First Case of Osteomyelitis Due to *Shewanella algae*

E. Botelho-Nevers,1 F. Gouriet,2 C. Rovery,1 P. Paris,3 V. Roux,2 D. Raoult,2 and P. Brouqui1*

**Service de Maladies Infectieuses et Tropicales, Hôpital Nord, Marseille, France; Laboratoire de Microbiologie, Timone, Marseille, France; and Service de Chirurgie Orthopédique, Clinique Beauregard, Marseille, France**

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*Shewanella* spp. are infrequently recovered from clinical specimens. We report here on the first case of osteomyelitis due to *Shewanella algae*. This bacterium, at first misidentified by phenotypic tests as *Shewanella putrefaciens*, was subsequently identified correctly as *S. algae* by 16S rRNA gene sequence analysis.

**CASE REPORT**

A 45-year-old woman who lived in Turkey and who had a history of depression tried to commit suicide in June 2003 by throwing herself into an open well. She broke her right tibia, resulting in an open fracture. Treatment of the fracture included osteosynthesis. No information about the initial prophylactic antibiotic therapy was available for this report. Following treatment, the initial outcome was favorable and the patient was capable of walking within the expected time frame. Ten months later, however, she presented with severe pain and limited use of her leg. She had a low-grade fever (temperature, 38°C). Her right leg was inflamed, but no fistula was present. Biochemical tests showed that her C-reactive protein level was elevated (47 mg/liter; normal range, <10 mg/liter) and her erythrocyte sedimentation rate was elevated (72 mm; normal range, <10 mm) at presentation. Radiographs of the affected leg revealed delayed consolidation of the bone with pseudarthrosis. An osteotomy and intramedullary pinning of the tibia were performed, and specimens of the removed bone and intramedullary tissue were cultured. A direct Gram stain was performed on this material but did not reveal any bacteria. Samples were inoculated onto chocolate agar plates (bioMérieux Inc., Marcy l’Etoile, France) and incubated at 37°C in a 5% CO2 atmosphere. The samples were also inoculated onto 5% sheep blood agar (bioMérieux Inc.) and incubated under anaerobic conditions at 37°C, although a prereduced medium was not used for anaerobic cultures. Fungal and mycobacterial cultures were performed but showed no growth. After 2 days, small brown mucoid colonies were observed on both agar plates (approximately 104 CFU per plate) and exhibited beta-hemolysis on the sheep blood agar. On a Gram stain, the isolate was found to be a gram-negative rod. The bacteria were isolated from the osteomyelitis tissue in pure culture and were identified as *Shewanella putrefaciens*, to which they had 99% similarity, by using the API 20NE system (bioMérieux Inc.). To further characterize the isolate, we extracted the nucleic acids from harvested colonies using a MagNA Pure kit (LC Roche, Paris, France). PCR amplification of the 16S rRNA-encoding gene was performed by using the universal primer pair ID1 and rp2 (18). The PCR products obtained were purified by MultiScreen PCR (Millipore, Molsheim, France) and were sequenced with a DNA sequencing kit (BigDye Terminator cycle sequencing, version 2.0, ready reaction kit; Perkin-Elmer Biosystems, Foster City, CA), according to the manufacturer’s instructions. The sequence products were purified, and electrophoresis was performed with a 3100 genetic analyzer (Applied Biosystem, Courtaboeuf, France). The 16S rRNA gene sequences were compared with sequences deposited in the GenBank database and were found to be 99% similar to that of *S. algae* (Fig. 1).

The bacteria were shown to be susceptible to ticarcillin, ceftazidine, ciprofloxacin, imipenem, colistin, and tobramycin by using the VITEK automated system (bioMérieux Inc.). The patient was treated for the first 2 weeks with 1,500 mg/day of oral ciprofloxacin, 3,000 mg/day of ceftazidine, and 200 mg/day of tobramycin and then with ciprofloxacin alone (3). Ciprofloxacin administration was continued for 8 months because, although it would have been preferred, the osteosynthesis material could not be removed due to the condition of the bone. During monthly follow-up appointments, bone consolidation was observed on radiographs and regression of the pain and inflammation was noted. Two months after the completion of treatment the patient was well.

