

Dialister pneumosintes Associated with Human Brain Abscesses

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Received 21 November 2001/Returned for modification 17 May 2002/Accepted 26 June 2002

In this report, we review two cases of brain infection due to *Dialister pneumosintes* in previously healthy patients. The bacterium was isolated from the first patient by blood culture and directly from a brain abscess in the second patient. In both cases, the infection was suspected to be of nasopharyngeal or dental origin. The patients had favorable outcomes following surgical debridement and antibiotic treatment. After *in vitro* amplification and partial sequencing of the 16S rRNA gene, two strains were classified as *D. pneumosintes*. However, traditional biochemical tests were not sufficient to identify the bacteria. In addition to causing periodontal and opportunistic infections, *D. pneumosintes*, contained in mixed flora, may behave as a clinically important pathogen, especially in the brain. In addition to phenotypic characterization, 16S rRNA partial sequencing was used to identify *D. pneumosintes* definitively.

CASE REPORTS

Case 1. A 17-year-old man was admitted to Strasbourg University Hospital for fever and dysphagia that had evolved over 1 week. Prior to admission, he had received amoxicillin and then clarithromycin. On physical examination, he presented with a high fever (40°C), right temporal headache, paralysis of the left arm, and swelling of the submandibular lymph nodes. Laboratory investigations revealed an inflammatory syndrome with a C-reactive protein level measuring 118 mg/liter and a leukocyte count of 13.3×10^9 cells/liter. Standard cerebrospinal fluid analysis as well as aerobic and anaerobic cultures did not reveal any abnormalities. Two aerobic and anaerobic blood cultures (BACTEC Plus Aerobic/F and BACTEC Plus Anaerobic/F; BD, Pont-de-Claix, France) were performed upon admission.

A brain computerized tomography (CT) scan revealed a subdural empyema compressing the right frontal lobe. Arteriography of the cerebral vessels revealed possible thrombophlebitis of the longitudinal median sinus. Antimicrobial treatment with cefotaxime at 12 g/day, metronidazole at 1.5 g/day, and acyclovir at 2.4 g/day was started. The general condition of the patient rapidly worsened, leading to sepsis and aggravation of his neurologic symptoms. On the second day after admission, a right frontal craniotomy was performed. A subdural empyema was evacuated and the frontal sinuses were drained. Thiamphenicol (750 mg) was injected into the operative cavity. Postsurgical treatment consisted of metronidazole, cefotaxime, ofloxacin, carbamazepine, and heparin.

Gram staining of the empyema revealed numerous polymorphonuclear cells and small gram-negative rods. Rare colonies of *Streptococcus anginosus* grew on chocolate agar under aer-

obic conditions and also on blood agar supplemented with hemin and vitamin K₁ under anaerobic conditions. Rare colonies of *S. anginosus* and coagulase-negative *Staphylococcus* were isolated from the frontal sinus sample. On the fourth day of hospitalization, *Dialister pneumosintes* (IBS 708/99) was obtained from one of the blood cultures done on the day of admission. All other blood cultures done remained negative during the rest of the patient's hospitalization.

The patient became afebrile 3 days after surgical evacuation. Metronidazole and cefotaxime were continued for an additional 19 days. The patient was discharged from the hospital in good condition with instructions to take oral antibiotics (amoxicillin at 6 g/day and ofloxacin at 400 mg/day) for 21 more days.

Case 2. A 66-year-old man was admitted to the same hospital for right-side tonic-clonic seizures. On physical examination, the patient was afebrile but was postictal and had right-side hemiparesis, right-side extensor plantar response, and expressive aphasia. He had moderate gingivitis and periodontolysis of three teeth.

Laboratory investigations revealed a moderate inflammatory syndrome: C-reactive protein measured 15.6 mg/liter, fibrinogen measured 4.83 g/liter, and the patient's leukocyte count measured 15.4×10^9 cells/liter with 87.9% neutrophils. A brain CT scan revealed a left posterior frontal lesion surrounded by edema. Preinterventional magnetic resonance imaging revealed a possible infectious etiology to the lesion.

