Two Cases of Infections Due to Multidrug-Resistant *Bacteroides fragilis* Group Strains

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*Bacteroides fragilis* group strains are still considered susceptible to most antimicrobial agents used for the treatment of infections caused by anaerobic organisms. We describe two cases of infections due to isolates simultaneously resistant to clindamycin, tetracycline, cefoxitin, pipercillin-tazobactam, and imipenem and, in one of the two cases, to metronidazole. Such infections, although still rare, do exist and tend to complicate treatment.

**First case report.** A 71-year-old man was admitted to the internal medicine ward of Laikon General Hospital, a university-affiliated, tertiary-care hospital in downtown Athens, Greece, with a temperature of up to 39°C and diarrhea for 8 days. The patient’s medical history included Crohn’s disease; stomach lymphoma, which had led to a total gastrectomy 15 years earlier; common variable immunodeficiency; and cytomegalovirus (CMV) colitis.

The patient’s blood cell count was within the normal range, and serum biochemistry revealed only an elevated C-reactive protein level (280 mg/liter; normal values, <50 mg/liter). Computed tomography scanning of the upper and lower abdomen was negative, while a colonoscopy revealed findings consistent with CMV colitis. Blood samples for culture were taken on admission; and after 2 days of incubation, the anaerobic bottle (BacT/ALERT 3D; bioMérieux, Marcy l’Étoile, France) yielded a gram-negative anaerobic rod that was identified as *Bacteroides fragilis* by the special-potency disk method; growth in the presence of 20% bile (Bacteroides-bile esculin agar plates; Bioprepare, Gerakas, Greece); and the biochemical profile (ID32ANA system; bioMérieux), including a negative reaction for indole (7). Cultures of stool samples were negative for pathogens.

Upon receipt of the preliminary finding of bacteremia caused by an anaerobic gram-negative organism, antimicrobial treatment was initiated with metronidazole three times daily at 500 mg intravenously (i.v.) and cefotaxime three times daily at 2 g i.v., after which the fever subsided but the diarrhea persisted, because of his underlying disease. His condition deteriorated during the following weeks, as he developed liver cirrhosis, ascites, and CMV pulmonary infiltrations. The patient died after 3 months of hospitalization due to pulmonary edema.

Susceptibility testing of the *B. fragilis* isolate was performed by the Etest method (AB Biodisk, Solna, Sweden), according to the manufacturer’s instructions, on brucella blood agar plates supplemented with vitamin K and hemin. The resulting MICs were >256 mg/liter for benzylpenicillin, pipercillin-tazobactam, ticarcillin-clavulanic acid, cefoxitin, and clindamycin; >32 mg/liter for imipenem and ertapenem; 128 mg/liter for tetracycline; and 0.5 mg/liter for metronidazole. The strain was positive for beta-lactamase production by the nitrocefin disk method (Cefinase; BBL, Becton Dickinson and Co., Franklin Lakes, NJ). The imipenem, ertapenem, and metronidazole MICs were confirmed by the agar dilution method (11). All plates used for susceptibility testing were incubated at 37°C for 48 h in a BacTron 1.5 anaerobic chamber (Cheldon Manufacturing, Cornelius, OR). Interpretation of the MIC results was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical and Laboratory Standards) (11). Strains *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used for quality control (11).

The presence of the cfiA gene, which encodes for a metallo-beta-lactamase which hydrolyzes carbapenems, was established by using a previously described PCR protocol (8). Direct DNA sequencing of the amplification product confirmed a 100% homology with the cfiA gene under GenBank accession number AY372696, as well as with cfiA genes with various other GenBank accession numbers.

