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Molecular Detection of Panton-Valentine Leukocidin (PVL) Toxins in Clinical Isolates of *Staphylococcus aureus* from Maitama District Hospital, Abuja, Nigeria

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Abstract

Pathogenic *Staphylococcus aureus* is the most frequently isolated Gram positive bacterium from clinical specimens; and it is among the leading cause of infection in man. *S. aureus* has gained significant interest in recent years as an important nosocomial pathogen – owing to its multidrug resistant nature which is associated to several virulence factors of the organism including Panton-valentine leukocidin (PVL). PVL is part of the toxins produced by pathogenic *S. aureus* – which help the organism to exacerbate their pathogenicity/virulence in the phase of an infection. This study evaluated the prevalence of PVL-positive *S. aureus* from clinical specimens – owing to the dearth of information on this subject matter in Nigeria. Out of the 118 non-consecutive *S. aureus* isolates employed for this study, only 56 isolates were biochemically confirmed as pathogenic *S. aureus*. The antibiogram showed that the *S. aureus* isolates were most susceptible to gentamicin, vancomycin, ciprofloxacin, erythromycin and linezolid. However, they were highly resistant to the cephamycin, cefoxitin (82.1%). The *S. aureus* isolates also showed reduced susceptibility to tigecycline (71.4%), clindamycin (66.1%), chloramphenicol (48.2%), amoxicillin-clavulanic acid (53.6%) and sulphamethoxazole-trimethoprim (48.2%). The prevalence of PVL genes in this study was 10.7%. Only 6 isolates of *S. aureus* (10.7%) were confirmed by PCR to harbour the PVL genes; and these *S. aureus* isolates were from wound samples, abscess and urine samples. The occurrence of pathogenic *S. aureus* harbouring drug resistant genes such as PVL genes in the hospital environment pose serious health and therapeutic challenges especially in choosing antimicrobial therapy for treatment. *S. aureus* isolates with PVL genes could also disseminate with high propensity within the hospital environment; and this could result in the outbreak of nosocomial infections. Continues antibiotic stewardship in our hospitals will help in the control and prevention of the emergence and dissemination of drug-resistant microbes in both the community and hospital environment.

Keywords: Panton-Valentine Leukocidin (PVL); *Staphylococcus aureus*; Nosocomial infections; Resistance; Antibiotics; Nigeria

Introduction

Staphylococcus aureus is a non-sporulating, nonmotile Gram positive opportunistic pathogen that is usually carried asymptomatically on the body of humans and animals, and they cause a plethora of pathological/disease conditions in humans including skin infections, meningitis, pneumonia, arthritis, endocarditis and osteomyelitis. Pathogenic *Staphylococcus aureus* still remains an important nosocomial and community-acquired pathogen because of its multidrug resistant nature and its association in several clinical episodes including skin infections and blood-borne infections [1,2]. *Staphylococcus aureus* has evolved from a once susceptible pathogen to becoming a multidrug resistant organism; and some strains of *S. aureus* including methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *S. aureus* (VRSA) now occur in the community and hospital environments [3,4]. Antibiotic-resistant bacteria pathogens cause serious therapeutic challenge to physicians, patients and other healthcare providers, and the continued intense and misuse of antibiotics (for both clinical and non-clinical purposes) are undoubtedly amongst the many reasons for their emergence and spread in the community and hospital environment [5-7]. *Staphylococcus aureus* (which is notorious in evolving resistance to β-lactam drugs) accounts for many hospital- and community-acquired infections including skin infections, bacteraemia, and pneumonia, and some strains of *S. aureus* are resistant to penicillins and some β-lactams. Both hospital acquired and community-acquired stains of pathogenic *S. aureus* produce different toxins that help to exacerbate their pathogenicity/virulence in the phase of an infection e.g. Panton-Valentine leukocidin (PVL), and Toxic-shock-syndrome-toxin-1 (TSST-1) [4,8-10]. The presence of PVL has been extensively described for methicillin-resistant *S. aureus* (MRSA), specifically in association with staphylococcal cassette chromosome mec (SCCmec) type IV and also SCCmec type V genes – upon which mecA genes (responsible for) are found [6,11,12]. According to Amini et al., there are five (5) types of SCCmec genes – which are Types I, II and III (found in hospital-associated MRSA strains) and Types IV and V (which are mostly found in community-acquired MRSA strains). Nevertheless, some reports are of the view that there are eight types of SCCmec genes (Types I-VIII). The emergence and spread of antibiotic resistant

strains of pathogenic *S. aureus* especially those harbouring PVL toxins should be kept under check through accurate and prompt monitoring, evaluation, and detection of resistant pathogenic strains of *S. aureus* from suspect specimens in both the community and hospital environment. This study evaluated the occurrence of pathogenic *S. aureus* harbouring Panton valentine leukocidin (PVL) toxins from clinical samples.

