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Genetics of bi-component leukocidin and drug resistance in nasal and clinical *Staphylococcus aureus* in Lagos, Nigeria

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ABSTRACT

Background: Resistant and virulent *Staphylococcus aureus* is a global public health challenge. Staphylococcal Bi-component leukotoxins are cytolytic to immune cells and evolve to disarm the innate immunity during infections, hence the severity of the disease.
Objective: We studied drug resistance profile and the occurrence of bi-component leukocidin in clinical and nasal *S. aureus* in Lagos, Nigeria.
Method: Ninety-two *S. aureus* (70 clinical and 22 nasal) strains were characterized by conventional and molecular methods.
Result: Of the resistance profiles generated, no isolate was resistant to fosfomycin, fusidic acid, teicoplanin, vancomycin, linezolid, mupirocin, nitrofurantoin and tigecycline. Twelve MRSA carrying staphylococcal cassette chromosome *mec*A gene types I, III, and IV elements were identified only in the clinical samples and type I dominated. High rates of *luk*E/D (100% among MRSA) and *luk*PV (dominated MSSA) were recorded among the nasal and clinical isolates. *Staphylococcus aureus* harboring only *luk*E/D (from clinical & colonizing MSSA) and combined *luk*E/D and *luk*PV (mostly from clinical MSSA, colonizing MSSA and clinical MRSA) toxins were found.
Conclusion: Although, *mec*A resistant genes were found only among clinical MRSA, the occurrence of other bi-component leukocidin genes in a large proportion among the isolates from both community and clinical settings is a major concern. The need for effective resistance and virulence factor surveillance, re-enforcement of antibiotic stewardship and good infection control policy, to prevent dissemination of epidemic strains is highlighted.

1. Background

Staphylococcus aureus is one of the most important and well-kown human pathogens. While some strains could be common commensals, some are leading causes of hospital and community associated infections [1]. The major factors associated with its pathogenicity are acquired antibacterial resistance and production of several virulent factors. The development of resistance is precipitated principally by indiscriminate and increasing use of antimicrobials. Initially, multidrug resistant (MDR) and methicillin resistant *S. aureus* (MRSA) strains predominate in hospitals, but, in the past few decades, several epidemics of community infections have been reported in apparently healthy populations [2]. From the available reports, about five community associated (CA)-MRSA clones causing outbreaks have been reported [3]. Universally, *S. aureus* causes a variety of infections including skin and soft tissue infections (SSTIs) and life-threatening systemic complications such as bactereamia [4].

The seriousness of the infections associated with this organism reflects its distinct abilities to escape the immune response, using multiple virulence factors including a group of pore-forming toxins known as bicomponent leucotoxins [5]. The bi-component pore forming toxins (BCPFTs) include the phage-encoded Panton–Valentine leucocidin (PVL; *lukPV*), the chromosomally encoded *luk*E/D and *luk*A/B and gamma-hemolysins (*hlgA* and *hlgCB*) genes. The BCPFTs are made up of two subunits of proteins designated as S and F. When the S subunit binds to cellular receptor, it forms heterodimer with the F component which is followed by multi-merization with subsequent pore formation on red blood cells and polymorphonuclear cells [6,7]. Additionally,

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individual subunits from different BCPFTs could form new functional toxins [6]. A report showed the interference of *lukS-PV* with the binding of three antibodies to human complement C5a receptor, an important step in sensing bacterial infection by phagocytes [8].

The binding of *luk*S-PV to C5aR causes the inhibition of neutrophils activation by displacing the natural ligand [8]; thereby signifying that *luk*S-PV alone may mediate immune evasion in *S. aureus* infections. Also, the individual subunits of these toxins could cross, combine and cause distinct lysis. Morinaga et al. [9] provided evidence that the S and F subunits between *luk*E/D and *hlg* are basically switchable and a combination of S subunit of PVL (*luk*S-PV) with *luk*D may form heamolytic toxin [6]. Thus, different toxins could be formed by cross-combination of leucotoxin subunits. A significant association has also been demonstrated between *luk*E/D expression and *S. aureus* invasive infections [10] especially those linked with cutaneous and urinary tract [5]. Similarly, studies have underscored the potential role of *luk*E/D as a critical virulence factor of *S.aureus* from Human Immunodeficiency Virus (HIV) infected persons with skin dermatitis and furuncles [11], diabetic foot ulcers [12], impetigo [13], and *S. aureus*-associated diarrhoea [14]. In Iran, more than 73% of *S. aureus* screened for *luk*E/D possessed the gene [5]. Data from Hilla/Iraq, indicated that *luk*ED-containing strains were detected among 9 and 11 of 24 CA- and HA-MRSA isolates analysed [15].

Several investigators have, however, denoted that PVL is the core virulence factor for CA-MRSA [16] and indeed, it can be found in both MRSA and MSSA strains [17,18]. It has been shown that PVL produces cytolytic activity specifically, on human and rabbit cells and induce pro-inflammatory cytokines in human and murine macrophages [19]. Furthermore, it acts to target prophylaxis and immunotherapy. Panton–Valentine leucocidin (PVL) - related infections or colonization have been reported among healthy individuals [15], travelers [20], rural settlers [21] and hospital staff/students [18]. Most *pvl* positive - *S. aureus* are associated with furunculosis and severe pneumonia [22]. The West and Central African countries have particularly shown high prevalence of PVL-positive MSSA isolates [23,24].

