Ventilator-Associated Pneumonia Caused by Dolosigranulum pigrum

A. Hoedemaekers, T. Schülin, B. Tonk, W. J. G. Melchers, and P. D. J. Sturm*

Department of Intensive Care and Department of Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Received 19 May 2006/Returned for modification 15 June 2006/Accepted 26 June 2006

Dolosigranulum pigrum is an unusual gram-positive catalase-negative coccus. It was isolated, only after prolonged incubation, from bronchial secretions from a patient with ventilator-associated pneumonia. The patient responded well to antimicrobial therapy. Identification was done by 16S rRNA DNA sequence analysis, but it can be done with relatively simple phenotypic tests.

CASE REPORT

An otherwise-healthy 51-year-old man was admitted to the intensive-care unit (ICU) after severe aneurysmal subarachnoid hemorrhage with respiratory failure. Prior to endotracheal intubation, the patient aspirated gastric contents and was empirically treated with amoxicillin-clavulanate. Gram stain and cultures of bronchial secretions were negative, and the antibiotic treatment was stopped after 48 h. The patient received selective oral decontamination according to the local protocol using topical polymyxin E, tobramycin, and amphotericin B during his entire stay in the ICU. Ten days after admission, the patient developed clinical signs of ventilator-associated pneumonia (VAP) with increasing sputum production, fever (38.8°C), leucocytosis (14.8 × 10⁹/liter), and new infiltrates on the chest X ray. Gram stain of bronchial secretions showed many polymorphonuclear leukocytes, many gram-positive cocci in clusters resembling staphylococci, and no squamous epithelial cells. Based on these microscopy findings, the patient was started on flucloucinil intravenously 1,000 mg every 6 h. Bronchial secretions were cultured semiquantitatively on 5% sheep blood and chocolate agar. Overnight incubation at 37°C in 5% CO₂ did not show any significant growth. After 36 h, the culture was interpreted as mixed oropharyngeal flora. In the absence of growth of Staphylococcus aureus, the antibiotics were changed after 48 h to teicoplanin intravenously 400 mg every 24 h. Reexamination of the culture after another 48 h of incubation showed an almost pure culture of alpha-hemolytic colonies at a concentration of 5 × 10⁵ CFU/ml. Gram stain of the colonies revealed gram-positive cocci in clusters. When significant growth of the unknown isolate was observed, teicoplanin was stopped and the patient continued treatment with amoxicillin-clavulanate for another 3 days to complete antibiotic treatment for 7 days. The patient became afebrile after 3 days of antibiotic treatment, with normalization of the white blood cell count and was successfully weaned off the ventilator and discharged from the ICU. The patient died 3 weeks after admission to the hospital after rebleeding from his cerebral aneurysm while rehabilitating in the neurological medium-care unit.

* Corresponding author. Mailing address: Department of Medical Microbiology, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Phone: 0031243614356. Fax: 0031243540216. E-mail: P.Sturm@mmb.umcn.nl.
control. According to the Clinical and Laboratory Standards Institute criteria for *Streptococcus* spp. other than *S. pneumoniae*, the isolate was susceptible to penicillin (MIC, 0.015 μg/ml), amoxicillin (MIC, 0.03 μg/ml), meropenem (MIC, 0.015 μg/ml), tetracycline (MIC, 0.125 μg/ml), clarithromycin (MIC, 0.25 μg/ml), clindamycin (MIC, 0.06 μg/ml), and vancomycin (MIC, 0.125 μg/ml).

Gram-positive, catalase-negative cocci are increasingly recognized as human pathogens. Phenotypic characterization of these bacteria is often cumbersome. Several new clinically relevant species have been described using 16S rRNA DNA sequence analysis and have been reviewed recently (7).

In 1993, two isolates of Gram-positive, catalase-negative cocci were described as a new genus and species, *Dolosigranulum pigrum* (1). One of the isolates was cultured from frozen spinal cord tissue from a patient with acute multiple sclerosis in 1988. The other isolate was cultured in 1991 from a bandage contact lens and an eye swab from a patient with a neurotropic cornea with complaints of blurred vision and discomfort. *D. pigrum* was found to be phylogenetically most closely related to *Aerococcus* and *Globicatella*.

Since this first description, *D. pigrum* has been described in only two case reports. In one case, it was cultured from the blood of a patient with rheumatoid arthritis and immunosuppressive therapy with clinical signs of synovitis of multiple joints (2). More recently, a patient with gallstones and acute cholecystitis and pancreatitis was described as having a positive blood culture with *D. pigrum* (5). In addition, a collection at the Centers for Disease Control and Prevention (CDC) of 27 isolates of *D. pigrum* was described (3). These isolates were cultured predominantly from blood, which probably represents the behavior of clinical laboratories when sending unknown isolates to the CDC for identification rather than the nature of infections that *D. pigrum* causes. Interestingly, 13 isolates were from the eye (n = 6), nasopharynx (n = 4), sinus (n = 1), sputum (n = 1), and stomach (n = 1), suggesting that the upper respiratory tract is the natural habitat of *D. pigrum*, as was noted by LaClaire and Facklam (3). The case of ventilator-associated pneumonia reported here further supports this suggestion.

While early-onset VAP, occurring within 5 days postintubation, is usually caused by members of the normal oropharyngeal flora, late-onset VAP is mainly caused by gram-negative bacteria and *S. aureus*. At the time of this case, an investigative trial of selective oral decontamination was ongoing in our ICU using topical polymyxin E, tobramycin, and amphotericin B. The oral application of these topical antimicrobials may have contributed to the development of late-onset VAP due to *D. pigrum*. In this case, *Dolosigranulum* (meaning a deceptive small grain) was indeed misleading when it appeared as staphylococci in the Gram stain of the sputum, suggesting VAP due to *S. aureus* (1).

*Dolosigranulum pigrum* can be recognized in the laboratory as gram-positive catalase-negative cocci in pairs and small clusters of 4 to 32 cells. The colonies resemble viridans streptococci. It shows poor growth after 24 h of incubation at 37°C in 5% CO2, with colonies of various sizes suggesting a mixed culture. The growth requirements are facultatively anaerobic, as described recently (6). Other important phenotypic characteristics to identify *D. pigrum* are vancomycin susceptibility; positive results for pyrrolidonyl arylamidase, leucine aminopeptidase, and esculin hydrolysis; and growth in 6.5% NaCl broth (4). All the results of the phenotypic tests with the current isolate were in keeping with the literature. Importantly, esculin hydrolysis was initially negative for our isolate using the API 20 Strep. Esculin hydrolysis is a key reaction to distinguish *D. pigrum* from other catalase-negative, gram-positive cocci in clusters that are PYR and LAP positive and that grow in the presence of NaCl. It was reported previously that it may take up to 14 days of incubation before the esculin hydrolysis reaction becomes positive (2). With our isolate, the esculin hydrolysis was unequivocally positive within 48 h, using a heart infusion agar slant. Possibly, if the respiratory tract is the source of the isolate, this may be another clue to the identification of these bacteria. With the API 20 Strep, Voges-Proskauer, PYR, LAP, and alkaline phosphatase were the only positive reactions. This resulted in an excellent, although wrong, identification of *Gemella* spp.

Similar morphological characteristics and possibly the same natural habitat for *D. pigrum* and *Gemella haemolysans* may have led to misidentification of previous isolates of *D. pigrum* as *G. haemolysans*. The introduction of 16S rRNA DNA sequence analysis for bacterial identification and the increasing number of immunocompromised patients due to modern treatment modalities are contributing to the recognition of bacteria such as *D. pigrum* as pathogens.

REFERENCES