Materials and Methods

Collection of isolates

A total of 78 non-duplicate *Staphylococcus aureus* strains were obtained from the culture collection unit of the Medical Microbiology laboratory of Maitama District Hospital in Federal Capital Territory (FCT), Abuja, Nigeria. The isolates were collected over a period of six (6) months (November 2014 to April 2015), and transported to the laboratory of Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Kaduna state, Nigeria for further processing.

Purification and re-identification of isolates

To purify the *S. aureus* isolates, each of the collected isolates was inoculated on nutrient broth overnight at 37ºC overnight; and a loopful of the turbid culture was streaked on Nutrient agar plates (Oxoid, UK) and incubated at 37ºC for 18-24 hrs. Identification of the bacterial isolates was carried out using standard microbiological procedures as was previously described including catalase test, coagulase, Gram staining, colony morphology and fermentation of mannitol [13,14].

Antibiogram

Antibiotic susceptibility testing was determined by the Kirby-Bauer disk diffusion method as per the Clinical Laboratory Standard Institute (CLSI) criteria using single antibiotic disks including gentamicin (10 µg), ciprofloxacin (5 µg), cefoxitin (30 µg), vancomycin (30 µg), erythromycin (15 µg), tigecycline (15 µg), clindamycin (2 µg), Sulphamethoxazole- trimethoprim (25 µg), chloramphenicol (30 µg), linezolid (10 µg), amoxicillin/clavulanic acid (20/10 µg). Briefly, an overnight culture of the test isolates (adjusted to 0.5 McFarland turbidity standards) was aseptically swabbed on Mueller-Hinton (MH) agar plates (Oxoid, UK), and single antibiotics disks were aseptically placed on each of the plates. The sensitivity plates were then allowed again to stay for 30 minutes for pre-diffusion time before they were incubated at 37ºC for 18-24 hours. All the plates were examined for zones of inhibition and the isolates were classified as "resistant", "intermediate", and "sensitive" based on the standard antibiotic breakpoints of CLSI [12,15].

PCR detection of PVL genes

The details of the primer sequences and other thermal cycler PCR conditions are summarized in Table 1. The PCR reaction mixture was made up to 12.5 µl, and this contained 1.0 µl of each primer (100 pmol; PROMEGA, USA), 2.5 U Taq polymerase, 2.5 mM $Mg^{2+,}$ and 2.5 µl PCR buffer. Finally, 4 µl template DNA preparations were added to the PCR reaction mixture. The PCR programme included an initial denaturation step at 94°C for 3 min, followed by 30 cycles of 94°C for 30 sec; annealing step at 56°C for 30 sec and extension step at 72°C for 1 min. A final extension step was done at 72°C for 5 min. The PCR reaction mixture was subjected to thermal cycling; and the PCR products were determined by agarose gel electrophoresis technique (using 1.5% agarose gel with Tris-acetate buffer, TAE, at a volume of 4.0 mmol/l Tris, 1 mmol/EDTA, and pH 8.0), and this was run at a voltage of 160V for 40 mins. The PCR products were visualized under UV light in the presence of ethidium bromide solution [9,12,16].

Gene	Primer sequence	Amplicon size (bp)	Reference
LukS-PVL –F	5'CATGCCATGGATAACAATATTGAGAAT 3'	500	Lina et al. (1999)
LukS-PVL –R	5'CCGCTCGAGTCAATTATGTCCTTTCA3'		
LukF-PVL –F	5'CATGCCATGGCTCAACATATCACAC3'	936	Lina et al. (1999)
LukF-PVL –R	5'CCGCTCGAGTTAGCTCATAGGATTTTTTTCC3'		

Table 1: Oligonucleotide primer sequences and PCR conditions used for detection of PVL genes.

