CASE REPORT

A 73-year-old man was hospitalized in December 2006 in the Pulmonary Diseases Unit of the European Hospital Georges Pompidou in Paris, France, for acute respiratory insufficiency. The medical history of this patient, a heavy smoker, included chronic respiratory insufficiency caused by chronic obstructive pulmonary disease and by consequences of pulmonary tuberculosis contracted during childhood and treated then. The patient also suffered from essential thrombocythemia treated with hydroxyurea.

A bilateral pulmonary embolism was initially diagnosed (day 1) using chest computed tomography, and the patient was subsequently treated with heparin. He developed fever (day 13) and his respiratory state worsened, with the onset of septic shock (day 15). He was referred to the intensive care unit for supportive care, and endotracheal intubation became necessary. Because of suggestive clinical signs and bilateral opacities on the chest X-rays, bronchoalveolar lavage was performed (day 15). The analysis of the lavage fluid showed gram-positive cocci in pairs, tetrads, and clusters (1). Biochemical tests using the API Strep System (bioMérieux, Marcy l’Etoile, France) identified the organisms as belonging to the genera *Staphylococcus* and *Aerococcus* (day 15). The analysis of the lavage fluid showed gram-positive cocci of two distinct morphotypes, with one round type and the other more ovoid in shape, in clusters, pairs, or tetrads. The fluid contained 250 leukocytes per mm³ (with 86% polymorphonuclear neutrophils, 27% of which had intracellular cocci). Initial treatment was with vancomycin and polymyxin B and gentamicin. At this time, a first set of cultures (using aerobic and anaerobic BacT/Alert media; bioMérieux, Marcy l’Etoile, France) from blood collected on day 13 yielded only *Staphylococcus aureus*, which was present at 2 × 10⁴ CFU/ml. Subculture of blood samples yielded only *Gemella*, as identified above. Its susceptibility was tested in vitro using disk diffusion on Mueller-Hinton agar with 5% sheep blood according to French guidelines (9; http://www.sfm.asso.fr/). The isolate was susceptible to all beta-lactam antibiotics tested, including amoxicillin, and to macrolides, aminoglycosides, glycopeptides, and sulfonamides; it was resistant to trimethoprim. The *S. aureus* isolate was a methicillin- and aminoglycoside-susceptible penicillinase producer. This motivated a switch to treatment with amoxicillin-clavulanic acid (during which gentamicin was continued for 2 days). A second set of cultures from blood collected on day 15 yielded only *S. aureus* with the same phenotype as that described above. The state of the patient improved slowly; he was extubated himself after 18 days (day 33). Unfortunately, he developed a second episode of nosocomial pneumonia 1 month later, in this case due to *Klebsiella oxytoca* and *Serratia marcescens*, which proved to be fatal (day 39).

Because the biochemical techniques used were not sufficiently accurate for identification of the presumptive *Gemella* isolates to the species level, 16S rRNA gene amplification and sequencing were performed. Bacterial DNAs were extracted using IntraGene Matrix (Bio-Rad) according to the manufacturer’s instructions. A 475-bp segment of the 16S rRNA gene was amplified using the universal primers p91E [5'-GGAATT-GGAATTGACGCGGC3'-] and p13B (5'-CGG ATCCAGGCCCCGGAACGTATTCC3'-) (7). The nucleotide sequence was determined with a 3700 DNA analyzer following a BigDye Terminator cycle sequencing ready reaction (Applied Systems). The search for similarities was carried out using NCBI BLAST and BiPhy phylogenetic tools (http://phil.univ-lyon1.fr/). Sequence analysis of the fragment led to the identification of *Dolosigranulum pigrum* in both bronchoalveolar lavage and blood samples. The nucleotide homology was 100% with respect to the corresponding sequence of the reference strain of the species (X70907).

Dolosigranulum pigrum Causing Nosocomial Pneumonia and Septicemia

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We report a case of non-ventilator-associated nosocomial pneumonia and septicemia due to *Dolosigranulum pigrum*, a rare gram-positive opportunistic pathogen. The organism was isolated from bronchoalveolar lavage fluid and blood of a debilitated patient. *D. pigrum* was identified after 16S rRNA gene sequencing.
chemical tests using conventional commercial kits were not conclusive, but the isolates appeared to be related to Gemella species. However, the arginine dehydrogenase test was positive and there was no immunological reaction between polyvalent antisera raised against the unknown isolates and type strains of Gemella. Finally, 16S rRNA gene sequencing revealed no close relation to previously known bacteria, and a new genus and species were established. The most closely related species, based on 16S rRNA gene sequences, are Aerococcus species, with 89% homology (1).

