First Isolation of \textit{Bacteroides thetaiotaomicron} from a Patient with a Cholesteatoma and Experiencing Meningitis

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A 45-year-old man with a cholesteatoma experienced purulent meningitis. Microbial analysis of cerebrospinal fluid yielded in pure culture a gram-negative bacillus. Phenotypic methods were suggestive of \textit{Bacteroides distasonis} or either \textit{Bacteroides thetaiotaomicron} or \textit{Bacteroides ovatus} infection. The isolate was identified by 16S rRNA gene sequence analysis as \textit{B. thetaiotaomicron}. This is the first case of \textit{B. thetaiotaomicron} meningitis in pure culture.

CASE REPORT

A 45-year-old man suffered from right-ear hearing loss and otorrhea for many years. The patient’s ear discharge became profuse, and he became febrile 2 weeks thereafter. He complained of right earache, mastoid pain, and gait ataxia without headache, nausea, or photophobia. A general practitioner prescribed pristinamycin (500 mg, four times daily) and ofloxacin (200 mg, twice daily) for 5 days, which led to defervescence. An otoscopic examination discovered epidermic fragments associated with otorrhea and suggestive of a cholesteatoma. An audiogram indicated total deafness in the right ear. Computed tomography (CT) scans focused on the petrous part of the temporal bone demonstrated a large invading cholesteatoma on the right side with external semicircular fistulae, fallopian canal erosion, and ossicular destruction (Fig. 1). The patient was admitted to an ear, nose, and throat department. He suffered from headache, nausea, and a fever of 38°C; results from a neurological examination were normal. Amoxicillin and clavulanic acid (500 mg, three times daily) combined with ciprofloxacin (500 mg, twice daily) were prescribed, and the patient underwent a surgical procedure (typanomastoidectomy). A histological examination confirmed the cholesteatoma.

The day thereafter, he experienced a fever of 39°C, vomiting, and unusual headache with cervical pain and had nuchal rigidity and Brudzinski’s sign; there was no other neurological deficit. His white blood cell count was 21,300/mm\(^3\), with 90% of neutrophils and unusual headache with cervical pain and had nuchal rigidity and Brudzinski’s sign; there was no other neurological deficit. His white blood cell count was 21,300/mm\(^3\), with 90% of neutrophils.

Cerebrospinal fluid (CSF) obtained by lumbar puncture was turbid, contained 10,000 leukocytes/mm\(^3\) (70% PMN), and had a protein concentration of 2.85 g/liter. The glucose level was 1.3 mmol/liter (serum glucose, 5.6 mmol/liter). Direct examination of CSF gave negative results. Intravenous amoxicillin (4 g, three times daily), cefotaxime (4 g, three times daily), and fosfomycin (4 g, three times daily) were started.

CSF cultured in 5% sheep blood agar under an anaerobic atmosphere yielded a gram-negative bacillus susceptible to amoxicillin associated with clavulanic acid (4:1; the MIC was 30 mg/liter), imipenem (30 mg/liter), metronidazole (20 mg/liter), and clindamycin (6 mg/liter) but resistant to penicillin G (6 mg/liter), cefotaxim (6 mg/liter), and vancomycin (6 mg/liter). The API-20A system (BioMérieux, Marcy-l’Etoile, France) profile was 46554370, consistent with \textit{Bacteroides distasonis} (percentage of identification was 45.1%; \( t \) index was 0.83) or either \textit{Bacteroides thetaiotaomicron} or \textit{Bacteroides ovatus} (percentage of identification was 49.1%; \( t \) index was 0.77). Another API-20A gallery was analyzed, the profile of which was 56554370, suggestive of \textit{B. thetaiotaomicron} or \textit{B. ovatus} (percentage of identification was 99.9%; \( t \) index was 0.87).

In order to firmly identify this isolate, we determined its 16S rRNA gene sequence and further compared the sequence to those available in the GenBank and EMBL databases by using the Clustal W program with the BLAST package (http://www.ncbi.nlm.nih.gov/BLAST/BLAST.cgi). Over 1,487 bp, the isolate gene sequence shared 100 and 96.3% sequence similarities with \textit{B. thetaiotaomicron} strain VPI-5482 (GenBank accession number NC 004663) and \textit{B. ovatus} JCM 5824\(^T\) (GenBank accession number AB050108), respectively.

