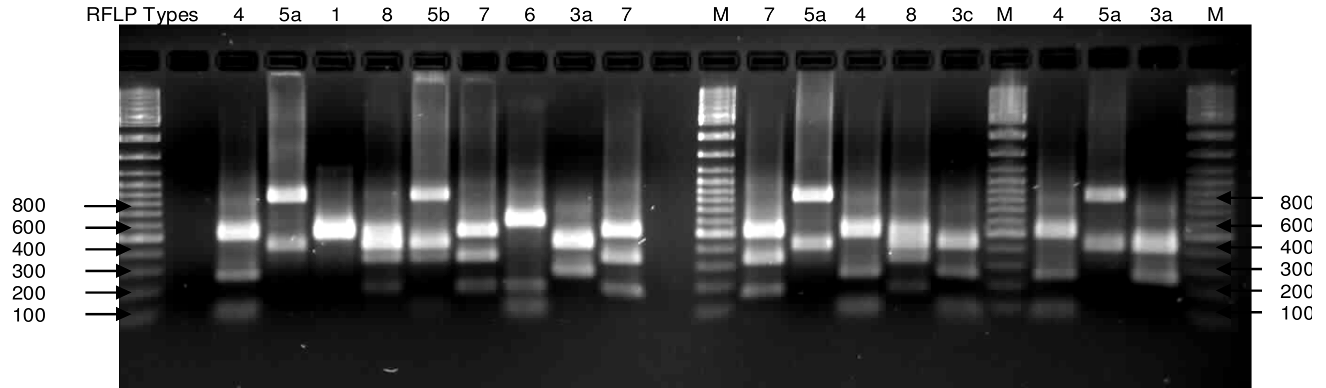

Figure 1. PCR-RFLPs of the *S. aureus* isolates from Nigeria. Lanes 1, 13, 19, 23: 100 bp molecular weight markers. Lanes 2 and 12: Negative control; Lane 3: Type 4; Lane 4: 5a; Lane 5: 1; Lane 6: 8; Lane 7: 5b; Lane 8: 7; Lane 9: 6; Lane 10: 3a; Lane 11: 7; Lane 14: 7; Lane 15: 5a; Lane 16: 4; Lane 17: 8; Lane 18: 3c; Lane 20: 4; Lane 21: 5a; Lane 22: 3a.

