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Pathogenic features of clinically significant coagulase-negative staphylococci in hospital and community infections in Benin

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ABSTRACT

In West Africa, very little consideration has been given to coagulase negative Staphylococci (CNS). Herein, we describe the features contributing to the pathogenicity of 99 clinically-significant independent CNS isolates associated with infections encountered at the National Teaching Hospital Center of Cotonou (Benin). The pathogenic potentials of nosocomial strains were compared with community strains. *S. haemolyticus* (44%), *S. epidermidis* (22%) and *S. hominis* (7%) were the most frequently isolated while bacteremia (66.7%) and urinary tract infections (24.2%) were the most commonly encountered infections. Most strains were resistant to multiple antibiotics, including penicillin (92%), fosfomycin (81%), methicillin (74%) and trimethoprim-sulfamethoxazole (72%). The most frequently isolated species were also the most frequently resistant to methicillin: *S. hominis* (100%), *S. haemolyticus* (93%) and *S. epidermidis* (67%). Screening of toxic functions or toxin presence revealed hemolytic potential in 25% of strains in over 50% of human erythrocytes in 1 h. Twenty-six percent of strains exhibited protease activity with low (5%), moderate (10%) and high activity (11%), while 25% of strains displayed esterase activity. Three percent of strain supernatants were able to lyse 100% of human polymorphonuclear cells after 30 min. Polymerase chain reaction and latex agglutination methods revealed staphylococcal enterotoxin C gene expression in 9% of *S. epidermidis*. A majority of hospital-associated CNS strains (68%) had at least one important virulence feature, compared with only 32% for community-acquired strains. The present investigation confirms that these microorganisms can be virulent, at least in some individual cases, possibly through genetic transfer from *S. aureus*.

1. Introduction

Staphylococci are among the most commonly encountered bacteria in hospital settings and are involved in various infections. *Staphylococcus aureus* is considered as the major species associated with severe staphylococcal infections for which several virulence factors responsible for the symptoms and severity of infections have been identified (Durupt et al., 2007; Baba-Moussa et al., 2008; Fluit et al., 2001). Meanwhile, several studies have emphasized the role of Coagulase-negative staphylococci (CNS) in various infection types (Delaunay et al., 2014; Piette and Verschraegen, 2009). Their growing role as pathogens has been demonstrated in human infections, especially in immunocompromised patients, preterm infants and in patients with implanted devices (Becker et al., 2014).

The most frequent CNS infections associated with humans are primarily nosocomial in nature and are due to *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* isolated from bloodstream infections, peritonitis, prosthetic valve-related endocarditis, and foreign body infections (Ertem et al., 2010; Falcone et al., 2004; Schoenfelder et al., 2010). *S. lugdunensis* and *S. saprophyticus* are involved in arthritis and in urinary tract infections among adolescent females, respectively (Lo et al., 2015; Peel et al., 2015). *S. warneri*, *S. capitis*, *S. saccharolyticus*, *S. caprae*, *S. schleiferi*, *S. hominis* and *S. auricularis* can also be associated with severe infections (Delmas et al., 2008).

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The pathogenicity of CNS and hospital-acquired CNS isolates has been difficult to establish, since the virulence factors of these microorganisms are poorly defined (Becker et al., 2014). Several CNS may carry at least parts of genes encoding exo-enzymes such as proteases, lipases and toxins, which are factors that can impact the severity of the infections (Von Eiff et al., 2002; Pinheiro et al., 2015). *S. epidermidis* produces several adhesion factors, proteins and exo-polymers involved in immune evasion and biofilm production (Otto, 2012). Recently, pathogenicity islands bearing enterotoxin SEC3 and SE1L have been described in *S. epidermidis* (Madhusoodanan et al., 2011). In addition, most CNS display a high antibiotic resistance profile, particularly to methicillin which remains a major challenge for clinicians to find alternative molecules with low grade toxicity (Diekema et al., 2001).

In most African clinical microbiology laboratories, CNS characterization is usually limited to a simple coagulation assay to distinguish *S. aureus* from non-*aureus* isolates. However, because of their involvement in human infections, it now remains essential that clinically significant CNS be identified to the species level in order to establish a correlation between clinical signs and identified bacteria, as well as to identify risks. In Benin and West Africa, the prevalence, pathogenicity and resistance profile of CNS are poorly known because of the lack of such species identification.

The present study was undertaken to characterize clinically significant CNS strains isolated from various samples at the National Teaching Hospital Center, Hubert Koutoukou Maga (NTHC-HKM), in Cotonou, Benin. In this analysis, the variability, resistance profile to antimicrobial agents and virulence factors of isolates were determined and the pathogenic potentials of nosocomial- and community-based CNS were compared.

2. Materials and methods

2.1. Ethical statement

This work was approved by the National Ethics Committee of Benin under protocol number 2015/006. All staphylococcal isolates evaluated in this study were submitted by physicians treating patients for infectious syndromes. Buffy coats from fresh human blood from healthy donors were purchased to the « Etablissement Français du Sang de Strasbourg, France », for which all information remains confidential.