Cerebral CT scans performed before and after iodine injection did not reveal any concomitant abscess or thrombophlebitis. Based on the antimicrobial susceptibility pattern, amoxicillin-clavulanic acid was sustained and cefotaxime and fosfomycin were replaced by metronidazole (500 mg, three times daily) for 15 days. The patient became asymptomatic and apyretic within 5 days. A second lumbar puncture produced a less cloudy CSF sample containing 900 leukocytes/mm\(^3\) (20% PMN) and having a protein concentration of 1.7 g/liter and a glucose level of 2.8 mmol/liter (serum glucose, 4.1 mmol/liter). Aerobic and anaerobic cultures remained negative. The patient was discharged in good condition after the completion of...
antibiotics on the 15th postoperative day. Follow-up at 2 months was unremarkable.

We present the case of a patient suffering from a cholesteatoma and experiencing purulent meningitis. Routine inoculation of CSF in anaerobic media yielded an isolate of the \textit{Bacteroides fragilis} group, which was further unambiguously identified as \textit{B. thetaiotaomicron} by 16S rRNA gene sequence analysis.

\textit{B. thetaiotaomicron} is a gram-negative, nonsporulated, anaerobic bacillus (11, 14). The genus \textit{Bacteroides} represents one of the most important groups of human commensal anaerobes, which are constitutive of the normal floras of the gastrointestinal tract, oral cavity, vagina, and respiratory tract (11, 14). Although anaerobes are less frequent components of the usual ear population (9), chronic otitis media, including the presence of a cholesteatoma, may lead to aeration impairment and the multiplication of commensal anaerobic flora (12), due to a decrease in local redox potential (7). Anaerobic bacteria are present in almost 50% of chronic otitis media and 95% of cholesteatomas, where \textit{Peptostreptococcus} spp., \textit{Prevotella} spp., \textit{Porphyromonas} spp., \textit{Bacteroides} spp., and \textit{Fusobacterium} spp. are predominant (2). Anaerobes may be implicated in various infectious diseases whenever they invade sterile body tissue and fluid (in particular, CSF) by hematogenous spreading or contiguous extension of their preferential living sites. Thus, \textit{Bacteroides} meningitis has been described for patients with digestive bacterial proliferation directly breaking into subarachnoidal space (17) and for patients after the spread of ear infection (16).

Meningitis occurring during ear infection may be difficult to diagnose. Indeed, nausea and fever may be present in the course of noncomplicated acute otitis and in the acute stage of chronic otitis, including that with a cholesteatoma. Moreover, meningeal syndrome and hyperthermia may be masked by previous antalgic, antibiotic, or antipyretic treatments. Finally, clinical features are usually misleading in children and the elderly (3, 18), the age groups in which the incidence of bacterial meningitis is highest (20). Meningitis should be suspected every time a patient suffering otitis experiences new symptoms (such as headache) or a worsening, sustainment, or recurrence of previous symptoms despite appropriate antibiotics; this suspicion should lead to the performance of lumbar puncture.

In our patient, CSF examination revealed a purulent anaerobic meningitis. Anaerobic meningitis is thought to occur rarely (6, 14), so many clinical laboratories do not routinely inoculate CSF under an anaerobic atmosphere (14). Schlech and colleagues have determined an anaerobic-organism etiology for only 5 out of 18,642 cases of bacterial meningitis in the United States from 1977 to 1981 (19). However, systematic inoculation of CSF into both aerobic and anaerobic media led us to find an unexpected anaerobic-organism etiology for 17 out of 178 cases of bacterial meningitis from 1998 to 2002, most of them (76.5%) being \textit{Propionibacterium acnes} or \textit{Propionibacterium} spp. in patients with CSF-shunting devices or other organisms belonging to the \textit{Bacteroides}, \textit{Peptostreptococcus}, or \textit{Actinobacillus} genera (L. Feuillet, unpublished data). The identification of anaerobes requires preliminary appropriate collection, transport, and culture of the CSF samples. The difficulties which may be encountered in fulfilling these conditions in clinical practice suggest that anaerobic-organism meningitis may remain underdiagnosed (16). Anaerobic culture performed in our case yielded a gram-negative bacillus belonging to the \textit{B. fragilis} group. Although \textit{Bacteroides} spp. are thought to be the main agents of anaerobic meningitis (13), fewer than 100 cases of \textit{Bacteroides} meningitis have been reported in the English-language literature, and they occur primarily in the pediatric population (13).