PFGE of clinical isolates of *S. aureus* from Ekiti State, Nigeria

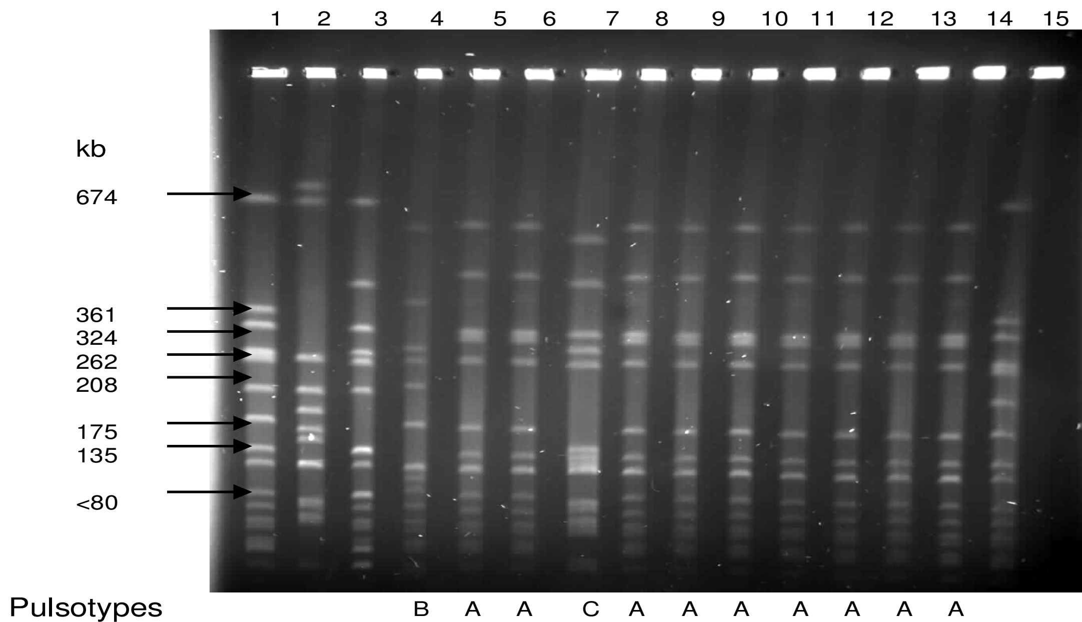

Figure 2. PFGE profiles of *S. aureus* isolates from Nigeria. Lanes 1 and 15: *S. aureus* NCTC 8325; Lane 2: 28IDA (MRSA from a previous study in Nigeria) Lane 3: THCD (MRSA from a previous study in Nigeria); Lane 4: EK32; Lane 5: EK22; Lane 6: EK34; Lane 7: EK44; Lane 8: EK48; Lane 9: EK49; Lane 10: EK25; Lane 11: EK29; Lane 12: EK 31; Lane 13: EK13; Lane 14: EK20. EK - Ekiti State.

nant type 4 and 19 of the 25 multiresistant MSSA isolates clustered in this group (Table 1).

There was good correlation between the two molecular typing methods (PFGE and PCR-RFLP of the coagulase gene). The MSSA isolates grouped in the predominant type 4 (PCR-RFLP) were classified in pulsotype A by PFGE (Figures 2 and 3) indicating that they were closely related. This dominant clone was identified among isolates obtained from clinical samples in Ekiti State and nasal samples of health care personnel in Ondo State. However, MSSA isolates from nasal samples of medical personnel in Ekiti State did not belong to this clone but were identified as pulsotypes E and F (Figure 3). This observation suggests that healthcare workers (HCWs) in Ondo State could serve as important reservoirs for strains capable of colonization and spread to patients in Ekiti State. Furthermore, isolates grouped in type 5a were classified in pulsotype F and type 3a, 3c in PFGE type E. The banding pattern of the MRSA isolate (PFGE) was different from the MSSA isolates as observed for the

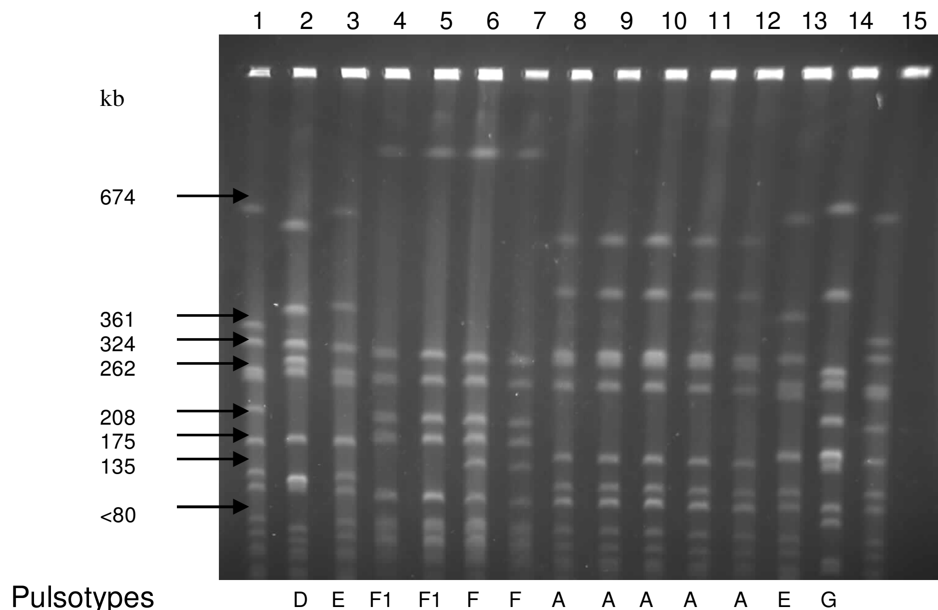

Figure 3. PFGE profiles of *S. aureus* isolates from Ekiti and Ondo State, Nigeria. Lanes 1 and 15: *S. aureus* NCTC 8325; Lane 2: EK1*; Lane 3: AK67*; Lane 4: EK2*; Lane 5: EK3; Lane 6: AK65*; Lane 7: AK66*; Lane 8: EK5; Lane 9: AK62*; Lane 10: AK63*; Lane 11: ND118*; Lane 12: ND120*; Lane 13: EK9*; Lane 14: EK7. AK and ND: Ondo State; * nasal isolates.