2.2. Study design and clinical relevance assessment of strains

Strains were collected prospectively from March 2015 to January 2016 at the NTHC-HKM, Cotonou, Benin. Clinically significant CNS isolates were retrieved from various specimens from both in- and out-patients. The clinical significance of CNS strains was determined by qualified medical personnel using the clinical records of each patient containing the demographic, clinical and laboratory data. The isolates were considered to be of clinical significance when the following general criteria were fulfilled: 1) strains obtained in bacterial pure culture; 2) presence of infection risk factors (immunosuppressive and/or front door); 3) clinical signs of infection (hyperthermia >38 °C, hypotension, tachycardia, tachypnea); 4) identification of a site of infection (respiratory, digestive, bone and joint, skin and soft tissues) (Mohammad et al., 2014). In the present study, only strains with clinical relevance were considered.

2.3. Identification of CNS

CNS Strains were phenotypically identified by standard microorganism identification methods in the Microbiology Laboratory of the NTHC-HKM. Methods were based on colony morphology, Gram staining, catalase positivity (ID color Catalase; bioMérieux, Marcy l'Etoile, France), non-agglutination of colonies with the Pastorex Staph Plus test (Bio-Rad, Marnes la Coquette, France), and free staphylocoagulase non production with lyophilized rabbit plasma. Identification at the species level was carried out using Api STAPH (bioMérieux, Marcy l'Etoile, France), according to the Manufacturer's instructions, and species identification was further confirmed for species identification with the MALDI-TOF Biotyper™ (Bruker Daltonics) method at the Institute of Bacteriology, University of Strasbourg, France. For two *S. epidermidis* isolates, 16 s rDNA sequencing was achieved (GATC Ltd, Basel, Switzerland) (Ghebremedhin et al., 2008).

2.4. MALDI-TOF mass spectrometry assay for bacterial identification

CNS strains were grown on Columbia agar with 5% (v/v) sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated for 24 h at 37 °C. One colony of each strain was placed in duplicate on a 96-spot polished target plate and allowed to dry at room air. Next, 1 μl of matrix (3 mg/mL alpha-cyano-4 hydroxycinnamic acid in 50% [v/v] acetonitrile–2.5% trifluoroacetic acid [v/v]) was overlaid onto the sample and allowed to dry. The acquisition of protein mass spectra was performed on a Microflex LT™ instrument using the flexControl™ 3.0 software (Bruker Daltonics), with a mass-to-charge ratio (m/z) range of 2–20 kDa. Automated data analysis of raw spectra was performed with MALDI Biotyper™ RTC 3.1.2.0 software (Bruker Daltonics). An identification score of $\geq$2 was considered as high-confidence identification at the species level, whereas scores comprised between 1.7 and 1.99 were considered as intermediate confidence genus-level identification only. Scores of <1.7 were considered as an unacceptable identification, according to the Manufacturer's recommendations, and were excluded from the analysis if any.

2.5. Antibiotic susceptibility and methicillin resistance testing

Antimicrobial susceptibility and methicillin resistance testing of the CNS isolates were performed with the VITEK 2 system (bioMérieux, Marcy l'Etoile, France) according to the recommendations of the Committee for Antimicrobial Susceptibility of the French Society for Microbiology (CA-SFM)-2015. The antibiotics tested were cefoxitin (FOX), benzylpenicillin (BEN), oxacillin (OXA), gentamicin (GEN), kanamycin (K), tobramicin (TM), ofloxacin (OFX), clindamycin (CL), erythromycin (E), lincomycin (L), pristinamycin (PT), linezolid (LIZ), teicoplanin (TEC), vancomycin (VA), tetracycline (TET), fosfomycin (FOS), nitrofurantoin (FT), fusidic acid (FA), rifampicin (RIF) and trimethoprim-sulfamethoxazole (SXT). Methicillin-resistance was assessed by the resistance of the strains with cefoxitin and oxacillin at 37 °C.

2.6. Screening for proteolytic activity

After identification, each strain was first cultured on Mueller Hinton (MH) agar enriched with 5% sheep blood for 18 h at 37 °C after which a clone was selected and inoculated into 100 ml of Brain Heart Infusion (BHI) broth. The suspension was then incubated with shaking at 37 °C for 18 h and centrifuged at 10,000 × g for 10 min. The culture supernatants were collected and filtered with a 0.22 μm filter. Each filtrate was stored at −20 °C until use. Protease activity of the strains was investigated using azocasein (Sigma-Aldrich, France) as substrate (Vandecandelaere et al., 2014). Briefly, 600 μl of buffer (0.2 M Tris HCl, 3 mM $CaCl_2$, pH 8.0), 200 μl of azocasein (25 g/ml) and 200 μl of each strain culture supernatant were incubated at 37 °C. After 3 h, 200 μl of 100% (w/v) trichloroacetic acid (TCA) were added and the new mixture was centrifuged for