Microbiological Characteristics, Presumptive Identification, and Antibiotic Susceptibilities of Staphylococcus lugdunensis[∇]

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This study validated abbreviated methods for the presumptive identification of *Staphylococcus lugdunensis* and studied the antibiotic susceptibilities of 106 isolates. The combination of positive responses to ornithine and pyrrolidonyl arylamidase identified all *S. lugdunensis* isolates. Resistance to penicillin and methicillin was detected in 27 and 5% of isolates, respectively.

Staphylococcus lugdunensis, a coagulase-negative staphylococcus, has clinical characteristics that resemble those of the coagulase-positive *Staphylococcus aureus*. Infections attributed to *S. lugdunensis* include infective endocarditis (19), bacteremia, meningitis (6), bone and joint infections (7), and soft-tissue infections (16). *S. lugdunensis* generally is susceptible to anti-staphylococcal antibiotics (18), but increasing penicillin resistance has been reported (9, 13). Meanwhile, oxacillin susceptibility breakpoints for *S. lugdunensis* were changed in 2005 (3), and oxacillin disc breakpoints were revised in 2005 and subsequently replaced by cefoxitin disc breakpoints in 2006 (2). The current reference method for the identification of coagulase-negative staphylococci (1) is labor-intensive. Screening for *S. lugdunensis* by detecting clumping factor (15, 21) or using a limited number of biochemical tests (5, 12, 15) has been proposed, but these methods have been evaluated only against small numbers of *S. lugdunensis* isolates.

The aims of this study were to evaluate the use of simple screening strategies for the identification of *S. lugdunensis*, to comprehensively describe the microbiological characteristics of *S. lugdunensis*, and to evaluate the antibiotic susceptibility of clinical isolates.

The accuracy of three abbreviated identification protocols and two commercial identification kits (ID 32 Staph and Vitek ID-GP; both from bioMérieux, France) was evaluated against a collection of coagulase-negative staphylococci that were isolated from clinical samples. Isolates from the collection were identified using a panel of 30 phenotypic and biochemical tests (1) and included *Staphylococcus capitis* (n = 7), *Staphylococcus caprae* (n = 5), *Staphylococcus cohnii* (n = 2), *Staphylococcus epidermidis* (n = 14), *Staphylococcus haemolyticus* (n = 24), *Staphylococcus hominis* (n = 3), *Staphylococcus lugdunensis* (n = 46), *Staphylococcus saprophyticus* (n = 2), and *Staphylococcus warneri* (n = 3). The identification of 10 isolates (belonging to *S. haemolyticus*, *S. cohnii*, *S. epidermidis*, and *S. lugdunensis*) was further confirmed by sequencing a 457-bp sequence of the 16S rRNA gene (8). For the three abbreviated identification protocols, isolates were tested for the presence of pyrrolidonyl arylamidase (PYR) (Oxoid, United Kingdom), ornithine decarboxylase (BioMedia, Malaysia), and urease (BioMedia, Malaysia); trehalose utilization; alkaline phosphatase activity; and mannose utilization (the last three substances were from Rosco, Denmark). In the first identification protocol, isolates that were positive for both PYR and ornithine decarboxylase were identified as *S. lugdunensis*. In the second identification protocol, isolates that were positive for PYR, ornithine decarboxylase, and mannose utilization were identified as *S. lugdunensis*. The third identification protocol identified an isolate as *S. lugdunensis* if it was positive for trehalose and ornithine decarboxylase and negative for alkaline phosphatase (12). The first and second identification protocols correctly identified all 46 isolates of *S. lugdunensis* (sensitivity, 100%), while the third testing protocol identified 42 isolates of *S. lugdunensis* (sensitivity, 91%). None of the other staphylococcal species was misidentified as *S. lugdunensis* by the three protocols (specificity, 100%). ID 32 Staph correctly identified 45 (98%) isolates of *S. lugdunensis*, and Vitek ID-GP correctly identified 43 (93%) isolates of *S. lugdunensis*.

The 46 strains of *S. lugdunensis* were subcultured onto trypticase-soy agar plates with 5% sheep blood and incubated in ambient atmosphere at 35°C for 3 days. Colonial morphology was observed after 24, 48, and 72 h of incubation. Colonies were 1 mm in diameter after 24 h of incubation and increased to 3 mm after 48 h at 35°C. Forty (87%) isolates showed a narrow border of beta-hemolysis after 24 h of incubation. After 48 h of incubation, 39 (85%) isolates showed significant beta-hemolytic activity, and 4 isolates (9%) showed slight beta-hemolysis. Colonies at 24 h typically were opaque white, with a glossy sheen (n = 40; 87%). At 48 and 72 h, 13 isolates (28%) developed a yellow-white hue resembling that of *S. aureus*. *S. lugdunensis* isolates had a characteristic sweet, hay-like odor resembling that of the screwpine leaf, and this was prominent in 38 (79%) isolates after 48 h of incubation.

