Clinical Significance of Staphylococcus lugdunensis Isolated from Routine Cultures

Elizabeth Kleiner, Alastair B. Monk, Gordon L. Archer, and Betty A. Forbes

1Department of Medicine, Division of Infectious Diseases, 2Department of Pathology, Virginia Commonwealth University Medical Center, Medical College of Virginia Campus, Richmond, Virginia

Over 1 year, 42 Staphylococcus lugdunensis isolates, identified by phenotypic and genotypic testing, were recovered from clinical specimens. Thirty-six (86%) were clinically significant pathogens, mostly from healthy outpatients; 16 (44%) of 36 were isolated in pure culture; and 30 (83%) of 36 were from skin and soft-tissue infections.

Staphylococcus lugdunensis, a coagulase-negative staphylococci (CoNS) species, has emerged as an important human pathogen [1, 2]. Given the pathogenic potential and wide spectrum of clinical disease associated with S. lugdunensis, this study assessed the clinical significance and examined phenotypic and genotypic characteristics of isolates of S. lugdunensis identified from consecutive specimens processed during 1 year in a large teaching hospital.

Methods. From May 2006 through June 2007, all isolates of CoNS (n = 576) that were the only or predominant bacteria isolated from consecutive sterile body fluids and wounds were screened for S. lugdunensis. CoNS species were identified on the basis of Gram stain, colony morphology, a positive catalase test, and a negative tube coagulase test with rabbit plasma. S. lugdunensis was differentiated from other CoNS species on the basis of a positive pyrrolidonyl arylamidase test (Remel) and the presence of ornithine decarboxylase activity (Remel). Minimal inhibitory concentrations for various antimicrobial agents were determined with the VITEK-1 system with use of the GPS-standard (bioMérieux); oxacillin and interpretative breakpoints. Laboratory identification was verified by polymerase chain reaction amplification and sequence analysis of 16S ribosomal DNA (rDNA) and rpoB [3]. In addition, sodA was polymerase chain reaction amplified using primers sodAF (5'-AATGGAAA-TCCATCATGATAAACA-3') and sodAR (5'-TAGGTTTTT-GCCTTCAGTTATGG-3'), both designed for this study on the basis of GenBank accession number AJ343952 (S. lugdunensis partial gene for sodA strain NEM2014), with subsequent sequence analysis. Pulsed field gel electrophoresis was performed and interpreted for 35 isolates of S. lugdunensis according to the technique of Bannerman et al [4].

The ability to form biofilms was measured as described elsewhere [5]. Two independent observers then read the stained wells and rated the biofilm as weak, medium, or strong, compared with the following test controls: S. aureus MN8M (strong), Staphylococcus epidermidis RP62A (medium), S. aureus MN8MΔica (weak), or TSB (negative).

A retrospective review of patient records was conducted for all patients whose clinical specimens yielded S. lugdunensis isolates. Clinical significance of S. lugdunensis isolates was determined by the clinical presentation of the patient and direct Gram stain results. Charts were reviewed for demographic data, clinical presentation, site of infection, comorbidities, previously documented infections, use of prior antimicrobial therapy, and response to therapy. The study was reviewed by the institutional review board, and conditions for waiver of documentation of consent were met.

Results. During the 12-month period, 42 S. lugdunensis isolates were identified from sterile body fluids and wounds, representing 7.3% of all CoNS (42 of 576). All isolates produced a brilliant white pigment, and 36 (85.7%) of 42 were β-hemolytic. Sequence-based methods gave 100% identity hits with use of BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST) to their respective GenBank entry as follows: S. lugdunensis 16S rRNA gene (GenBank accession number EF442311; S. lugdunensis 16S rDNA partial sequence strain G-17050), sodA (GenBank accession number AJ343952; S. lugdunensis partial gene for sodA, strain NEM2014), and rpoB (GenBank accession number AF325870.1; S. lugdunensis RNA polymerase partial gene sequence). For 16S rDNA, the next species after S. lugdunensis were uncultured Staphylococcus species with a differential of 1%. The differential for sodA and rpoB was 11% and up to 14%, respectively, with the closest alternate species being Staphylococcus hominis and Staphylococcus haemolyticus in both...
cases; the predominance of hits were to genome-based fragments rather than gene identification. Thus, genotypic testing confirmed the accuracy of the phenotypic screen for identification of *S. lugdunensis*.

All isolates were susceptible to gentamicin, levofloxacin, lincomycin, dalbopristin/quinupristin, rifampin, trimethoprim/sulfamethoxazole, and vancomycin. Three (9%) of 35 isolates were erythromycin resistant, whereas 1 (3%) of 35 were clindamycin resistant. Only 1 isolate (3%) was resistant to oxacillin; the presence of the *meca* gene was confirmed by polymerase chain reaction. Sixteen (45%) of 35 isolates were resistant to penicillin and ampicillin, and only 1 isolate was resistant to cefazolin.

Of the 35 *S. lugdunensis* isolates analyzed by pulsed field gel electrophoresis, 10 clustered into 1 group of isolates which were either indistinguishable or closely related. None of these 10 clustered isolates was temporally or geographically related to the others. Among the remaining isolates, there were several smaller groups of 2, 3, or 4 isolates, but they had little similarity to each other or the larger pulsed field gel electrophoresis group of 10 isolates. There was no association among the grouped isolates with the type or severity of clinical disease.

The amount of biofilm formed varied greatly among the isolates in this study. Nevertheless, all isolates were able to form at least a weak biofilm (16 [48%] of 33), whereas 8 (24%) and 9 (27%) of 33 were medium and heavy biofilm formers, respectively.