Results

This study evaluated the occurrence of Panton-Valentine Leukocidin (PVL) toxin in clinical isolates of *Staphylococcus aureus* (n=118) clinical isolates from Maitama district hospital, Abuja, Nigeria. Out of the 118 clinical isolates of *S. aureus* employed for this study, only 56 isolates were biochemically confirmed as pathogenic *S. aureus*; and these 56 isolates of *S. aureus* were tested for their antimicrobial susceptibility. Figure 1 shows the percentage distribution of the *S. aureus* isolates according to the specimens they were isolated from. Most of the isolated *S. aureus* came from abscess (29%), wound (11%), sputum (25%), urine (18%), swabs (5%), blood (5%), and semen (2%). The highest number of *S. aureus* isolates was from abscess samples (29%) while semen samples produced the lowest number of *S. aureus* isolates (2%). The antimicrobial susceptibility patterns of the isolated *S. aureus* are shown in Table 1.

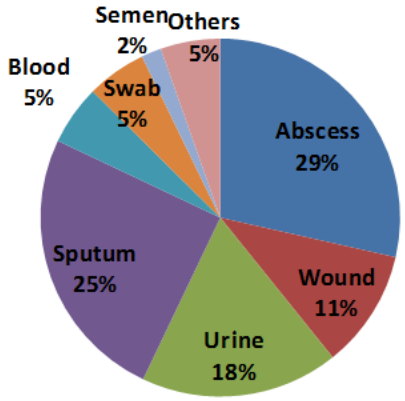

Figure 1: Percentage distribution of confirmed *S. aureus* isolates.

Antibiotics (µg)	Susceptible n (%)	Resistant n (%)
Cefoxitin (30)	10 (17.9)	46 (82.1)
Gentamicin (10)	48 (85.7)	8 (14.3)
Linezolid (10)	35 (62.5)	21 (37.5)
Vancomycin (30)	45 (80.4)	11 (19.6)
Amoxicillin/clavulanic acid (20/10)	26 (46.4)	30 (53.6)
Tigecycline (15)	16 (28.6)	40 (71.4)
Chloramphenicol (30)	29 (51.8)	27 (48.2)
Ciprofloxacin (5)	35 (62.5)	21 (37.5)
Erythromycin (15)	30 (53.6)	26 (46.4)
Clindamycin (2)	19 (33.9)	37 (66.1)
Sulphamethoxazole-trimethoprim (25)	29 (51.8)	27 (48.2)

Table 2: Antibiogram of *S. aureus* isolates (n=56).

Most of the *S. aureus* isolates were susceptible to the tested antibiotics. The highest level of antibiotic susceptibility of the *S. aureus* isolates was observed against gentamicin (85.7%); and this was followed by vancomycin (80.4%), ciprofloxacin (62.5), linezolid (62.5) and erythromycin (53.6%). However, the *S. aureus* isolates still showed reduced susceptibility to some of the tested antibiotics – which are also vital for the treatment of infections caused by pathogenic *S. aureus*. The highest level of antibiotic resistance was observed against cefoxitin (82.1%), a cephamycin antibiotic with broad-spectrum of antimicrobial activity. Least susceptibility of the *S. aureus* isolates was also observed against tigecycline (71.4%), clindamycin (66.1%), amoxicillin-clavulanic acid (53.6%), sulphamethoxazole-trimethoprim (48.2%), chloramphenicol (48.2%) and erythromycin (46.4%) (Table 2). The result of the PCR amplification of Panton-valentine-leukocidin (PVL) genes in the isolated pathogenic *S. aureus* isolates is shown in Table 2. Figure 2 showed the amplicon of the PVL genes particularly LukS-PVL gene and LukF-PVL gene – which are responsible for PVL-mediated antimicrobial resistance and the virulence nature of pathogenic *S. aureus* isolates.

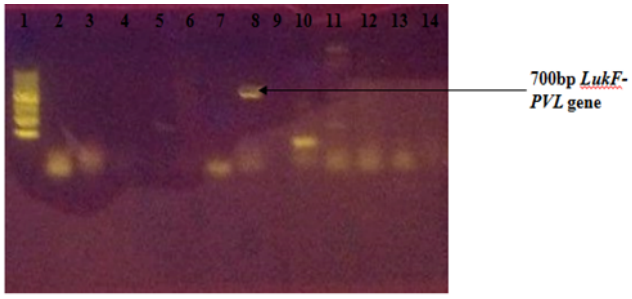

Figure 2: Amplicons of the LukF-PVL gene of *S. aureus* with a molecular size of 700 bp. Lane 1 is 100 bp molecular marker as a size marker, lane 2 is positive control, lanes 3-13 are different samples of the pathogenic *S. aureus*; and lane 14 is negative control.

