Septicemia Due to *Pasteurella pneumotropica*: 16S rRNA Sequencing for Diagnosis Confirmation

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Bacteremia due to *Pasteurella pneumotropica* occurs infrequently. We report a case of septicemia in a 72-year-old woman who had no underlying illness. The microorganism was isolated from 10 blood cultures and identified by conventional and molecular methods. This is the first reported case of *P. pneumotropica* septicemia in an immunocompetent patient.

**Case report.** A 72-year-old female patient was admitted to the hospital for an 18-day history of fever, chills, night sweats, and asthenia. The patient had a history of rheumatic mitral valvulopathy and had had a Saint-Jude valve transplantation operation in 1982. She underwent a permanent pacemaker implantation procedure in 1989 for sick sinus syndrome. The patient lived in poor social conditions in the company of five cats and one dog. She admitted that she was frequently scratched by the cats. On physical examination the patient was found to be febrile (temperature, 39.5°C) but was not hemodynamically unstable (blood pressure of 150/60 mm of mercury). Skin examination revealed no cutaneous lesion and no visible sign of animal scratch or bite. The white blood cell count was 18.8 × 10⁹/liter, with 91% of cells being polymorphonuclear. Other hematological assessments included the following: hemoglobin, 13.7 g/dl; hematocrit, 41.3%; and platelet count, 112,000/mm³. The level of C-reactive protein was 46.1 mg/liter at admission and reached 271.4 mg/liter the next day. Serum electrolyte levels were normal. A transesophageal echocardiography did not reveal any functional anomaly of the mechanical valve and did not show any vegetation. Ten sets of blood cultures were drawn, with seven being drawn on the day of admission and three on the next day. Each of these blood cultures yielded a gram-negative-bacillus pure culture. The other investigations, i.e., urine culture, analysis of cerebrospinal fluid, electrocardiography, chest radiograph, cerebral scan-ner, and abdominal echography, were normal. The pacemaker was functional and was not replaced.

The Gram stain from the culture showed small gram-negative coccobacilli, with some longer bacillary forms. Blood, chocolate, and MacConkey agar plates were inoculated. After 24 h of incubation in 5% CO₂, small, smooth, white-gray, transparent, nonhemolytic colonies were observed on the plates, but no growth was detected on MacConkey agar. The bacteria were facultatively anaerobic, nonmotile, and non-spore forming. Biochemical tests performed on the isolate showed that reactions for oxidase, catalase, indole, and urease were positive. The isolate was further found to reduce nitrates to nitrites. Phenotypic tests performed twice with the API 20NE system (bioMérieux, la Balme les Grottes, France) resulted in an identification of *P. pneumotropica* (96.9% certainty) (numerical code, 3200004), while the GNI card (bioMérieux Vitek, Inc., Hazelwood, Mo.) resulted in an identification of *Pasteurella haemolytica* (64% certainty) (numerical code, 40001400000). This discrepancy, which was possibly related to the lack of *P. pneumotropica* in the Vitek database, and the need for a diagnosis confirmation prompted us to perform 16S rRNA sequencing of the organism. An 803-bp portion of the 16S rRNA gene was amplified from plate-grown bacteria by using the primers 16S1, 5'-GAGAGTTTGATCCTGGCTC-3' and 16S2, 5'-GTGGACTACCAGGTATCTAAATC-3'. The 16S rRNA gene sequence was determined, after amplicon purification, in an automated DNA sequencer (Applied Biosystems Inc., Foster City, Calif.) by a dye-labeled deoxy termination method (Taq Dye-Deoxy Terminator Cycle sequencing kit; Applied Biosystems Inc.). The sequence was compared with National Center for Biotechnology Information GenBank entries by using the BLAST algorithm, which indicated 100% homology with *P. pneumotropica*. Meanwhile, the susceptibility of the isolate was tested in vitro by the disk diffusion method on Mueller-Hinton agar containing 5% horse blood. The organism was susceptible to ampicillin, pipercillin, cefotaxime, aminoglycosides, trimethoprim-sulfamethoxazole, and fluoroquinolones. The patient was empirically treated intravenously with cefotaxime (2 g/day) and netilmicin (300 mg/day) for 10 days. The treatment was subsequently switched to intra-
venous amoxicillin (4 g/day) and trimethoprim-sulfamethoxazole (2 g/day) for 10 days. Although the patient remained febrile for 12 more days, she subsequently made an uneventful recovery and was discharged 20 days later on oral amoxicillin (4 g/day) and trimethoprim-sulfamethoxazole (1.5 g/day) for 10 days.

