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Original Article

Prevalence and Antibiotic susceptibility pattern of Panton-Valentine Leucocidin(PVL) positive Staphylococcus aureus Strains from clinical specimens in Northeastern Nigeria

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Abstract

Panton-Valentine leucocidin (PVL), a synergohymentropic toxins encoded on S.aureus genes are associated with soft tissue infection and community-acquired staphylococcal infection. The purpose of the study was to determine the prevalence and antibiotic susceptibility of PVL-postive S.aureus isolates from clinical specimens.A total of 96 consecutive S.aureus isolates were examined. 12(12.5%) methicillin-resistant S.aureus strains(MRSA) and 84(87.5%)methicillin-sentive S.aureus(MSSA) identified by disc-diffusion and PCR assay methods. Screening of S.aureus isolates for PVL locus by PCR assay, 50(52.1%) amplified the PVL genes, 35(70.0%) were recovered from outpatient, 15(30.0) from inpatient. PVL positive S.aureus were isolated from wound specimens, 20 (40.0%); 9(18.0%) urine, 6(12.0%) and least 1(2.0%) each from blood culture and endocervical swab. Staphylococcal cassette chromosome mec typing by two standard multiplex PCR assay, revealed an uncharacterized resistance element. Overall antibiotic susceptibility pattern showed relatively high degree of susceptibility,however 1 isolate demostrated multidrug resistant pattern, 37(74.0%)resistant to only penicillin, 5 to one additional drug with penicillin, and 3 to two-additional drugs. The high prevalence of S.aureus PVL-positive strains posed dire clinical conquences, because co-existence of MRSA strains with MSSA PVL –positive strains could result in the emergence of MRSA PVL-positive strains, with propensity of rapid dissemination within the hospital environment in the study area.

Keywords; Panton-Valentine leucocidin, S.aureus , epidemiology, Northeastern Nigeria

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Introduction

Panton-Valentine Leucocidin (PVL), a bicomponent exotoxin was first described by Panton and Valentine (1932). It is a *S.aureus* specific exotoxin that forms part of the family of bicomponent synergohymenotropic toxins, which action directed towards cell membrane, particularly on the white blood cells (Gravet *et al.* 2001). The PVL components binds to neutrophil, induces release of chemotatic factors like IL-8 and leukotriene B4 as well as variety of inflammatory mediators responsible for cell death (Konig *et al.* 1995), and also soft tissue infection and community-acquired infection (Lina *et al.* 1999).

PVL locus is encoded by lukF-PV (939nucleotides) and lukS-PV (978nucleotides) genes, contiguous and co-transcribed and separated by only thymine and protein of 32 and 38kDa respectively (Prevost *et al.* 1995). These genes are located on four phages, φ108PVL, φPVL, φSLT, and φSa2mw (Ma *et al.* 2002, Feng *et al.* 2008).

The changing trend of MRSA epidemiology,showed the use of PVL locus detection as a marker of CAMRA isolates, alongside with nonmultiresistant pattern and SCCmec type IV or V (Deurenberg and Stobbering, 2009). Epidemiologically, prevalence of PVL locus in S.aureus strains varies with geographical location and tissue specimens (Goering *et al.* 2008). In Africa, high prevalence level had been reported from Mali (Aires de Sousa et al., 2006), Algeria (Ramdam-Bougnassa *et al.* 2006), and in Ibadan southwestern Nigeria (Ghebremedhin *et al.* 2009).

In Nigeria, available data on *S.aureus* PVL-positive strains is scanty, except the recent report from Ibadan, southwestern Ibadan on community-associated methicillin-resistant *S.aureus* (Ghebremedhin et al., 2009). Clinical presentation of staphylococcal infection ranged from superficial to systemic infection. However, because of paucity of information on *S.aureus* PVL-positive (Baba-Moussa et al., 2008) strains and its associated clinical implication, it is possible to speculate that significant proportion might be undiagnosed in our medical laboratories. Therefore, epidemiological information derivable from the study would form the baseline for further research area and subsequently shed light on the magnitude of clinical problem associated with staphylococcal infection relation to PVL-positive S.aureus strains and the needs for urgent intervention measures.

This study examined the phenotypic and molecular characterization of S.aureus PVL-positive isolates from clinical specimens in the northeastern, Nigeria.

Materials and Methods

The study area comprised of six administrative state of the northeastern Nigeria , boarded by three republics namely, Niger, Chad and Cameroon. The *S.aureus* isolates were recovered between January-December 2007 from the six tertiary hospitals in each of the administrative states. The hospitals are multidisciplinary in medical practices, bed size capacity ranged from 250-500. The University of Maiduguri

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Teaching Hospital , located in the capital of Borno state, doubles as the major referred center to the other tertiary hospital in the geographical zones. Ninety-six non-duplicate, consecutive *S.aureus* isolates were collected from the six tertiary hospitals in the study area. Epidemiological information collected with bacterial isolates includes, age, sex of the patient, type and source of clinical specimens. The source of clinical specimen were classified into inpatient, of patients on admission on the wards and outpatient , of those patient seen on outpatient basis.

