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ORIGINAL ARTICLE

Genotypes and phenotypes of *Staphylococcus lugdunensis* isolates recovered from bacteremia

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Background: *Staphylococcus lugdunensis* is a member of coagulase-negative staphylococci, which has the potential to cause serious infections, such as endocarditis, bone and joint infections, and septicemia. Differences in phenotypic/genotypic characterization may be linked to different diseases.
Methods: Genotypes of 11 *S. lugdunensis* isolates from bacteremia were determined by pulsed field gel electrophoresis and accessory gene regulator (*agr*) typing. The SCC*mec* elements in two oxacillin-resistant isolates were sequenced. Phenotypes were tested by antimicrobial susceptibility testing, biofilm formation assessments, and virulence factor analysis (hemolytic and protease activities).
Results: Among the 11 isolates, six pulsotypes were found, and seven isolates belonged to two major pulsotypes. Two *agr* types (*agr-1*$_{sl}$ or *agr-2*$_{sl}$) were found. The 11 isolates were susceptible to most antimicrobial agents tested. The SCC*mec* elements in two oxacillin-resistant

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isolates belonged to the SCC*mec* type V, but with additional *ccrAB2* genes. The *agr*-2_{sl} isolates ($n = 7$) displayed higher hemolytic and protease activities than the *agr*-1_{sl} isolates. All isolates contained the *icaA* gene but with variable biofilm activities. The results suggest that protein might play an important part in *S. lugdunensis* biofilms, possibly through an *ica*-independent pathway. Of the 11 patients with *S. lugdunensis* bacteremia, one patient had a community-onset infection, and others had a hospital-acquired infection, which were mostly central venous catheter-related infections.

Conclusion: The 11 *S. lugdunensis* bacteremia isolates displayed various genotypes and phenotypes. Two oxacillin-resistant isolates contained SCC*mec* type V and carried additional *ccrAB2* genes. Correlation of genotypes and phenotypes with infections needs further studies.

Introduction

Staphylococcus lugdunensis, first described in 1988,[1] is a member of the coagulase-negative staphylococci (CoNS) and has been recognized as an important human pathogen.[2–5] In contrast to other CoNS, this microorganism is very similar to *S. aureus* and causes serious infections such as endocarditis, bone and joint infections, and septicemia.[6,7] Unlike other CoNS, *S. lugdunensis* is generally susceptible to many antimicrobial agents.[8,9] Although the resistance of *S. lugdunensis* to methicillin (*mecA* positive) has been reported previously, only limited reports on the SCC*mec* structure of *S. lugdunensis* are available.[4,10,11]

The staphylococcal accessory gene regulator (*agr*) locus is a quorum-sensing system that modulates virulence factors in *S. aureus*.[12] This locus encodes two divergent transcripts (RNAII and RNAIII), which are regulated by the promoters P2 and P3, respectively.[13] Two *agr* types (*agr*-1_{sl} and *agr*-2_{sl}) have been identified in *S. lugdunensis*,[13] and the *agr*-like locus (which has been named *agr*-1_{sl}) was correlated with hemolytic activity.[14] However, the correlation between the *agr* type and other virulence factors remains unknown.

Biofilm activity has been studied in *S. lugdunensis* isolates.[15,16] Although the *icaADBC*-encoded poly-*N*-acetylglucosamine (PNAG) is a major component of biofilm development in *S. aureus* and *S. epidermidis*,[17,18] several studies revealed that the biofilm formation of *S. lugdunensis* was mediated through the *ica*-independent pathway. Previous studies indicated that the *S. lugdunensis* biofilm consists of proteins, extracellular teichoic acids, and glucosamine but lacks dominant PNAG.[15,16,19] The biofilms were sensitive to proteinase K but not degraded by metaperiodate (a PNAG-degrading agent), which suggests that proteins may be the major component of biofilm formation in *S. lugdunensis*.

In this study, we examined the genotypes [pulsed field gel electrophoresis (PFGE) and *agr* typing] and phenotypes of 11 *S. lugdunensis* isolates causing bacteremia. The phenotypic characterizations were antimicrobial susceptibility testing, biofilm formation assessments, and virulence factor analyses (hemolytic and protease activities). The *agr*-2_{sl} isolates displayed higher hemolytic and protease activities than the *agr*-1_{sl} isolates. The SCC*mec* structure of two oxacillin-resistant *S. lugdunensis* isolates was also determined.

Materials and methods

Bacterial isolates and patients

Eleven *S. lugdunensis* isolates causing bacteremia were recovered from blood cultures between 2007 and 2008 in the Bacteriology Laboratory of the National Taiwan University Hospital, a university hospital with 2500 beds that is located in Northern Taiwan. *S. lugdunensis* was identified by BD Phoenix automated microbiology system (Becton-Dickinson Diagnostic Systems, Sparks, MD, USA) and 16S ribosomal RNA gene sequencing. Clinically significant bacteremia was defined as two or more sets of positive blood cultures or the coexistence of positive blood and central venous catheter (CVC) cultures.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by both disk diffusion and agar dilution according to the guidelines of the Clinical and Laboratory Standards Institute. The antimicrobial agents tested were oxacillin, fusidic acid, gentamicin, clindamycin, erythromycin, teicoplanin, minocycline, trimethoprim/sulfamethoxazole, and vancomycin.

agr typing

The *agr* types were determined by polymerase chain reaction (PCR; 3′ end of *agrB* to 5′ end of *agrC*) and sequencing.[13] Because the primers of AGR-1 and AGR-2, which were designed for *S. aureus*, did not completely match with the *agr* sequence of *S. lugdunensis*, we redesigned the primers SLagrF (5′-TGTGGCATTACCTTGGTCA-3′) and SLagrR (5′-CTTTGATGGCACGAACCTT-3′), based on the sequence from GenBank accession number AF173933.1, to obtain a 1333-bp amplification product.

Hemolytic activity

The hemolytic activity assay used in this study was slightly modified from the method reported by Majerczyk et al.[20] Bacteria were grown in 20-mL tryptic soy broth (TSB) medium to an initial optical density at a wavelength of 600 (OD_{600}) of 0.05 and incubated at 37°C in a shaker at 240 rpm. After 3, 6, 7, 8, and 10 hours, 0.5 mL of each