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Ventilator-Associated Pneumonia Caused by *Dolosigranulum pigrum*

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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 amoxicilline-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 (14.8×10^9/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 flucloxacillin 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% CO_2 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 5×10^5 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-clavunalate 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.

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Significant growth from the bronchial secretions was noticed only after prolonged incubation of the isolation media. After subculture, growth was better on 5% sheep blood agar than on chocolate agar. No growth was obtained with nutrient agar or MacConkey agar. Growth requirements were tested as recently described on Colombia blood agar with 5% sheep blood (6). Growth was best in ambient air and poorest under anaerobic conditions. After 24 h, the colonies were pinpoint. After 48 h, the colonies were alpha-hemolytic, dome shaped, and glistening grayish, with entire edges ranging in size from 1 to 2 mm. In 5% CO_2 and under anaerobic conditions, colony sizes were more variable, ranging from pinpoint to 2 mm, with the larger colonies showing umbonate morphology. The colonies were nonhemolytic under anaerobic conditions. Gram stain of a 24-hour broth culture (brain heart infusion) showed gram-positive cocci in pairs, tetrads, and small clusters. Catalase and oxidase reactions were negative. Disk tests for pyrrolidonyl arylamidase (PYR) and leucine aminopeptidase (LAP) (Remel) were positive, and growth was obtained in 6.5% NaCl. The isolate was susceptible to penicillin and vancomycin (zone sizes, >50 mm and 33 mm, respectively). This phenotypic profile was consistent with the rarely isolated bacteria *Ignavigranum* spp., *Alloiococcus* spp., *Facklamia languida*, and *Dolosigranulum pigrum* (7). The biochemical test results obtained with the API 20 Strep (API Laboratory Products, bioMérieux, France) were as follows: positive results for the Voges-Proskauer test, PYR, LAP, and alkaline phosphatase and negative results for hippurate and esculin hydrolysis, α-galactosidase, β-glucuronidase, β-galactosidase, and and arginine hydrolysis; no fermentation of ribose, L-arabinose, mannitol, sorbitol, lactose, trehalose, inuline, raffinose, and glycogen; and no starch hydrolysis. The API 20 Strep resulted in profile code 1160000, associated with excellent identification of *Gemella* spp. The uniform uptake of crystal violet and tolerance for 6.5% NaCl were not in keeping with this identification. The isolate was then further identified using 16S rRNA gene sequence analysis. The sequence obtained from a 520-bp product showed 99% similarity with *D. pigrum*. This identification was confirmed phenotypically by a positive result for esculin hydrolysis on a heart infusion agar slant after 48 h of incubation (4).

In vitro susceptibility testing was performed by Etest using Mueller-Hinton agar with 3% lysed horse blood, 0.5-McFarland inoculum, and 24 hours of incubation at 37°C in ambient air. *Streptococcus pneumoniae* ATCC 49619 was included for quality

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 neurotopic 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% CO_2, 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.

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