S.aureus was identified on the basis of colony and microscopic morphology, tube coagulase, catalase and DNase test. Antibiotic susceptibility testing of the S.aureus was determined by disc diffusion method according to CSLI guidelines, using the following antibiotics, oxacillin(OX)(1ug), cefoxitin(FOX) (30ug) penicillin(PEN) (10IU), cotrimoxazole (SXT) (25ug),erythromycin (ERY) (15ug), ciprofloxacin (CIP) (5ug), gentamycin(GEN) (10ug), vancomycin(VAN) (30ug),rifampicin(RP)(30ug), fusidic acid(FA) (10ug) mupirocin(MUP) (5ug). Methicillin resistance of S.aureus isolates was determined by using the CSLI breakpoint of oxacillin and cefoxitin discs and PCR assay for mecA gene. Staphylococcal Cassette Chromosome SCCmec typing were determined by multiplex PCR technique as previously described by Kondo *et al* (2007) and Oliveria *et al.* (2006).

Screening of PVL locus was determined by PCR assay according to method described by Lina *et al.* (1999). The primer sequence for the PVL gene were as follow; for luk-PV-1, 5'-ATCATTAGGTAAAATGTCTGGACATG ATCCA-3'; for luk-PV-2, 5'-GCATCAASTGTATTGGATAGCAAAAG C-3'.The amplification condition is as follow, intial denaturation set at 94^0C for 30 seconds, annealing at 55^0C for 30seconds and extension at 72^0C for 1minute, a total of 30cycles.The PCR products was resolved by electrophoresis through 1.5% agarose gel. The amplified PVL bands were stained with GelRed stains. Positive and negative control were included in the analysis.

Results

Of the 96 *S.aureus* isolates screened for PVL locus, 50 (52.1%) were scored PVL-positive isolates, all were methicillin-sensitIve S.aureus , and the 12 MRSA isolates were negative. Demographic information of the S.aureus PVL-positive strains (table 1), showed that the mean age of patient with staphylococcal infection was 27+19.7years, gender distribution of 33(66.0%) males and 17 (34.0%) females. High prevalence of PVL-positive strains were recovered from patient within age-group 30-39years (13.5%), followed by <10years (12.5%) and the least in 10-19 and >50years with 5(5.2%) each. Fifteen (30.0%) of 50S.aureus PVL-positive were isolated from inpatient, and 35(70.0) from outpatients. The dissemination of S.aureus PVL-positive strains within the hospitals

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studied, majority were recovered from UMTH 36(72.0), followed by FMC Azare 6 (12.0), Gombe 4 (8.0), Yola 3 (6.0) and the least from Jalingo 1 (2). Distribution of PVL-positve S.aureus isolates with clinical specimens are as follows; 20 (40.0) wounds, urine 9 (18.0),Ear swab 6 (12.0), HVS 6(12.0) urethral swab 3(6.0), 2(4.0) each from pus and semen and 1(2.0) each from blood culture and endocervical swab respectively. The result of both SCCmec typing revealed the presence of uncharacterized SCCmec types. Overall antibiotic susceptibility pattern of the S.aureus PVL-positive strains, showed high resistant pattern, with majority (37/50, 74.0) of isolates resistant to penicillin, 5 isolates were to penicillin and one-additional drugs, 3 to two-additional drug and 4 to three-additional drugs and one was susceptible to all the drugs tested

Table I: Demographic information of *S.aureus* PVL-positive isolates (n=50)

Age		27.15±15.05 years
Sex	Male	33(66.0)
	Female	17(34.0)
Distribution within age-group		
	<10years	12(12.5)
	10-19	5(5.2)
	20-29	8(8.3)
	30-39	13(13.5)
	40-49	7(7.3)
	>50	5(5.2)
Source of *S.aureus* isolates		
	Inpatient	15(30.0)
	Outpatient	35(70.0)

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Dissemination within the tertiary hospitals

UMTH	36(72.0)
Azare	6(12.0)
Nguru	-
Gombe	4(8.0)
Jalingo	1(2.0)
Yola	3(6.0)

Distribution of the isolates within clinical specimens analysed

Wounds specimens	20(40.0)
Urethral swab	3(6.0)
Pus	2(4.0)
Semen	2(4.0)
HVS	6(12.0)
Blood	1(2.0)
Urine	9(18.0)
Ear swab	6(12.0)
Endocervical swab	1(2.0)