Out of the 56 isolates of pathogenic *S. aureus* used in this study, only 6 isolates of *S. aureus* (10.7%) were confirmed by PCR to harbour the PVL genes (Table 3). These 6 isolates of *S. aureus* tested positive for the presence of LukS-PVL gene (n=2) and LukF-PVL gene (n=4) as shown in Table 3. The LukF-PVL gene was more prevalent than the LukS-PVL gene is this study. A closer look at these 6 isolates of *S. aureus* that harboured the PVL genes in this study showed that 1 *S. aureus* was recovered from outpatients while the remaining 5 isolates of *S. aureus* was from inpatients. The PVL-positive *S. aureus* isolates were isolate from wound specimen (n=3), abscess (n=2) and from urine specimen (n=1).

Resistance marker	Number of positive isolates	Percent of positive isolates
LukS-PVL	2	3.6
LukF-PVL	4	7.1
Total	6	10.7

Table 3: Prevalence of virulence-associated (PVL) genes amongst the *S. aureus* isolates (n=56).

Discussion

Pathogenic *Staphylococcus aureus* is responsible a plethora of nosocomial and community-acquired infections including wound burn infections, skin infections and urinary tract infections (UTIs); and this organism is a commonly isolated pathogen that may harbour some multidrug resistant genes that allow them to ward-off potent antimicrobial onslaught. This study determined the frequency of Panton-Valentine Leukocidin production (PVL) in clinical isolates of *S. aureus* clinical isolates from Maitama district hospital, Abuja, Nigeria. A total of 118 non-consecutive isolates of *S. aureus* was employed for this study, and only 56 isolates from wound infections, urine, abscesses, semen, blood, swabs and sputum specimens were biochemically confirmed to be pathogenic *S. aureus* isolates. All the *S. aureus* isolates were susceptible to a handful of the tested antibiotics. Overall, the *S. aureus* isolates were most susceptible to gentamicin (85.7%); and this was followed by vancomycin (80.4%), ciprofloxacin (62.5), linezolid (62.5) and erythromycin (53.6%). Vancomycin,

erythromycin and linezolid are typical examples of antibiotics used for the treatment of infections caused by Gram positive bacteria, and the susceptibility of the *S. aureus* clinical isolates used in this study to these antimicrobial agents show that these antibiotics are still reliable for the treatment of infections caused by these pathogens. The susceptibility of the *S. aureus* isolates to these antibiotics is similar to the report of Terry et al. [12] and Shuaihua et al. [17] who reported similar susceptibility of *S. aureus* isolates to these antibiotics. Also in this study, the *S. aureus* isolates were also found to be highly resistant to the cephamycin, cefoxitin (82.1%). Reduced susceptibility was also recorded against tigecycline (71.4%), amoxycillin-clavulanic acid (53.6%), clindamycin (66.1%), sulphamethoxazole-trimethoprim (48.2%) and chloramphenicol (48.2%). The resistance of the *S. aureus* isolates to these antibiotics were however slightly different from the report of Suleiman et al. and Amini et al. [13]. Ghamba et al. [18] also reported lower resistance of *S. aureus* isolates to chloramphenicol, erythromycin, ciprofloxacin and gentamicin. The resistance of pathogenic *S. aureus* isolates to some readily available antibiotics is of immense clinical importance – owing to the fact that some of these organisms may be me multiply resistant, thus remaining undaunted even in the face of potent antimicrobial agents from different classes. The prevalence of PVL toxin genes in this study was 10.7%; and these *S. aureus* isolates that harboured the PVL toxin genes were from wound samples, abscess and urine samples. The prevalence of PVL toxin gene in this study is lower than the study of Shittu et al. [19] who reported that the prevalence of PVL toxin gene amongst Methicillin-Susceptible S. aureus (MSSA) was 40%. PVL-positive *S. aureus* isolates have been described from Germany, Ireland and from Australia as reported in this study [8-10,20,21]. This study conclusively reported a lower incidence of PVL-positive *S. aureus* of nosocomial origin in a local hospital in Nigeria; and it is thus critical to screen clinical isolates for the presence of these organisms in order to assuage any possible disease outbreak due to PVL-positive bacteria in this environment. The occurrence of PVL toxin gene in pathogenic *S. aureus* portends serious clinical implications – since most of these organisms are known to be multidrug resistant in nature. Thus, it is vital to lookout for PVL-positive *S. aureus* from clinical samples so that therapy can be properly guided.

Competing Interests

No competing interests exist.

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