CASE REPORTS

Osteomyelitis Caused by *Staphylococcus schleiferi* and Evidence of Misidentification of This *Staphylococcus* Species by an Automated Bacterial Identification System

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We report a case of sternal osteomyelitis due to *Staphylococcus schleiferi* in a patient who underwent thoracic surgery. This constitutes the first documented case of osteomyelitis caused by this *Staphylococcus* species. We also relate our experience in the utilization of commercially available MicroScan panels for the identification of this microorganism.

CASE REPORT

A 67-year-old male was admitted to our hospital in January 1996 because of chest pain. His history included non-insulindependent diabetes mellitus and an aortic valve replacement due to aortic insufficiency 3 months prior to admission.

On admission, the patient was febrile. He had a tender and reddened centrothoracic mass. Laboratory studies were unremarkable. A computed tomography scan of the chest showed a sternal dehiscence with irregular margins. Mediastinitis was ruled out. A gallium 67 scan showed pathological uptake in the sternal body and xiphoid appendix. Drainage of the mass was performed, and the exudate culture yielded gram-positive cocci, β-hemolytic on Columbia agar with 5% sheep blood (bioMérieux, Marcy l'Etoile, France), that were catalase positive, consistent with Staphylococcus species. They were positive for latex slide agglutination (Pastorex Staph-Plus; Sanofi Diagnostics Pasteur, Marnes-la-Coquete, France). These results were confirmed several times with repeated samples. No additional microorganisms were isolated. The patient was started on intravenous cloxacillin plus gentamicin. After 2 weeks of treatment, no improvement was observed, and the patient underwent a surgical reexploration. Sternal osteomyelitis was confirmed (biopsy sample), and a piece of braided polyester tape (Cervix-set; Braun, Melsungen, Germany) was disclosed in the inferior third of the sternum. Local debridement was performed, and the sternal tape was removed. A few days later, the wound was healed without evidence of infection. After 4 weeks of intravenous treatment with cloxacillin, the patient was discharged. Due to gastrointestinal intolerance to cloxacillin, the treatment was continued with oral amoxicillin for an additional 2 weeks. Two years later, the patient remained asymptomatic.

From this patient, 12 clinical specimens that yielded *Staph*ylococcus schleiferi, including the polyester tape, were recovered. The microorganism was first identified as penicillin-susceptible Staphylococcus aureus by MicroScan Combo Pos 4I panels (Dade International Inc., West Sacramento, Calif.), with a code profile of 317343 and a 99.8% certainty. However, there was a result in the panels, a positive result for pyrrolidonyl- β -naphthylamide hydrolysis, which is not consistent with S. aureus identification according to standards (12). Other results reported by the MicroScan system, such as the absence of production of acid from mannitol and lactose, were also unusual for the S. aureus biochemical profile. These and other results present in the panels (Table 1) were, nevertheless, consistent with those for S. schleiferi. This species was afterwards confirmed by the results of other reactions: coagulase tube test negative (Difco Laboratories, Detroit, Mich.), DNase positive (Merck Laboratories, Darmstadt, Germany), ornithine decarboxylase negative (Difco Laboratories), and polymyxin (300-U disk; Sanofi Diagnostics Pasteur) susceptible. The identification was also verified by the API ID 32 Staph biochemical gallery (bioMérieux), which yielded the result S. schleiferi with 99.9% certainty. At that time S. schleiferi was not recognized by our MicroScan system software, until December 1998, when new software that identifies more bacterial species, including S. schleiferi, was incorporated to our equipment. Although we did not identify the isolates to the subspecies level, they were considered to belong to the subspecies schleiferi since they were urease negative and tube coagulase negative (S. schleiferi subsp. coagulans is urease positive and tube coagulase positive) (19).

S. schleiferi was described in 1988 by Freney and coworkers (6). It is commonly found living on carnivores, but may be transferred from pets to their owners or handlers (10). Recent studies suggest that this microorganism is a member of the human preaxillary skin flora (2), but it is not known if carriage is persistent or transient. Since its first description, only a few data have been published in the literature on its pathogenicity. In studies of abscess formation in mice, *S. schleiferi* was shown to be more virulent than other coagulase-negative *Staphylococcus* species (4, 14). Although it has been implicated as the

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 TABLE 1. Characteristics displayed by 64 clinical isolates of S.

 schleiferi in MicroScan Combo Pos 4I panels

Test	Results for case report strain	% of isolates for which test was positive
Resistance to:		
Crystal violet	_	0
Bacitracin (0.05 µg/ml)	+	100
Novobiocin (1.6 µg/ml)	_	0
Optochin	+	100
Nitrate reduction	+	100
Acetoin production	+	100
Indoxil phosphatase	+	a
Alkaline phosphatase	+	100
Bile esculin (40%)	_	0
Urease	_	0
Pyrrolidonase	+	100
Arginine dehydrolase	+	100
β-Lactamase	_	0
β-Glucuronidase	_	0
β-Galactosidase	+	100^{b}
Acid production from:		
Lactose	_	0
Trehalose	+	19
Mannose	+	100
Mannitol	_	0
Raffinose	_	0

^{*a*} Inconsistent results, usually positive by manual reading or by autoScan 4 and negative by WalkAway 96. Uncertain meaning, but manufacturers comment that the test is usually positive for coagulase-positive and DNase-positive staphylococci.