**Second case report.** A 75-year-old man was admitted to the general surgical ward of a secondary-care hospital in Athens, Greece, due to gastric carcinoma that had been diagnosed several weeks earlier. During the surgical procedure, a total gastrectomy and esophageojunostomy were performed. Two leakages in the esophageojunostomy were detected on the 8th and the 16th postoperative days. The leakages were treated surgically, and the drainage was placed. A low-grade postoperative fever persisted which was attributed to a minor atelectasis of the left lower lung. Ten days later, however, the patient developed dyspnea, tachypnea, tachycardia, and a high temperature and was transferred to the intensive care unit (ICU). The patient’s blood cell count and serum biochemistry were within the normal ranges, and samples of the drainage fluid and blood were also negative by culture. The patient was treated symptomatically; and antimicrobial chemotherapy with...
levofloxacin once daily at 500 mg i.v., piperacillin-tazobactam three times daily at 4.5 g i.v., and fluconazole twice daily at 100 mg i.v. was initiated. His clinical condition improved, and after 12 days in the ICU he was transferred to the general surgical ward of Laikon General Hospital for further treatment. Six days later, however, and without any additional surgery performed, he was transferred to the ICU of Laikon Hospital due to respiratory insufficiency and was placed on mechanical ventilation. The blood cell count revealed a leukocyte count of 33,1 \( \times 10^9/\text{liter} \) and a proportion of polymorphonuclear cells of 92%; and serum biochemistry revealed an elevated aspartate aminotransferase level (4,840 U/liter; normal range, 8 to 33 U/liter), an elevated alanine aminotransferase level (175 U/liter; normal range, 4 to 36 U/liter), and an elevated creatinine level (371 \( \mu \text{mol/liter} \); normal range, 53 to 106 \( \mu \text{mol/liter} \)). Drainage fluid, blood, and bronchial secretion specimens were taken for culture; and the antibiotic treatment was changed to imipenem three times daily at 500 mg i.v. and fluconazole twice daily at 200 mg i.v. However, the patient died 2 days later due to irreversible septic shock arising from ruptures at the site of the anastomoses.

The aerobic and anaerobic cultures of the drainage fluid yielded two different gram-negative rods. The first isolate was identified as *Klebsiella pneumoniae* by use of the ID 32E system (bioMerieux), while the second one was identified as *Bacteroides vulgatus* by use of the methodology described above in the first case report. Cultures of the bronchial secretion were negative for pathogens, and blood cultures yielded a *Candida parapsilosis* isolate.

Susceptibility testing of the *K. pneumoniae* isolate, which was performed by the Kirby-Bauer disk diffusion method, with the results interpreted according to the guidelines of the CLSI (3), revealed a strain that produced extended-spectrum beta-lactamasess and that was resistant to ciprofloxacin, gentamicin, tobramycin, and amikacin. Susceptibility testing of the *B. vulgatus* isolate was performed, and the results were confirmed as described above in the section on the first case report; the MICs detected were >256 mg/liter for benzylpenicillin, piperacillin-tazobactam, ticarcillin-clavulanic acid, cefoxitin, clindamycin, tetracycline and metronidazole and >32 mg/liter for imipenem and ertapenem. The isolate was also negative for beta-lactamase production, as determined by the nitrocefin disk method.

Previously described PCR assays were used for detection of the *nim* class of genes, which confer resistance to metronidazole (17), and the *cfiA* gene, which confers resistance to carbapenems (8). The strain was negative for the *nim* class of genes and positive for the *cfiA* gene. Confirmation of the identity of the *cfiA* PCR product was performed by DNA sequencing, as described above in the section on the first case report.

Isolation of *Bacteroides* sp. strains from clinical specimens is a frequent as well as a significant finding, since these species are capable of causing diseases associated with increased mortality (13). Such infections may be easily mistreated, because susceptibility testing of anaerobic bacteria is not usually performed in everyday clinical practice, due to the belief that resistance among anaerobes is predictable. Large multicenter studies indicate that metronidazole, carbapenems and beta-lactam-beta-lactamase inhibitor combinations are still effective against these species (6, 9, 14), although reports from different parts of the world call attention to the emergence of resistance, especially to metronidazole (2, 5, 6, 8, 12, 15, 16). Nevertheless, infections due to multidrug-resistant *Bacteroides* sp. isolates are still extremely rare, and as far as we know, only two case reports of such infections are available in the literature (18, 19). Both strains presented here were resistant to almost all antimicrobial agents considered for use for the treatment of infections caused by anaerobic organisms, including cefoxitin, imipenem, ertapenem, piperacillin-tazobactam, ticarcillin-clavulanic acid, clindamycin, and tetracycline. In addition, one of the two isolates was also resistant to metronidazole. In that respect both isolates met the definition of multidrug resistance.