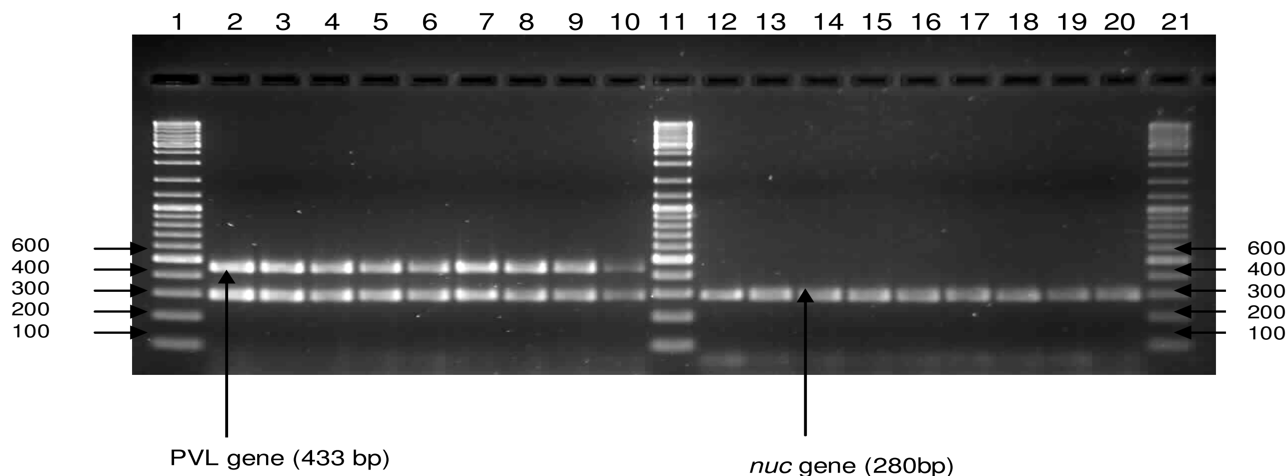

Figure 4. Detection of the *nuc* and PVL genes of *S. aureus* isolates from Nigeria. Lanes 1, 11 and 21: Molecular weight marker (100bp); Lane 2: EK38; Lane 3: EK43; Lane 4: EK7; Lane 5: AK19; Lane 6: AK57; Lane 7: AK31; Lane 8: AK27; Lane 9: AK28; Lane 10: WW149; Lane 12: EK33; Lane 13: AK56; Lane 14: AK67; Lane 15: EK37; Lane 16: EK 44; Lane 17: WW148; Lane 18: AK39; Lane 19: ND120; Lane 20: EK 48. EK - Ekiti State; AK, ND and WW - Ondo State.

PCR-RFLP of the coagulase gene.

The Panton-Valentine Leukocidin (PVL), which is encoded by *lukS-PV-lukF-PV* is a component of the phage genome in the chromosome which was first reported in reported in 1932 (Panton et al., 1932). The PVL is a bicomponent cytotoxin that is preferentially linked to furuncles, cutaneous abscess and severe necrotic skin infections (Lina et al., 1999). Although the detection of the *lukPV* gene and its association in community-acquired MRSA is controversial, it is generally accepted as a common virulence marker in community-associated MRSA (Strommenger et al., 2008). In this study, nine of the 50 (18%) isolates were PVL positive (Figure 4) which was lower than recent studies conducted in Nigeria

(Ghebremedhin et al., 2009; Okon et al., 2009). It is worthy of note that all the seven MSSA isolates in type 8 were PVL positive, indicating that most of the PVL gene-positive MSSA strains were distinct from PVL-gene negative MSSA strains, and suggesting that the PVL genes is distributed to certain specific populations of *S. aureus* clones. Although the number of isolates investigated was limited and sequence-based methods like *spa* and multilocus sequence typing (MLST) were not employed, the study established the importance of confirming phenotypic identification of *S. aureus* by molecular methods. Secondly, a predominant MSSA clone was identified in Ondo and Ekiti States based on phenotypic and molecular typing methods. Thirdly, PVL positive MSSA from nasal and clinical samples was detected in this study. The investigation provided baseline information on the need for establishing effective infection control measures in health-care institutions in South-Western Nigeria. More studies are needed in understanding the epidemiology of *S. aureus* in this region.

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