Sepsis with an *Atopobium*-Like Species in a Patient with Fournier’s Gangrene

Matthijs Oyaert, Piet Cools, Joke Breyne, Gert Heyvaert, Anne Vandewiele, Mario Vaneechoutte, Steven Vervaeke, Emmanuel De Laere

Department of Microbiology, AZ Delta, Roeselare, Belgium; Laboratory of Bacteriology Research, Department of Clinical Chemistry, Microbiology and Immunology, Faculty Medicine & Health Sciences, Ghent University, Ghent, Belgium; Department of Molecular Diagnostics, AZ Delta, Roeselare, Belgium; Department of Surgery, AZ Delta, Roeselare, Belgium

*Atopobium* species are Gram-positive, anaerobic, catalase-negative, fastidious bacteria belonging to the family *Coriobacteriaceae*. We report the isolation of an *Atopobium*-like species in a patient with Fournier’s gangrene and highlight the role of 16S rRNA gene sequencing in the identification of fastidious organisms in the clinical laboratory.

CASE REPORT

A 43-year-old mentally retarded, obese woman, without relevant medical history, was admitted to the hospital with fever and an extensive perianal abscess with spontaneous drainage during 1 week. Clinical examination showed an erythematous, swollen, warm perianal region with ulcers and regional lymphadenopathy. Hematological investigations revealed a white blood cell (WBC) count of 34.73 × 10^3 cells/µl (reference range of 3.90 × 10^3 to 11.0 × 10^3/µl) with 91% neutrophils (reference range of 46 to 64%), a hemoglobin level of 11.5 g/dl (reference range of 11.8 to 14.8 g/dl), and a platelet count of 418 × 10^3/µl (reference range of 150 × 10^3/µl to 450 × 10^3/µl). C-reactive protein was increased up to 448.0 mg/liter (reference range of 0 to 7.0 mg/liter). Biochemical testing revealed increased glucose (154 mg/dl [reference range of 65 to 110 mg/dl]), decreased serum iron (13 µg/dl [reference range of 50 to 150 µg/dl], and decreased serum transferrin (194 mg/dl [reference range of 200 to 360 mg/dl]). Liver function tests showed increased alkaline phosphatase (178 U/liter [reference range of 42 to 98 U/liter]), gamma-glutamyltransferase (65 U/liter [reference range of 7 to 32 U/liter]), aspartate transaminase (AST) (51 U/liter [reference range of 10 to 35 U/liter]), and alanine aminotransferase (ALT) (56 U/liter [reference range of 10 to 35 U/liter]). There were signs of prerenal failure (urea, 93 mg/dl [reference range of <50 mg/dl]; creatinine, 1.70 mg/dl [reference range of 0.51 to 0.95 mg/dl]).

Before empirical antibiotic treatment with amoxicillin-clavulanic acid (1 g every 6 hours [q6h] intravenously [i.v.]) was initiated, two aerobic and two anaerobic blood culture bottles (Bectec FX; Becton, Dickinson, Sparks, MD) were drawn, and the patient was transferred to the operation room for incision of an ischiorectal abscess. During this procedure, a diagnosis of Fournier’s gangrene (FG) was made, followed by an extended surgical debridement of all necrotic and infectious tissue. Purulent fluid was obtained and sent to the laboratory for microbiological examination. Gram staining of the pus showed predominantly Gram-positive rods and cocci. Specimens were plated onto homemade Columbia sheep blood agar and commercial chocolate agar (bioMérieux, Marnes-la-Coquette, France), both incubated in anaerobic (80% N2, 10% H2, 10% CO2) and aerobic conditions at 37°C. After 48 h of incubation, catalase-negative, anaerobic, Gram-positive rods and two types of Gram-positive cocci were isolated. The cocci were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Microflex; Bruker, Bremen, Germany) as vancomycin-susceptible *Enterococcus faecalis* and *Staphylococcus epidermidis*. After a mean incubation time of 82.7 h at 35°C, both anaerobic blood culture bottles yielded short, rod-shaped, Gram-positive, catalase-negative bacteria. At this time, antibiotic therapy was switched to meropenem (2 g q8h i.v.). The strain was tested using the commercially available API RAPID 32A test (bioMérieux), which produced a presumptive identification of *Bacillus* species with poor probability (53.1%) of correct identification. MALDI-TOF MS identification of the Gram-positive rods revealed no clear match with any of the species in the database, with the best identification score being 1.304 for *Bacillus cereus*.