The detection of clumping factor/protein A using three commercial latex agglutination kits, Pastorex Staph (Bio-Rad, France), Staphaurex, and BactiStaph (both from Remel, United States), was performed on overnight cultures. Rapid and clear agglutination was classified as a positive result, while slower and less distinct agglutination was classified as a weak

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TABLE 1. Antibiotic susceptibilities of *S. lugdunensis* strains based on disc susceptibility testing[a]

Antibiotic	No. of isolates tested	No. (%) sensitive	No. (%) resistant
Cefoxitin	106	101 (95.3)	5 (4.7)
Ciprofloxacin	106	105 (99.1)	1 (0.9)
Clindamycin	106	104 (98.2)	2 (1.8)
Cotrimoxazole	106	106 (100)	
Erythromycin	106	104 (98.2)	2 (1.8)
Penicillin	106	77 (72.6)	29 (27.4)
Tetracycline	106	94 (88.7)	12 (11.3)

[a] The interpretative criteria are from reference 4.

TABLE 2. No. of strains positive or negative for the *mecA* gene according to oxacillin MICs[a]

mecA status	No. of strains with oxacillin MIC (μg/ml) of:							
	0.125	0.25	0.5	0.75	1	2	16	≥32
Negative	2	13	53	2	29	2		
Positive							4	1

[a] Methicillin resistance is defined as an oxacillin MIC of ≥4 μg/ml.

positive result. The interpretation of a positive agglutination test was always made with reference to the manufacturer's instructions. All isolates were tested for the presence of free coagulase using a rabbit plasma tube coagulase test method. The three latex agglutination kits showed different performance characteristics. BactiStaph and Pastorex Staph showed strongly positive agglutination for 49 and 42% of *S. lugdunensis* isolates, respectively, while strongly positive agglutination was present in only 9% of isolates that were tested by Staphaurex. For both positive and weak-positive agglutination reactions, the test kit positivity was 73, 76, and 17% for BactiStaph, Pastorex Staph, and Staphaurex, respectively. All isolates were negative for free coagulase.

Antibiotic susceptibility testing was performed on a separate collection of 106 isolates of *S. lugdunensis* that were collected prospectively from clinical specimens from 2004 to 2006 and identified using the first identification protocol. Antibiotic susceptibilities were performed by disk diffusion and were interpreted using CLSI guidelines (4), except for oxacillin, for which the last applicable guidelines from 2005 were applied (3). MICs of penicillin and oxacillin were determined by agar dilution. All isolates were screened for the *mecA* gene (20), and *mecA*-positive isolates were tested for the presence of *pbp2′* by latex agglutination (Oxoid, United Kingdom) using previously described methods (10). The species identification of all *mecA*-positive *S. lugdunensis* isolates was confirmed by 16S rRNA gene sequencing. The antibiotic susceptibilities of 106 clinical isolates are listed in Table 1. Twenty-nine isolates (27%) were resistant to penicillin by agar dilution. The modal oxacillin MIC was 1 μg/ml (Table 2), which is higher than those for most other staphylococcal species but similar to that for *S. saprophyticus* and *S. cohnii* (11, 13). The oxacillin MICs for five isolates were ≥4 μg/ml, and all were positive for both *mecA* and *pbp2′*. Current cefoxitin disk breakpoints accurately detected *mecA*-positive isolates, as opposed to oxacillin disk breakpoints (susceptibility breakpoint, ≤13 mm; resistance breakpoint, ≥10 mm), which had poor sensitivity (80%) and specificity (65%).

This study comprehensively examined the use of abbreviated protocols for the presumptive identification of *S. lugdunensis*. Our results show that the use of clumping factor may not be a reliable screening method to identify this organism, as results appear to be kit dependent. The two-test protocol that utilized ornithine decarboxylase and PYR accurately identified all of our *S. lugdunensis* strains. The suggested addition of mannose utilization to differentiate *S. haemolyticus* from *S. lugdunensis*

(15) was not required to improve the specificity of this protocol. Of the commercial kits, the ID 32 Staph kit performed marginally better than the Vitek ID-GP card.

S. lugdunensis generally has been characterized as being susceptible in vitro to most antibiotics. Early studies reported penicillin resistance rates of <4% (14), while more recent studies report penicillin resistance rates of 12 to 15% (9, 13). Only one methicillin-resistant isolate has been reported (17). Over a quarter of clinical isolates of *S. lugdunensis* in our population were resistant to penicillin, while methicillin resistance was present in 5% of study isolates. However, other than that for tetracycline, resistance to non-beta-lactam antibiotics was low. Current CLSI breakpoints for oxacillin MIC and cefoxitin disc testing clearly differentiate *mecA*-positive strains of *S. lugdunensis*. The latex detection of *pbp2* also is a viable alternative.

This study validates a simple screening strategy to differentiate *S. lugdunensis* from other coagulase-negative staphylococci. This will improve the recognition of clinical disease and the surveillance of antibiotic resistance in *S. lugdunensis*.

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