In most laboratories, \textit{Bacteroides} identification currently relies on phenotypic tests (11). The second biochemical profile and the antimicrobial susceptibility pattern were here consistent with either \textit{B. thetaiotaomicron} or \textit{B. ovatus} infection. \textit{B. thetaiotaomicron} and \textit{B. ovatus} have each been reported...
once before as causing meningitis, but in those cases, both were isolated within a mixed flora (8, 17). The unique previous case of meningitis due to well-identified *B. thetaiotaomicron* occurred in association with *B. distasonis* and *B. fragilis* species in a woman who experienced bowel impaction (17). The unique previous case of *B. ovatus* meningitis was described to occur with the growth of *Streptococcus intermedius* in a 19-month-old female presenting with an occult congenital dermal sinus communicating with an intraspinal infected dermoid cyst (8). We found neither relevant bowel dysfunction in our patient’s medical history nor a cutaneous opening overlying the spine but rather a cholesteatoma.

Although the cholesteatoma was destructive, invading the deep petrous bone structures (Fig. 1), the relationship between the patient’s chronic ear infection and his meningitis could not be definitively assessed. Indeed, we did not collect the purulent sample from ear discharge because both aerobic and anaerobic cultures might have displayed various commensal floras instead of the real etiological agent of meningitis, potentially leading to a false-positive identification (16). Moreover, we report here the first case of *B. thetaiotaomicron* meningitis in pure culture. Since bacterial proliferation is often polymorphic in chronic otitis (1), and with cholesteatoma in particular, meningitis of mixed etiologies might also have occurred in our patient as described previously after digestive contamination (17).

Although conventional biochemical tests are well established and standardized for the identification of anaerobes in particular (10), they remain labor-intensive, time-consuming, and cumbersome (7). Inexpensive and rapid identification microsystems such as API-20A are best suited for fast-growing anaerobes, such as members of the *B. fragilis* group (7, 11). However, identification to the species level may be, as in our case, not precise enough and also may be incorrect or not possible (15).

With various pathogenic conditions, genetic analysis may help in the identification of anaerobes (4). Moreover, 16S rRNA gene sequence analysis has been found more reliable than phenotypic methods in accurate species-level identification (5). When the method is available, analysis of the 16S rRNA gene sequence is suggested to characterize the organism to the species level, since accurate and definitive identification should be obtained for isolates coming from blood, organs, or body cavities and CSF (11). 16S rRNA gene sequence analysis allowed us to finally identify the clinical isolate as *B. thetaiotaomicron*.

*B. thetaiotaomicron* and *B. ovatus* were individualized in taxonomy as distinct species about 30 years ago. Since their first descriptions, misdiagnosis at the species level may be due to the exclusive use of phenotypic methods. In fact, further molecular investigatory methods to characterize species might have been unavailable for most older reports or not used in more recent ones when species identification was considered too expensive and/or not relevant enough in clinical practice (11).

We report on the first case of meningitis due to *B. thetaiotaomicron* as a unique etiological agent. Isolation has been made possible by the systematic inoculation of the CSF sample in anaerobic media. This isolate was further identified by 16S rRNA gene sequencing, illustrating the usefulness of this method for identification to the species level. This well-defined identification allowed us to describe the natural history and the clinical and biological features of our patient’s meningitis and, finally, the antibiotic susceptibility of the etiologic agent, *B. thetaiotaomicron*.

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**REFERENCES**