^b Sometimes a weak reaction was displayed and was read as a negative result by WalkAway 96. Nevertheless, the reaction was always estimated to be positive by manual reading.

causative agent of several human infections (1, 2, 5, 9, 13, 15, 17; M. Latorre, P. M. Rojo, M. J. Unzaga, and R. Cisterna, Letter, Clin. Infect. Dis. 16:589-590, 1993), including a case of bacteremia with possible vertebral infectious localization (5), this is, to the best of our knowledge, the first confirmed case of osteomyelitis due to S. schleiferi. It is well-known that coagulase-negative Staphylococcus species are commonly isolated from wounds of patients after median sternotomy, having an important impact on cardiothoracic surgery-related morbidity (16). Sternal wound infections occur in 1 to 3% of patients who undergo open-heart surgery, and they can range from superficial infections to open mediastinitis with invasion of deep structures (3). In our patient a piece of polyester sternal tape probably acted as a pathogenic factor. In this regard, it is tempting to speculate that specific adhesins and slime produced by this organism could have favored its growth as a biofilm adherent to the polyester surface of the sternal tape, starting and maintaining the S. schleiferi infection (11). On the other hand, growth as a biofilm could have protected the staphylococci from antibiotics, and, therefore, the patient experienced a clear improvement only when the foreign body was removed.

The low presence of *S. schleiferi* in human flora (10) could explain the low frequency of infections due to this microorganism. However, it has been suggested that the real occurrence of these infections is underreported due to the erroneous identification of *S. schleiferi* as *S. aureus* in routine laboratory testing (13). Both strains exhibit beta-hemolysis and are morphologically similar on blood agar. Moreover, *S. schleiferi* subsp. *schleiferi*, like *S. aureus*, produces both clumping factor and thermonuclease.

Although a tube coagulase test could be helpful to discrim-

inate S. aureus from other staphylococci, this practice is, at present, practically in disuse because it has been replaced by the easier and more rapid latex slide agglutination tests, which detect clumping factor and protein A. Nevertheless, one should be mindful that neither tube coagulase nor slide agglutination can be considered a definitive test to differentiate S. aureus from other staphylococci. A positive result for clumping factor in the absence of coagulase may indicate coagulasenegative S. aureus, S. lugdunensis, or S. schleiferi subsp. schleiferi, and, therefore, in some situations additional tests can be necessary to resolve the identification (F. Vandenesch, M. Bes, C. Lebeau, T. Geenland, Y. Brun, and J. Etienne, Letter, Lancet 342:995–996, 1993). Various easy schemes to identify S. schleiferi are published elsewhere (7, 8, 15, 18). In our case, tests present in MicroScan conventional panels, such as production of pyrrolidonase and alkaline phosphatase, acid production from mannitol, and the Voges-Proskauer reaction, were sufficient to discriminate between latex slide agglutination-positive species. However, special care should be take when rapid slide agglutination tests are used for S. schleiferi due to its variability depending on the culture medium and the commercial kit (9).

Finally, our experience illustrates how a former MicroScan database for automated bacterial identification has also contributed to the S. schleiferi confusion with S. aureus. From January 1996, when we first diagnosed the described case of S. schleiferi infection, until the acquisition of the new MicroScan identification database (December 1998), we manually read the biochemical tests in panels where S. aureus was identified, especially from those isolates that displayed susceptibility to all antibiotics. The presence of a pyrrolidonyl-B-naphthylamide positive test indicated suspicion that an isolate was S. schleiferi, and the isolate's identity was confirmed by other biochemical characteristics present in MicroScan panels (Table 1) and by the tube coagulase test (negative) and tests for the production of DNase (positive) and ornithine decarboxylation (negative). In this way, a total of 42 isolates from 20 patients were recovered after the first case, and four distinct biochemical profile codes were defined by the MicroScan system: 307343, 317343, 307341, and 317341. Additionally, we accomplished a retrospective search to find S. schleiferi reported as S. aureus, looking in our database for results displaying the mentioned code profiles that correspond to S. schleiferi. Following this, another nine patients infected with S. schleiferi (10 isolates) were revealed. These isolates were recovered from a variety of sources, including wound exudate (17 patients), blood culture (5 cases), catheter tip (4 patients), ear exudate (3 cases), pleural fluid (1 patient), corneal exudate (1 patient), biliary drainage (1 patient) and urine (1 patient).

In summary, this report highlights the importance of the careful identification of *S. schleiferi* in the clinical microbiology laboratory and expands the clinical spectrum of this microorganism as a causative agent of human infections.