Some authors have expressed concerns about *S. aureus* strains possessing unique combinations of antibiotic resistance determinants and toxins. For instance, the genetic analysis of CA-MRSA in France described the co-occurrence of *pvl* and *luk*E/D genes in all the isolates [22]. Vandenesch et al. [25] also revealed the presence of both *pvl* and *hlg* sequences in MRSA from Oceania. In Nigeria, however, particularly the Southwest region, high occurrence of *pvl*-positive *S. aureus* has been described in both clinical and nasal isolates of *S.aureus* [26–28]. Most of these investigators have also insisted that the increasing resistance of our isolates to antibiotics remains a challenge [27,28]. Even though, the results of a study in 2009 showed the occurrence of *luk*E/D genes in CA-MRSA [28], information on Nigerian *S. aureus* for their bi-component leucotoxin contents other than PVL is extremely inadequate. Thus, the significance of these toxins necessitated the design and their investigation amongst invasive and colonizing methicillin sensitive and resistant strains. Efforts was also directed at determining the co-occurrence of the toxins in the isolates screened and the resistance status of the isolates to enhance the implementation of drug restriction/control policy needed for effective management of staphylococcal infections.

2. Materials and methods

2.1. Ethics and study site

The study approval was obtained from the Institutional Review Board (IRB) of the College of Medicine, University of Lagos, Nigeria (ref. No: CM/COM/8/VOL.XIX). Between June 2007 and April 2009, clinical samples were collected from Lagos University Teaching Hospital (LUTH), Idi-araba and National Orthopaedic Hospital, Igbobi (NOHI). Nasal swabs were also obtained from non-hospitalised and apparently healthy individuals in six local council areas of Lagos state (Mushin, Surulere, Mainland, Alimosho, Ikeja and Agege). All participants consented and filled the informed consent form.

2.2. Study population

The cohort comprised of 200 adults (male and female of 18 years and above) within hospital settings that had various clinical conditions (septicemia, urinary tract infection, wound infection, urogenital infection and respiratory infection). One hundred apparently healthy volunteers were screened for carriage of *S. aureus* in the anterior nares using sterile swabs.

2.3. Inclusion and exclusion criteria

Patients included in this study were those hospitalised and specimens were collected for bacterial aetiology after admission (clinical). Patients not on admission and those on the first day of admission were also excluded (clinical). For determination carriage status, apparently healthy volunteers with no history of recent hospitalization In addition, samples were not collected from individuals with any form of facial wounds, rhinitis, catarrh and skin conditions.or antibacterial consumption (Community/colonizing) were screened. Children and all those who refused consent were excluded.

2.4. Sample processing and identification of Staphylococcus aureus

The primary laboratory isolation of bacterial organisms was carried out at the Nigerian Institute of Medical Research, Yaba. Isolation of *Staphylococcus* was achieved using appropriate media [30]. Suspected *S. aureus* isolates were identified based on standard bacteriological procedures including Gram reaction, catalase test, tube coagulase test, DNase test and confirmed with VITEK 2 system ID-GP card (BioMérieux, Marcy Etoile, France). A single isolate was selected per sample. The molecular characterization of *S. aureus* was carried out at Microbiology laboratory of Otto-von-Guericke Universitat, Magdeburg, Germany.

2.5. Antimicrobial susceptibility testing

Susceptibility to twenty (20) different antimicrobial agents (penicillin, cefoxitin, oxacillin, clindamycin, erythromycin, fosfomycin, Fusidic acid, gentamycin, levofloxacin, linezolid, moxifloxacin, mupirocin, vancomycin, nitrofurantoin, rifampicin, teicoplanin, tetracycline, tigecycline, tobramycin and trimethoprim/sulfamethoxazole) was performed using VITEK 2 system, AST P580 card (BioMérieux, Marcy l'Etoile, France). *S. aureus* ATCC 29213 was used as control strain. The VITEK 2 Minimum Inhibitory Concentration (MIC) results were interpreted using the Advanced Expert System of the VITEK 2 system. Multi-Drug Resistance (MDR) was defined as resistance to one or more antibiotics in three or more categories of drugs [31].

2.6. DNA extraction

Two to three colonies of overnight blood agar culture of *S. aureus* was pre-treated with lysostaphin (QIAgen, Hilden, Germany) (20 μg/ml) in 160 μl of TE buffer (10 mmol of Tris HCl/liter, 1 mmol of EDTA/liter, pH 8.0) at 37 °C for 30 min. The cells were harvested and DNA was extracted using a DNeasy tissue kit as recommended by the manufacturer (Qiagen, Hilden, Germany). The concentration of DNA was estimated spectrophotometrically.

2.7. Determination of SCCmec type

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types were determined using multiplex PCR as previously described [32]. The eight