A craniotomy was performed, and greenish pus was evacuated from the frontal lesion. Gram staining of this sample revealed numerous polymorphonuclear cells and gram-positive rods. No antibiotics were given prior to performance of the craniotomy. Aerobic cultures were negative for growth, and anaerobic cultures yielded only rare colonies of *D. pneumosintes* (IBS 18607/00). The patient was treated intravenously for 21 days with cefotaxime at 8 g/day, fosfomicin at 16 g/day, and metronidazole at 1.5 g/day. The patient did well; thoracic and abdominal CT scans as well as heart and liver ultrasonography results were normal. The patient was discharged in good

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TABLE 1. Percentage of similarity of 610 bp from the 16S rRNA genes of *D. pneumosintes* and other related bacteria

Isolate or bacterium	% Similarity to:										
	<i>D. pneumosintes</i> IBS 708/99	<i>D. pneumosintes</i> IBS 18607/00	<i>D. pneumosintes</i> ATCC 33048	<i>D. pneumosintes</i> strain SC3D	<i>Dialister</i> sp. oral clone BS095	<i>Dialister</i> sp. oral clone GBA27	<i>Dialister</i> sp. oral clone BS016	<i>M. elsdenii</i> ATCC 25940	<i>V. parvula</i> DSM 2008	<i>V. dispar</i> DSM 20735	<i>V. atypica</i> DSM 20739
<i>D. pneumosintes</i> IBS 708/99	100										
<i>D. pneumosintes</i> IBS 18607/00	100	100									
<i>D. pneumosintes</i> ATCC 33048	99.8	99.8	100								
<i>D. pneumosintes</i> strain SC3D	99.3	99.3	99.5	100							
<i>Dialister</i> sp. oral clone BS095	89	89	89.1	89.2	100						
<i>Dialister</i> sp. oral clone GBA27	89	89	89.1	89.2	97.5	100					
<i>Dialister</i> sp. oral clone BS016	88.3	88.3	88.5	88.5	90.1	89.8	100				
<i>Megasphaera elsdenii</i> ATCC 25940	87.5	87.5	87.6	87.5	81.5	80.3	81.1	100			
<i>Veillonella parvula</i> DSM 2008	85.4	85.4	85.5	85.5	80.8	81.3	79.3	83.6	100		
<i>Veillonella dispar</i> DSM 20735	85.3	85.3	85.4	85.4	80.9	81.5	79.1	83.8	99.2	100	
<i>Veillonella atypica</i> DSM 20739	81	81	81.3	84.9	80.8	81.3	79.6	83.3	96.6	96.3	100

condition with instructions to take oral antibiotics (ofloxacin and metronidazole) and intramuscular teicoplanin for 3 weeks.

Microbiology. Standard phenotypic tests for anaerobic bacteria (11) of both strains revealed gram-negative rods which were strictly anaerobic, nonpigmented, nonmotile, non-spore-forming, and nonsaccharolytic. The usual biochemical tests were negative. This was consistent with an identification of *D. pneumosintes* but also with several other nonsaccharolytic gram-negative anaerobic rods (11).

Antibiotic susceptibilities were determined by agar diffusion (E test; AB Biodisk, Solna, Sweden) with brucella blood agar in accordance with the recommendations of the National Breakpoint Committee of the French Society for Microbiology (2). Both isolates were susceptible to amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, imipenem, chloramphenicol, and metronidazole, and both were resistant to erythromycin and vancomycin.

Bacterial DNA was extracted as previously described (4), and the 16S rRNA gene was amplified according to the previously published procedures (1, 12). The amplified fragments were purified with a CHROMA SPIN 100 column (Clontech, Palo Alto, Calif.) according to the manufacturer's recommendations. Partial sequencing (of 610 bp) of a variable region was performed on both coding and complementary strands by using two primers that were 5' end labeled with fluorescein isothiocyanate (Eurogentec, Seraing, Belgium), namely, primer P8 (5'-AGAGTTTGATCTGGCTCAG-3') and primer Pc804 (5'-GTGGACTACCAGGGTATCTAATCC-3'). By using the CLUSTAL method (9), the DNA sequences obtained were compared with those from other *D. pneumosintes* strains and other members of the *Sporomusa*, a subbranch of the *Clostridium* subphylum cluster IX, which contains closely interrelated bacteria that belong to the genera *Dialister*, *Megasphaera*, and *Veillonella* (20), sequences which are available from

GenBank (Table 1). The two isolated strains, IBS 708/99 and IBS 18607/00, displayed exactly the same 16S rRNA partial gene sequences, which were very similar to those of *D. pneumosintes* strain SC3D (99.3% similarity) and *D. pneumosintes* ATCC 33048 (99.8%) (14). These data indicate that the two isolates are very closely related or identical to the *D. pneumosintes* species, in accordance with previously established criteria (18).