The most widely studied metronidazole resistance mechanism is the one associated with genes of the *nim* class (*nimA* to *nimF*) (2, 5, 10, 17). These genes have been detected in resistant as well as intermediate and susceptible (MICs as low as 1 mg/liter) *Bacteroides* sp. isolates, and the high MICs of the former have been associated with insertion sequences (IS) that may be triggering the phenotypic expression of resistance. This has been duplicated in vitro, where exposure to metronidazole of *nim*-positive, susceptible isolates resulted in nonreversible elevated MICs (5, 10). The *B. vulgatus* isolate described in the second case report was resistant to metronidazole, but the PCR assay used did not detect the *nim* genes, thus indicating that another resistance mechanism may be responsible. A similar report of a high-level metronidazole-resistant but *nim*-negative *Bacteroides* strain, isolated after prolonged treatment with metronidazole, has been presented (19). In addition, it has been shown that in vitro exposure to metronidazole of *nim*-negative, susceptible isolates resulted in the production of metronidazole-resistant mutants with significant changes in their protein profiles (4). In that respect, it seems that the *nim* genes may be part of a more general and diverse metronidazole resistance mechanism (4, 5); and considering that this agent remains the cornerstone of antimicrobial chemotherapy for infections caused by anaerobic organisms, more studies are urgently required to clarify the exact nature of resistance, as well as the true clinical significance of the presence of resistance mechanisms with the simultaneous presence of phenotypic susceptibility.

The major known mechanism of resistance to carbapenems in anaerobic bacteria involves the production of a zinc-dependent metallo-beta-lactamase encoded by the *cfiA* gene (8). This gene may also be silent or expressed at various degrees, resulting in a wide range of carbapenem MICs. Up to 7% of susceptible strains possess the silent gene, and these strains can be converted spontaneously into strains with high-level resistance via the insertion of various IS elements (9). It has been suggested that this conversion can take place in vivo after prolonged exposure to imipenem during treatment (18). Nevertheless, imipenem resistance rates among *Bacteroides* sp. strains worldwide remain low (0 to 2%), indicating that this conversion may be more diverse in its mechanism. In addition, *cfiA*-negative strains with elevated carbapenem MICs have been isolated (15), proving that other carbapenem resistance mechanisms also exist. Both isolates from the two cases presented here were found by PCR to possess the *cfiA* gene.
Prolonged treatment with metronidazole and/or carbapenems before isolation of the two strains was not recorded in the two cases described here. In that respect, the resistant strains were either already carried in the intestinal microflora of the patients before admission or were spread from other patients, as a result of contamination or via the hands of personnel. The latter hypothesis is far less likely to have occurred, as infections due to *Bacteroides* sp. strains are considered endogenous in origin. Nevertheless, at least one study, which used molecular genotypic methods, has reported patient-to-patient dissemination of resistant *Bacteroides* sp. isolates (1). In contrast, the first hypothesis may be more accurate, as at least the first case patient, due to his underlying conditions, should have had multiple hospital admissions, coupled with antibiotic treatment.

The two cases described here highlight the need for susceptibility testing of anaerobes isolated from specimens from usually sterile sites, from critically ill patients, or from patients who do not respond to empirical therapy. Health care personnel should be aware that multiresistant *Bacteroides* sp. isolates are still very rare but do exist in clinical settings and tend to complicate treatment. Molecular methods are also essential for discovering and understanding their mechanisms of resistance.

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REFERENCES