Because conventional identification was inconclusive, 16S rRNA gene sequencing was performed. Bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed as described in previous studies (1) using abNOT (forward primer) (5’-GGTATCGCTGCTGCTCTGC-3’) and omegaMB (reverse primer) (5’-AGCCACGTACCCAGGTGC-3’) as the PCR and sequencing primers (2). The obtained sequence was compared with the publicly available bacterial 16S rRNA gene sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) software (www.ncbi.nlm.nih.gov/BLAST/). There was only 95% nucleotide identity between the 16S rRNA gene sequence of the isolate and that of *Atopobium minutum*. Because the closest relatives (95%) were found to be *Atopobium minutum* strains, all available complete 16S rRNA gene sequences of *Atopobium minutum*, *Atopobium fassorum*, *Atopobium parvulum*, *Atopobium rima*, and *Atopobium vaginae* were aligned with the obtained sequence and with the 16S rRNA sequence of *Coriobacterium glomerans*, the closest relative of the genus *Atopobium* (3), as an outgroup using the online Clustal Omega Software (http://www.ebi.ac.uk/Tools/msa/clustalo) with default parameters. After alignment, the gaps at the 5’ and 3’ ends of the alignment

Received 22 August 2013 Returned for modification 10 September 2013 Accepted 17 October 2013 Published ahead of print 23 October 2013

Editor: K.C. Carroll

Address correspondence to Emmanuel De Laere, edelae@azdelta.be.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

were omitted from further analysis. The alignment was subsequently used to calculate evolutionary distances by Kimura’s two-parameter model (4) using the MEGA 5.2.2 software package (http://www.megasoftware.net) (5). Phylogenetic trees were constructed using the neighbor-joining method (6), maximum parsimony (7), and maximum likelihood (8) methods, utilizing Coriobacterium glomerans PW2T (9) as the outgroup.

Antimicrobial susceptibility of this Atopobium species was determined according to the protocol described by De Backer et al. (10). Briefly, an inoculum taken from Columbia agar was suspended in 0.5 ml of physiological water and adjusted to a turbidity of 1 McFarland standard. Before the plates were inoculated and testing was done for the described antibiotics, plates were reduced in an anaerobic environment for 24 h prior to the Etests being set up. EUCAST clinical breakpoints for Gram-positive anaerobic bacteria were applied (http://www.eucast.org). The following MIC results were obtained: penicillin, 0.016 μg/ml, susceptible (clinical breakpoint, 0.25 μg/ml); meropenem, 0.012, susceptible (clinical breakpoint, 2 μg/ml); metronidazole, 24 μg/ml, resistant (clinical breakpoint, 4 μg/ml); clindamycin, <0.016 μg/ml, susceptible (clinical breakpoint, 4 μg/ml); vancomycin, 0.5 μg/ml, susceptible (clinical breakpoint, 2 μg/ml); cefotaxime, 0.016 μg/ml (no clinical breakpoint available).

The patient responded very well after surgery with meropenem and topical application of povidone iodine. Clinical and biochemical signs of infection and fever disappeared gradually, and the patient was discharged in good health 9 weeks after admission. A checkup after 25 weeks revealed the patient to be symptom free.

FG is a fulminant form of infective necrotizing fasciitis of the perineal, genital, or perianal regions. Men are more frequently affected than women and children (11). Early surgical debridement of necrotic tissues and administration of antibiotics are fundamental in the treatment of FG. Mostly, a polymicrobial infection of genitourinary or perianal source is found in these patients, but the portal of entry is often difficult to establish. Microbial invasion usually occurs either through direct injury or through a direct spread from the urogenital tract or from a perforated viscus like the colon, rectum, or anal orifice. In a meta-analysis, the portal of entry was found to be dermatological in 24%, colorectal in 21%, and urological in 19% of cases, whereas in 36% of the cases no definite portal of entry was established (12). The portal of entry in our case was thought to be perirectal.