Discussion. *D. pneumosintes* is a small, nonfermentative, gram-negative anaerobic rod which was first described by Olisky and Gates as *Bacterium pneumosintes* and obtained from the nasopharyngeal secretions of patients with influenza during the epidemics of 1918 through 1921 (17). After several phylogenetic reclassifications over the last several years, it was reclassified as *D. pneumosintes* (10, 14, 20). Precise identification of the bacterium cannot be performed by morphological and traditional biochemical means and therefore requires nucleotide sequencing of the 16S rRNA gene (20). Very little clinical and microbiological information is available about human diseases caused by *D. pneumosintes* because of the lack of an accurate biochemical identification method. Before the use of molecular methods, *D. pneumosintes* was suspected to be associated with brain infections alone or in association with *S. anginosus* (10). This assumption was never firmly established because of the poor results of both the phenotypic characterization and analysis of the short-chain fatty acids by direct gas-liquid chromatography (8, 10). Our two cases firmly establish for the first time that *D. pneumosintes* may be associated with cerebral abscesses.

In the first case, *D. pneumosintes* was found in mixed flora associated with *S. anginosus*. It is most likely that the gram-negative bacilli visualized by direct microscopic examination of the subdural samples from the patient were *D. pneumosintes* and that the related bacteremia was caused by the subdural empyema. The most likely source of the infection was the frontal sinuses from which *S. anginosus* was isolated.

In the second case, the monomicrobial culture of *D. pneumosintes* from the brain abscess proved the involvement of the microbe in the infectious process. It is possible that the gram-positive rods visualized by the Gram stain and which did not grow in the culture were fastidious bacteria, since these kinds of organisms are not uncommon in brain abscesses. We believe that their gram-positive appearance was likely due to inadequate decolorization of the Gram stain, since only one type of bacteria was seen on direct examination of the pus collected from the craniotomy. Gram staining could not be repeated because all of the material was used in the culture. As reported previously (13), the absence of fever and the moderate inflammatory syndrome may not exclude the diagnosis of brain abscess. The patient's poor dental hygiene could be a predisposing factor for *D. pneumosintes* infection.

On the basis of the phenotypic characterization, *D. pneumosintes* was supposed to be a commensal organism of the oral cavity (mainly of the gingival crevices) (10) and to be normally present in nasopharyngeal, intestinal, and vaginal flora (6, 10). *D. pneumosintes* was also suspected to be a periodontal pathogen responsible for gingivitis (15, 16). On the basis of genotypic characterization, *D. pneumosintes* has been detected in severe periodontitis, gingivitis, and dentoalveolar abscesses in association with other anaerobic species (3, 5, 19).

Apart from periodontal infections, this bacterium was previously suspected to be responsible for various supradiaphragmatic infections (in the respiratory tract, head, and neck) (10) and could be isolated from bite wound infections (7), blood (10), and amniotic fluid and placentas of pregnant women with premature rupture of the amniotic sac (6). In all of these cases, the identification was performed only on the basis of phenotypic characteristics.

During our 2-year study, using the same molecular methods, we documented two additional cases of *D. pneumosintes* bacteremia in two patients with decompensated liver cirrhosis. The bacteria may have originated in the gut. These two patients died 1 and 2 weeks, respectively, after the diagnosis of bacteremia, but the primary cause of death was not *D. pneumosintes*.

Our data show that *D. pneumosintes* contained in mixed flora may behave as a deadly pathogen, especially in the brain, in addition to causing periodontal or opportunistic infections.

We are indebted to Timothy Quan for his helpful discussion and review of the manuscript.

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