REFERENCES

- Célard, M., F. Vandenesch, H. Darbas, J. Grando, H. Jean-Pierre, G. Kirkorian, and J. Etienne. 1997. Pacemaker infection caused by *Staphylococcus schleiferi*, a member of the human preaxillary flora: four case reports. Clin. Infect. Dis. 24:1014–1015.
- Da Costa, A., H. Lelièvre, G. Kirkorian, M. Célard, P. Chevalier, F. Vandenesh, J. Etienne, and P. Touboul. 1998. Role of the preaxillary flora in pacemaker infections. Circulation 97:1791–1795.
- Fariñas, M. C., F. Peralta, J. M. Bernal, J. M. Rabasa, J. M. Revuelta, and J. Gonzalez-Macías. 1995. Suppurative mediastinitis after open-heart surgery: a case-control study covering a seven-year period in Santander, Spain. Clin. Infect. Dis. 20:272–279.
- Ferguson, K. P., D. W. Lambe, Jr., J. L. Keplinger, and J. H. Kalbfleisch. 1991. Comparison of the pathogenicity of three species of coagulase-negative

Staphylococcus in a mouse model with and without a foreign body. Can. J. Microbiol. **37:**722–724.

- Fleurette, J., M. Bès, Y. Brun, J. Freney, F. Forey, M. Coulet, M. E. Reverdy, and J. Etienne. 1989. Clinical isolates of *Staphylococcus lugdunensis* and *S. schleiferi*: bacteriological characteristics and susceptibility to antimicrobial agents. Res. Microbiol. 140:107–118.
- Freney, J., Y. Brun, M. Bes, H. Meugnier, F. Grimont, P. A. D. Grimont, C. Nervi, and J. Fleurette. 1988. *Staphylococcus lugdunensis* sp. nov. and *Staphylococcus schleiferi* sp. nov., two species from human clinical specimens. Int. J. Syst. Bacteriol. 38:168–172.
- Hebert, G. D. 1990. Hemolysins and other characteristics that help differentiate and biotype *Staphylococcus lugdunensis* and *Staphylococcus schleiferi*. J. Clin. Microbiol. 28:2425–2431.
- Ieven, M., J. Verhoeven, S. R. Pattyn, and H. Goossens. 1995. Rapid and economical method for species identification of clinically significant coagulase-negative staphylococci. J. Clin. Microbiol. 33:1060–1063.
- Jean-Pierre, H., H. Darbas, A. Jean-Roussenq, and G. Boyer. 1989. Pathogenicity in two cases of *Staphylococcus schleiferi*, a recently described species. J. Clin. Microbiol. 27:2110–2111.
- Kloos, W. 1997. Taxonomy and systematics of staphylococci indigenous to humans, p. 113–137. *In* K. B. Crossley and G. L. Archer (ed.), The staphylococci in human disease. Churchill Livingstone, New York, N.Y.
- Kloos, W. E., and T. L. Bannerman. 1994. Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. 7:117–140.
- Kloos, W. E., and T. L. Bannerman. 1999. *Staphylococcus* and *Micrococcus*, p. 264–282. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

- Kluytmans, J., P. Steegh, F. Vandenesch, J. Etienne, and A. Van Belkum. 1998. Outbreak of *Staphylococcus schleiferi* wound infections: strain characterization by randomly amplified polymorphic DNA analysis, PCR ribotyping, conventional ribotyping, and pulsed-field gel electrophoresis. J. Clin. Microbiol. 36:2214–2219.
- Lambe, D. W., Jr., K. P. Ferguson, J. L. Keplinger, C. G. Gemmell, and J. H. Kalbfleisch. 1990. Pathogenicity of *Staphylococccus lugdunensis*, *Staphylococcus schleiferi*, and three other coagulase-negative staphylococci in a mouse model and posible virulence factors. Can. J. Microbiol. 36:455–463.
- Leung, M. J., N. Nuttall, M. Mazur, T. L. Taddei, M. McComish, and J. W. Pearman. 1999. Case of *Staphylococcus schleiferi* endocarditis and a simple scheme to identify clumping factor-positive staphylococci. J. Clin. Microbiol. 37:3353–3356.
- Mossad, S. B., J. M. Serkey, D. L. Longworth, D. M. Cosgrove III, and S. M. Gordon. 1997. Coagulase-negative staphylococcal sternal wound infections after open heart operations. Ann. Thoracic Surg. 63:395–401.
- Ozturkeri, H., O. Kocabeyoglu, Y. Z. Yergok, E. Kosan, O. S. Yenen, and K. Keskin. 1994. Distribution of coagulase negative staphylococci, including the newly described species *Staphylococcus schleiferi*, in nosocomial and community acquired urinary tract infections. Eur. J. Clin. Microbiol. Infect. Dis. 13:1076–1079.
- Schnitzler, N., R. Meilicke, G. Conrads, D. Frank, and G. Haase. 1998. *Staphylococcus lugdunensis*: report of a case of peritonitis and an easy-toperform screening strategy. J. Clin. Microbiol. 36:812–813.
- Vandenesch, F., C. Lebeau, M. Bes, G. Lina, B. Lina, T. Greenland, Y. Benito, Y. Brun, J. Fleurette, and J. Etienne. 1994. Clotting activity in *Staphylococcus schleiferi* subspecies from human patients. J. Clin. Microbiol. 32:388–392.