Table 2: Antibiotic resistance pattern of the *S.aureus* PVL-positive isolates

Susceptible to all antibiotics	1(2.0)	
PEN-	37(74.0)	
ERY	2(4.0)	
CIP	1(2.0)	
PEN,ERY	2(4.0)	
PEN,SXT	1(2.0)	
PEN,CIP		2(4.0)
PEN,SXT,CIP	2(4.0)	
PEN,ERY,CIP	1(2.0)	
PEN,ERY,SXT,CIP	1(2.0)	

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Discussion

The finding of our study had addressed salient aspect of S.aureus epidemiology that is lacking in the study area.There have been varied reports on the prevalence level of PVL-positive S.aureus isolates in Nigeria and other parts of Africa based on clinical conditions, geographical locations, and studied population. In our study, the prevalence of PVL-positive S.aureus strains of 52.1% may be considered high for an environment without no previous epidemiological data for comparsion. Nevertheless, high prevalence level had been reported in studies conducted elsewhere in Africa, for example prevalence level of 52.0% in Mali(Aires de Sousa *et al.* 2006) 72.0% in Algeria (Ramdam-Bourgnassi *et al.* 2006) and 20.0% in Ibadan(Ghebremedhin et al., 2009, Baba-Moussa *et al.* 2008), which were mainly among MRSA isolates as against MSSA isolates recorded in this study. Studies have shown that prevalence level of PVL locus varies with geographical location, and clinical specimen (Goering *et al.* 2008, Campbell *et al.* 2008). In Europe and US, the prevalence is relatively low(<5) (Kuchnert *et al*. 2006) while data from Asia had reported high up to 60% (Hsu *et al.* 2007, Afroz *et al.* 2008), prevalence of 40% was documented in Arkangia region of Russia(Vorobieva *et al.* 2008) and in Germany (50.0%) (Monecke *et al.* 2007). The close association between PVL positive S.aureus strain and tissue infection and community –acquired infections have been reported(Lina *et al.* 1999), this is consistent with finding of our study, as wound specimens accounted for 20(40.0) of the PVL-positive strains detected. In addition, to wound, pus and abscess, in which high proportion of PVL-positive S.aureus have been recovered as documented in most studies. Relatively high prevalence level have been reported in clinical specimens like urine (Baba-Moussa *et al.* 2008), trachea and CSF(Moroney *et al.* 2007).In this study, urine specimen was second in frequency of occurrence of PVL-positive S.aureus isolates, and futher affirmed S.aureus as one of the leading aetiological agent of UTI.

There was relatively wide dissemination of the PVL strains within the tertiary hospitals where the isolates were recovered from, which affirmed the possibility that these hospital might be serving as reservoir. This observation raises the question to what degree is good hospital hygiene and cleaning procedures carried out in these tertiary hospital involved in the study.The propensity of rapid dissemination of pvl genes might be facilitated by intra/ interhospital transmission between colonized or infected patients. The *pvl* gene are carried on temperate phage that allows for rapid dissemination especially in hospital environment (Narita et al.,2001) . The prevalence level of these strains might further increase in these hospital because of the relatively low level awareness of S.aureus PVL-positive strain, its associated clinical implication and non-effective infection control units. Clinical implication of the high level, is the co-existence with MRSA strains which could result in the acquisition of mecAgene and intergration of pvl gene into the MSSA lineage, therefore resulting in the emergence of MRSA PVL-positive strain that had been reported(Grebrehemdhin *et al.* 2009). Most documented report of PVL positive MRSA strains are associated with community-acqiured MRSA responsible for soft tissue infection and community – acquired pneumonia (Lina *et al.* 1999)

Overall antibiotic susceptibility pattern of PVL-positive S.aureus strain revealed

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relatively high degree of susceptibility of PVL-positive strains with drugs tested, suggestive that these drugs are still efficacious in the treatment of staphylococcal infection due to PVL-positive strains. In addition, the resistance pattern of S.aureus isolates to penicillin is unsurprising as similar pattern had been reported in most documented studies. In most sub-Saharan communities, antibiotic prescription without laboratory investigation and over-the-counter purchase practice is a common norm. In such situation, excessive usage of unprescribed antibiotic could exert selective pressure effect, which could encourage emergence of resistance strains.

In conclusion, the prevalence of S.aureus PVL-positive strain of 52.1% is high for geographical region without previous epidemiological data and is of serious clinical consideration. The reason is the close proximity with MRSA strain in the same hospital environment with MSSA PVL-positive strain that could facilitate rapid dissemination, integration into susceptible MSSA strain and possibly emergence of MRSA PVL-positive strain.

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