**Discussion.** *P. pneumotropica* is an aerobic and facultatively anaerobic gram-negative coccobacillus that was first isolated and characterized in 1950 by Jawetz (10). This microorganism is part of the commensal oropharyngeal floras of many animals, including dogs and cats. It is also frequently isolated from the normal upper respiratory floras of rodents and has been frequently reported as an infective agent in chronic subclinical or epidemic infections of laboratory animals. The infections of laboratory animals include pulmonary (10), uterine (2), ocular (17), and enteric (13) infections. The association of this organism with necrotizing pneumonia in mice led Jawetz to name it *P. pneumotropica* (10). Two biotypes, the Heyl and Jawetz biotypes, have been distinguished and found in epidemic infections of laboratory animals (12, 22). Because the biochemical identification of *P. pneumotropica* is time-consuming, enzyme-linked immunosorbent assay (14) and PCR-based detection methods (12, 18, 21) have been alternatively proposed for the diagnosis of infections in laboratory animals.

The present case report illustrates the clinical utility of molecular identification to confirm the role of exceptional pathogens in major cases of sepsis. Although the microorganism in this case was successfully identified by a conventional method, the API 20NE system, further identification was required to confirm the role of this unusual agent in this serious systemic infection. Such a confirmation can eventually be obtained by conventional means. In the present case, the critical biochemical test results that could have been used to differentiate *P. pneumotropica* from *P. multocida* included urease, maltose, and mannitol reactions (7). However, these conventional identification methods are laborious because they are time-consuming and require subjective evaluation. Different conventional automated systems can alternatively be used to confirm an identification and are easier to perform. Unfortunately, the Vitek 32GNI system, which was used in this study, does not include *P. pneumotropica* in its database and identified the organism as *P. haemolytica*, which is the principal microorganism responsible for bovine pneumonic pasteurellosis but which is not known to be a human pathogen. Thus, the apparent lack of consistency between the API 20NE system and the Vitek GNI card was related to the incompleteness of the Vitek database. In such a case of apparent discrepancy, molecular detection is the most reliable means to rapidly confirm a diagnosis (12, 18, 21). Moreover, molecular identification has been shown to be more accurate for distinguishing some isolates that were not clearly identified as *P. pneumotropica* by biochemical procedures (12). In the case of laboratory animal infections, the presence of *P. pneumotropica*, if suspected on the basis of results of conventional methods, can be easily confirmed by a specific PCR (12, 18, 21). In humans, *P. pneumotropica* infections occur very rarely, and so the performance of a specific PCR is not justified; instead, the identification of unusual invaders is confidently assessed by 16S rRNA sequencing. In our study, this strategy allowed an indisputable identification of the organism, since its nucleotidic sequence exhibited 100% identity with that of *P. pneumotropica* type Jawetz, which was accessible from GenBank.

The presence of *P. pneumotropica* is sometimes reported in the respiratory floras of persons who have pets, especially if these individuals have some underlying disease, such as cirrhosis, or a neoplasm (5, 19). In a review of the English-language literature, we found sporadic case reports of human infections with *P. pneumotropica*, such as endocarditis (3), cellulitis (1), bone and joint infections (8, 20), meningitis (16), skin infections (15), peritonitis in a dialysis patient (4), and pneumonia in a patient with AIDS (5). One case of septicemia in a patient receiving chemotherapy for myeloid leukemia was reported in 1973 (19). Another case, involving a patient who contracted meningitis following a dog bite, suggested the occurrence of bacteremia in the pathogenesis of the infection (16). To our knowledge, the present case documents the first case in the English-language literature of septicemia caused by *P. pneumotropica* in an immunocompetent elderly host. Serious systemic human infections with *Pasteurella* species are unusual, but they may occur in individuals with predisposing underlying illnesses. Because systemic *P. pneumotropica* is very rare, pathogenesis data are lacking. The most likely mechanisms of infection can be inferred from those of *P. multocida* infections. Systemic *P. multocida* infections may complicate the formation of an abscess following animal trauma when an underlying chronic disease is present (11). Systemic infections may also be associated with infection of the respiratory tract related to atraumatic animal exposure in patients with underlying chronic respiratory disease (6). In the present case, our patient had close contact with animals and was frequently scratched. Therefore, the most probable pathogenic mechanism of infection was either an animal trauma, which was likely healed at the time of admission and therefore not detected on physical examination, or the contact of minor skin lesions with cat or dog secretions. Another mechanism could be an oropharyngeal colonization by *P. pneumotropica*, as suggested by the report of pneumonia due to *P. pneumotropica* infection in a patient with AIDS (5). This hypothesis is unlikely because our patient did not present with respiratory symptoms and had a normal chest radiograph. However, we did not investigate the presence of *P. pneumotropica* in the commensal upper respiratory flora of the patient or the carriage of *P. pneumotropica* in the household pets.

In conclusion, *P. pneumotropica* is usually considered an exceptional and opportunistic human pathogen. The present case suggests that its ability as a primary invader should not be underestimated, especially in elderly patients who have contact with household pets. Since the database of commercial identification systems cannot be exhaustive, molecular identification is the most efficient way to confirm the role of such exceptional pathogens in major cases of sepsis.

**REFERENCES**