To our knowledge, this is the first case of osteomyelitis caused by *S. algae* to be reported. First identified as *S. putrefaciens* by phenotypic characterization, the isolate was only correctly identified as *S. algae* by 16S rRNA gene sequence analysis. No underlying conditions were found in our patient. The bacteria were isolated in pure culture from an osseous tissue sample obtained during a surgical procedure, and consequently, its pathogenic role in this situation is very likely. No other case of *Shewanella* sp. infection was reported in the literature (7, 17). Usually, *S. algae* and *S. putrefaciens* are both susceptible to gentamicin, piperacillin, and ceftaxime, whereas *S. algae* is tolerant of penicillin, ampicillin,
and tetracycline, contrary to *S. putrefaciens* (17). The use of the VITEK automated system for susceptibility testing has already been described (8, 14) and was validated with an antibiotic disk diffusion assay (Bio-Rad, Marnes-la-Coquette, France) (1). The outcome for our patient under prolonged and combined therapy was favorable.

Although *S. algae* has been described in seawater, stagnant water, such as that in an open well, is one of the environmental reservoirs of *Shewanella* spp. (5). It is therefore probable that the open tibial fracture in our patient was infected by direct inoculation of the bacteria living in the well, although it was not possible to obtain a sample of the well water for analysis to confirm this strong assumption. The patient apparently developed a chronic osteomyelitis with few symptoms, explaining the long period of time that elapsed before she presented with pain and inflammation following the initial treatment. This is a common finding in chronic bone infection with indwelling devices (10).

Cases of *S. algae* infection have been described in humans and are usually linked to contact with water. Bacteremia is rare and has been reported in only five patients. This condition was described in two patients with lower leg ulcers (5). Three other cases of bacteremia have been reported: a patient on hemodialysis developed a case of mixed bacteremia (*Escherichia coli* and *S. algae*) (7), an immunocompromised patient with multiple myeloma developed cellulitis in both forearms in association with *S. algae* bacteremia (9), and a patient suffered a rupture of a primary aneurysm infected with *S. algae* (13). Ear infections are more common, and a series of 65 cases has been described: patients, mostly children, had acute or chronic otitis media that occurred in the summer (6). In these patients, contact with seawater shortly before symptoms developed was reported (6).

Although *S. putrefaciens* is the predominant human pathogen described within this genus, several investigators have suggested that most clinical isolates of *S. putrefaciens* from humans might in fact belong to the separate species *S. algae* (first described as a tetrodotoxin-producing isolate recovered from red algae) (12). In a comparison of 49 strains of *Shewanella* spp., *S. putrefaciens* was more often associated with nonhuman sources (70%); in contrast, *S. algae* originated from clinical material in 92% of cases (8). Pathogenicity studies in mice indicate that *S. algae* appears to be more virulent than *S. putrefaciens*, possibly due to the production of a hemolytic
substance or exotoxins (8). By traditional phenotypic characterization, S. algae can easily be misidentified as S. putrefaciens, as occurred in this case. It is important that the API 20NE system does not distinguish between S. putrefaciens and S. algae (17). The two species can be differentiated only by use of the phenotypic characteristics proposed by Nozue et al. (12), whole-cell protein profiling, ribotyping, antimicrobial susceptibility, or 16S rRNA sequencing (17).

Two cases of arthritis due to S. putrefaciens species have been described (11, 16). The first was a patient with end-stage renal failure on chronic ambulatory peritoneal dialysis who presented with acute arthritis of his left wrist and right ankle in association with bacteremia due to S. putrefaciens (16). In this case, the method of identification of the bacteria was not reported. The second was a patient who developed arthritis of the second proximal interphalangeal articulation of his left foot after it was punctured by a sea urchin. The arthritis was associated with cellulitis of his foot due to S. putrefaciens, which was identified by use of the API 20NE system (bioMérieux Inc.) (11). One case of S. putrefaciens-associated osteomyelitis was also described: a young boy with bacteremia due to S. putrefaciens acquired sacroiliac osteomyelitis after receiving subcutaneous infusions for the treatment of thalassemia major. Again, in this case, the method of identification was not specified (15). Thus, it is not possible in these cases, to confirm that the causal agent was in fact S. putrefaciens. S. putrefaciens has also been involved in infections of chronic leg ulcers, ear infections, pneumonia, urinary tract infections, and septicemia (2, 4). When the species has been isolated, it has often been associated with other bacterial pathogens (60% of cases) (11). In most instances, isolation of S. putrefaciens has occurred in the absence of clinical disease and has been considered merely colonization rather than an active infection (2).

We report here on the first case of osteomyelitis due to S. algae after an open fracture that came into contact with stagnant water. S. algae was identified by 16S rRNA gene sequence analysis only. This report emphasizes the need for molecular typing of Shewanella spp. to obtain an accurate identification of the causal agent given the differences in pathogenecities of the two species of Shewanella described in human disease.

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