In practice, three types of necrotizing soft tissue infections are seen. Type I infections, such as FG, are polymicrobial in origin, and a combination of Gram-positive and Gram-negative bacteria along with anaerobes can be cultured. Type II infections are mono-microbial and are mostly caused by Streptococcus pyogenes (group A streptococi) but may be associated with Staphylococcus aureus. Type II is less common than type I and is usually seen in healthy, immunocompetent patients. Type III infections are usually caused by Vibrio vulnificus (13).

Predisposing factors for FG include diabetes mellitus, which is reported to be present in 20 to 70% of patients with FG, and chronic alcoholism, which is found in 25 to 50% of the patients (14). In our patient, neither of these conditions was present. Isolation of Atopobium species has only rarely been recognized in patients with necrotizing fasciitis (15). The lack of hygiene and social neglect together with morbid obesity and mental retardation are possible causes that have given rise to this case of FG. Our patient was undoubtedly septic, with the probable cause of the sepsis being the infected necrotic perianal ulcers. No single bacterial species was exclusively responsible for the sepsis. Interestingly, the Atopobium species was the only bacterial pathogen identified in both blood cultures and perioperatively obtained pus. Therefore, its virulence and pathogenic role in the clinical sepsis are strongly suggested.

Phylogenetic analysis based on the 16S rRNA sequences of Atopobium strain HHRM1715 and the 12 available Atopobium sequences indicate that Atopobium strain HHRM1715 forms a distinct phylogenetic lineage within the genus Atopobium (Fig. 1), most closely related to the lineage comprising Atopobium fossor and Atopobium minutum strains. Similar tree topologies were gen-

FIG 1 Phylogenetic tree based on 16S rRNA gene sequences of Atopobium species strain HHRM1715 and 16S rRNA gene sequences of the genus Atopobium. Coriobacterium glomerans, the closest relative of the genus, was used as an outgroup. Evolutionary distances were computed using Kimura’s two-parameter model. The tree was constructed using the neighbor-joining method. Bootstrap values are expressed as percentages of 1,000 replications. Scale bar represents 0.02 substitutions per nucleotide position. Accession numbers are shown between brackets, followed by strain name if applicable.
erated using the maximum likelihood and the maximum parsimony algorithms (trees not shown).

The genus *Atopobium* was described as a new bacterial genus in 1992 with reclassification of the species *Lactobacillus minutum, Lactobacillus rimae*, and *Streptococcus parvulus* based on their phylogenetic relatedness determined by 16S rRNA gene sequencing (16). The genus *Atopobium* species are found in human gingival crevices and rarely have been described in various human infections, including dental abscesses, abdominal wound infections, pelvic abscesses, and bacteremia. Mostly, these bacteria were found associated with other microorganisms (3, 17, 18). Currently, the genus consists of five species: *A. rimae, A. parvulum, A. minutum, A. fossor*, and *A. vaginale*. *A. vaginale* was first isolated from the vaginal flora in a healthy woman in 1999 (3). Subsequently, the bacterium has also been found in patients with bacterial vaginosis, usually cooccurring with other anaerobic bacteria like *Gardnerella vaginalis* (20, 21). As in our experience, identification and differentiation of *Atopobium* species from other non-spore-forming Gram-positive coccobacilli by the use of conventional phenotypic and biochemical tests, including the commercially available test kits, are often laborious and may carry the risk of misidentification (22–25). The identification process is especially difficult in the presence of coexisting colonizing organisms (25). Newer identification techniques, particularly 16S rRNA gene sequencing, have allowed the accurate and rapid identification of *Atopobium* species, like *A. vaginale* (23, 24, 26). MALDI-TOF MS has also been used with success in one previously reported case (24) but was inaccurate in our case, which was due to the fact that our species was not included in the databases.

In conclusion, this case demonstrated that *Atopobium* species must be considered a potential pathogenic microorganism that can lead to sepsis in patients with Fournier’s gangrene. Commercial identification as well as more advance identification systems may fail to identify or misidentify *Atopobium* species in such situations, 16S rRNA gene sequencing may be considered a useful alternative method for rapid and accurate identification of the organism.

**Nucleotide sequence accession number.** The GenBank accession number for the partial 16S rRNA gene sequence of *Atopobium* species strain HHRM171715 is KF537630.

**ACKNOWLEDGMENT.**

There are no conflicts of interests to declare.

**REFERENCES**