Clinical findings are summarized in Table 1. *S. lugdunensis* was judged to be a significant cause of infection in 36 of 42 patients (86%). Two blood isolates were obtained but were unlikely to be clinically significant given the clinical presentation. Due to incomplete patient records, the clinical significance of 4 superficial skin isolates was difficult to delineate. Therefore, these 6 isolates were excluded from further analysis. Of the clinically significant isolates of *S. lugdunensis*, the majority were obtained in the outpatient setting (26 [72%] of 36 isolates). More than 80% of cases occurred in patients aged 19–65 years (30 [83%] of 36 cases). The male to female distribution was nearly equal. Of note, many patients had no underlying comorbid illness (17 [47%] of 36); 11 (31%) of 36 had undergone recent surgery or trauma.

Soft-tissue infections, particularly skin abscesses, were the most common type of clinical presentation (30 [83%] of 36). Nearly every region of the body was involved in infections, but postoperative, lower extremity surgical sites and the breast were most common. Nearly one-half of the clinical specimens yielded *S. lugdunensis* in pure culture (16 [44%] of 36). Polymicrobial infections typically involved other skin commensal organisms. No previous documented infections were noted in the majority of patients (25 [69%] of 36).

**Discussion.** Most reports of *S. lugdunensis* have consisted of single case reports or small case series focusing on aggressive clinical infections. In our study, *S. lugdunensis* represented 7.3% of CoNS isolated from sterile body fluids and wounds. This finding is in contrast to the estimated frequency from a previous study in which *S. lugdunensis* comprised 1.3% of all staphylococcal clinical isolates [6].

Bacterial isolates were identified phenotypically and genotypically with excellent correlation. Of the 35 *S. lugdunensis* isolates analyzed for strain relatedness by pulsed field gel electrophoresis, 29% of isolates clustered into 1 group of highly related isolates. The absence of any obvious connection among these 10 patients by time of isolation or place of residence suggests that there is a high degree of genetic relatedness. Similar data suggest that a low degree of genetic diversity has been presented in other studies [7]. Although all our isolates were able to form at least a weak biofilm, the amount of biofilm formed by isolates was heterogeneous with poor correlation between clinical severity of disease and degree of biofilm formation.

Although *S. lugdunensis* remains highly susceptible to the majority of antimicrobial agents, the current study suggests that an-
tibiotic resistance is evolving. Only 45% of the tested isolates were susceptible to all antibiotics. These data differ from another study in which the majority of isolates (49 [68%] of 72) were pansusceptible [8]. A single isolate, resistant to clindamycin, ce-

fazolin, and oxacillin (minimal inhibitory concentration, >256 μg/mL), was found to have the mecA gene.

In our study, the majority of infections caused by this emerging pathogen occurred in healthy adults, primarily in the out-
patient setting. Of significance, nearly one-half had no signif-
ificant comorbid conditions, although one-third of the patients had experienced recent trauma or a surgical procedure. Only 17% received a diagnosis of underlying diabetes mellitus, which is in contrast to other studies [9, 10].

Cutaneous involvement was overwhelmingly the most common site of S. lugdunensis infection (30 [83%] of 36). This has been confirmed by other reports. Although other studies have documented a higher prevalence of inguinal area carriage and pelvic girdle infection [9, 10], the prevalence of infections above the waist exceeded those documented below the waist (53% vs 47%) in the current study.

Of particular interest was the increased rate of nonpuerperal breast abscesses, which are relatively uncommon [11]. All cases involved nonpregnant, nonlactating females. Prior recurrent breast infections were documented in 4 (80%) of 5; S. lugdu-
nensis was isolated in pure culture in 4 of the 5 cases. In the current study, a single case was associated with diffuse furuncu-
losis, but the other 4 cases had isolated breast involvement.

Finally, aggressive intravascular S. lugdunensis infections were not documented in the current series. Although other literature has emphasized the tendency of S. lugdunensis to cause ag-
gressive clinical infections [1, 2, 12], a retrospective analysis by Ebright et al [2] suggests that most cases of bacteremia were of short duration and not clearly related to clinical disease.

Given its broad spectrum of clinical infection, pathogenic potential, and current Clinical Laboratory Standards Institute recommendations to use S. aureus interpretative breakpoints for oxacillin, identification of S. lugdunensis is essential. On the basis of our data, accurate identification of S. lugdunensis from sterile body sites and wounds is easily accomplished by first using the pyrrolidonyl arylamidase test and, if positive, setting up an ornithine decarboxylase test on CoNS isolates. Our study also revealed several important epidemiologic factors of this pathogen, including its predilection to cause soft-tissue and skin infections in healthy adults.

Acknowledgments
We thank the staff of the clinical microbiology laboratory for their help in supporting this investigation.

Financial support. National Institute of Allergy and Infectious Diseases (Public Health Service grant 2RO1AI035705 to G.L.A.).

Potential conflicts of interest. G.L.A. has served on the Scientific Advisory Board for Cubist Pharmaceuticals. All other authors: no conflicts.

References
1. Frank, KL del Pozo JL, Patel R. From clinical microbiology to infection pathogenesis: how daring to be different works for Staphylococcus lug-

2. Ebright JR, Penugonda N, Brown W. Clinical experience with Staphylococcus lugdunensis bacteremia: a retrospective analysis. Diagn Mi-

3. Mellmann C, Becker, AK, von Eiff C, et al. Sequencing and staph-


5. Monk AB, Boundy S, Chu VH, et al. Analysis of the genotype and virulence of Staphylococcus epidermidis isolates from patients with in-


7. Hellbacher C, Tornqvist E, Soderquist B. Staphylococcus lugdunensis: clinical spectrum, antibiotic susceptibility and phenotypic and geno-


9. Bellamy R, Barkham T. Staphylococcus lugdunensis infection sites: pre-