Recently, molecular methods have led to the establishment of close to 15 genera of catalase-negative, gram-positive cocci in addition to Streptococcus and Enterococcus (8). The increasing rate of isolation of these unusual bacteria is probably due to their real emergence as novel opportunistic pathogens among patients with compromised immunity, not simply a consequence of the growing use of 16S rRNA gene sequencing in clinical laboratories.

The phenotypic characteristics of D. pigrum have been described (5, 8), but attempts to identify this species using commercial test strips lead to the determination of an “unacceptable identification” or to misidentification because the profiles generated by D. pigrum are not included in the databases provided by the manufacturers. If they were, identification with the API rapid ID32 STREP test strip system would be possible, provided by the manufacturers. If they were, identification with the test conventionally, before 16S rRNA gene sequencing, we were unable to identify D. pigrum. The 27 strains of D. pigrum were all susceptible to β-lactam antibiotics, and 52% were resistant to erythromycin, with one strain resistant to trimethoprim-sulfamethoxazole (4). The strain isolated in our case was susceptible to β-lactams and erythromycin but resistant to trimethoprim.

Little is known about the habitat of D. pigrum and its pathogenicity. Of the 27 isolates, 12 were from blood cultures, but bacterial sepsis was reported in only five cases. One was an isolate from a spinal cord after autopsy and one was from urine, while the remaining isolates were from the eye (n = 6), nasopharynx (n = 4), sinus (n = 1), sputum (n = 1), or stomach (n = 1), suggesting that D. pigrum may be a commensal mainly of the upper respiratory tract (4).

There are only three reports of D. pigrum-related infections. One is of multiple synovitis, with two sets of D. pigrum-positive cultures from blood but not from synovial aspirates (2). In this case, the patient had received parenteral cefazolin for 24 h before aspiration of the synovial fluid, and the treatment was continued beyond blood culture and susceptibility testing. However, the high level of white blood cells in the synovial fluid (>80,000/ml with 83% neutrophils) suggested septic arthritis. In this case, the patient was immunocompromised because he received prednisolone daily and methotrexate weekly for chronic rheumatoid arthritis, which could have facilitated the bacteremia and the secondary localization of D. pigrum in the synovial fluid. The second report is of a case of acute cholecystitis and pancreatitis potentially caused by D. pigrum (6). In this case, a set of blood cultures was positive, but a search for bacteria in the biliary tract was not carried out. The patient recovered after 2 weeks of appropriate antimicrobial therapy (empirical ampicillin-sulbactam, followed by oral cephalosporin after the blood culture results). There was no report of either chronic disease or immunodepressive treatment that could have explained infection with D. pigrum.

The third report is of a case of ventilator-associated pneumonia caused by D. pigrum (3). The patient was hospitalized in an intensive care unit and intubated for 10 days because of severe subarachnoid hemorrhage with respiratory failure when he developed pneumonia. He received oral decontamination with topical polymyxin E, tobramycin, and amphotericin B, which could have facilitated pneumonia due to D. pigrum. Bacteria were cultured in abundance from the bronchial aspirate but not from blood. In the three reports, D. pigrum was identified using 16S rRNA gene sequencing.

The case of nosocomial pneumonia reported here is the first in which D. pigrum was isolated from both blood and bronchoalveolar lavage fluid. Although S. aureus was also found in the latter, D. pigrum was present at a substantially higher concentration, with 7 × 10^8 as opposed to 2 × 10^4 CFU/ml. It should be noted that D. pigrum was found in blood cultures 2 days before the onset of septic shock and acute respiratory failure. The patient received hydroxy carbamid for essential thrombocytopenia, which, in itself, may have favored the supervening of an opportunistic pathogen such as D. pigrum. We excluded contamination with this bacterium for at least four reasons, including (i) its high abundance in the bronchoalveolar lavage fluid, (ii) the presence of D. pigrum in both the aerobic and anaerobic blood culture bottles, (iii) the fact that the blood and bronchoalveolar lavage samples were collected in the pulmonary disease and intensive care units, respectively, and (iv) the fact that the two samples were handled by two technicians at two different workstations.

Among the numerous catalase-negative gram-positive cocci considered to be emerging opportunistic pathogens, D. pigrum is one of the least frequently described. The use of appropriate molecular methods of identification should facilitate the determination of the true rate of its involvement in infectious diseases.

REFERENCES