

CASE REPORT

First case of pouchitis due to *Prevotella disiens* and *Morganella morganii* co-infection

Premier cas de pouchite due à une co-infection à *Prevotella disiens* et *Morganella morganii*

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Abstract

Objectives

We report a case of pouchitis due to *Prevotella disiens* and *Morganella morganii* in a 45-years-old woman patient with ulcerative colitis.

Patients and methods :

The pus sample was processed for aerobic and anaerobic culture. Identification of anaerobic bacteria and Gram-negative bacilli was realized respectively by ANC and GN cards in VITEK-2 system (BioMerieux, France). Susceptibility results were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

Results :

After 18–24 h of aerobic incubation on 5% sheep blood agar and Drigalski agar, growth of *Morganella morganii* was detected. After 72h of anaerobic incubation, very small greyish colonies grew on chocolate agar and were identified as *Prevotella disiens*. It was susceptible to β -lactams and ciprofloxacin.

Conclusion :

The majority of anaerobic infections are mixed infections, involving aerobic and anaerobic bacteria that act synergistically. The treatment of anaerobic bacterial infections is complicated because of their long culture and the polymicrobial nature. It must cover anaerobes, enterobacteria and enterococci.

Keywords : *Prevotella disiens*, *Morganella morganii*, pouchitis, anaerobic bacteria, ulcerative colitis

Résumé

Objectifs :

Nous rapportons un cas de pouchite due à *Prevotella disiens* et *Morganella morganii* chez une femme de 45 ans souffrant de rectocolite hémorragique.

Patients et méthodes :

L'échantillon de pus a été mis en culture aérobie et anaérobie. L'identification de la bactérie anaérobie et celle de bacilles à gram négatif a été réalisée par les cartes ANC et GN de l'automatesystème VITEK-2 (BioMerieux, France). La sensibilité aux antibiotiques a été interprétée selon les recommandations de l'European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Résultats :

Après une incubation de 18-24h en aérobie sur gélose au sang et sur gélose Drigalski, *Morganella morganii* a été isolée. Après 72h d'incubation en anaérobiose, de petites colonies grises ont poussé sur gélose chocolat qui ont été identifiées comme *Prevotella disiens*. Cette dernière était sensible aux beta-lactamines et à la ciprofloxacine.

Conclusion :

La majorité des infections à germes anaérobies sont en réalité des infections mixtes, qui impliquent des bactéries aérobies et anaérobies agissant en synergie. Le traitement des infections à bactéries anaérobies est compliqué à cause de la culture bactérienne longue et la nature poly-microbienne du prélèvement. Son spectre doit couvrir les bactéries anaérobies, les entérobactéries et les entérocoques.

Mots-clés : *Prevotella disiens*, *Morganella morganii*, pouchite; bactéries anaérobies, rectocolite hémorragique

INTRODUCTION

Pouchitis, a non specific inflammation of the ileal region, is the most frequent long-term complication following ileo-anal anastomosis in patients with inflammatory bowel diseases (IBD) (1,2). The physiopathology of pouchitis is still not well understood and involves several factors such as microbial overgrowth, colonic metaplasia, initial disease, immune dysregulation and faecal stasis (3). These different mechanisms alter the colic membrane and lead to inflammation favored by an imbalance in the production of pro and anti-inflammatory cytokines (1). Indeed, the intestinal microbiota of IBD patients are initially deregulated under the effect of multiple factors (4). This process is certainly associated with microbial proliferation (1). The involvement of strict anaerobic bacteria in the post-operative intra-abdominal infections process is evaluated at 75% to 100% (5). This could be explained by the synergy that exists between facultative aero-anaerobic bacteria and strict anaerobic bacteria: facultative aero-anaerobic bacteria consume the oxygen present in the medium for their own growth and will then create the ideal conditions for the growth of anaerobes (6). This report discusses a case of pouchitis due to

Prevotella disiens (*P. disiens*) and *Morganella morganii* (*M. morganii*) in patient with ulcerative colitis.

CASE REPORT

The patient, a 45-year-old woman with ulcerative colitis since 2007, underwent a subtotal colectomy with ileostomy and sigmoidectomy for severe acute colitis in February 2017. In August 2018 she received a restoration of the digestive continuity by means of an ileorectal anastomosis. After one year, due to recurrent flare-ups of proctitis that were resistant to medical treatment, a proctectomy with an ileal pouch-anal anastomosis was performed associated with a diverting ileostomy. She was treated successively with mesalazine, corticosteroid and then azathioprine-infliximab without a favorable response. Currently, the evolution was marked by a stenosis of the ileoanal pouch anastomosis which led to pouch related sepsis. She was hospitalized on February 2nd, 2021 for surgical operation. Colonoscopy prior to the surgery showed active pouchitis with mucosa characterized by diffuse redness, severe edema with erosions and ulcers, and an ileal pouch fistula (figure 1 and 2).

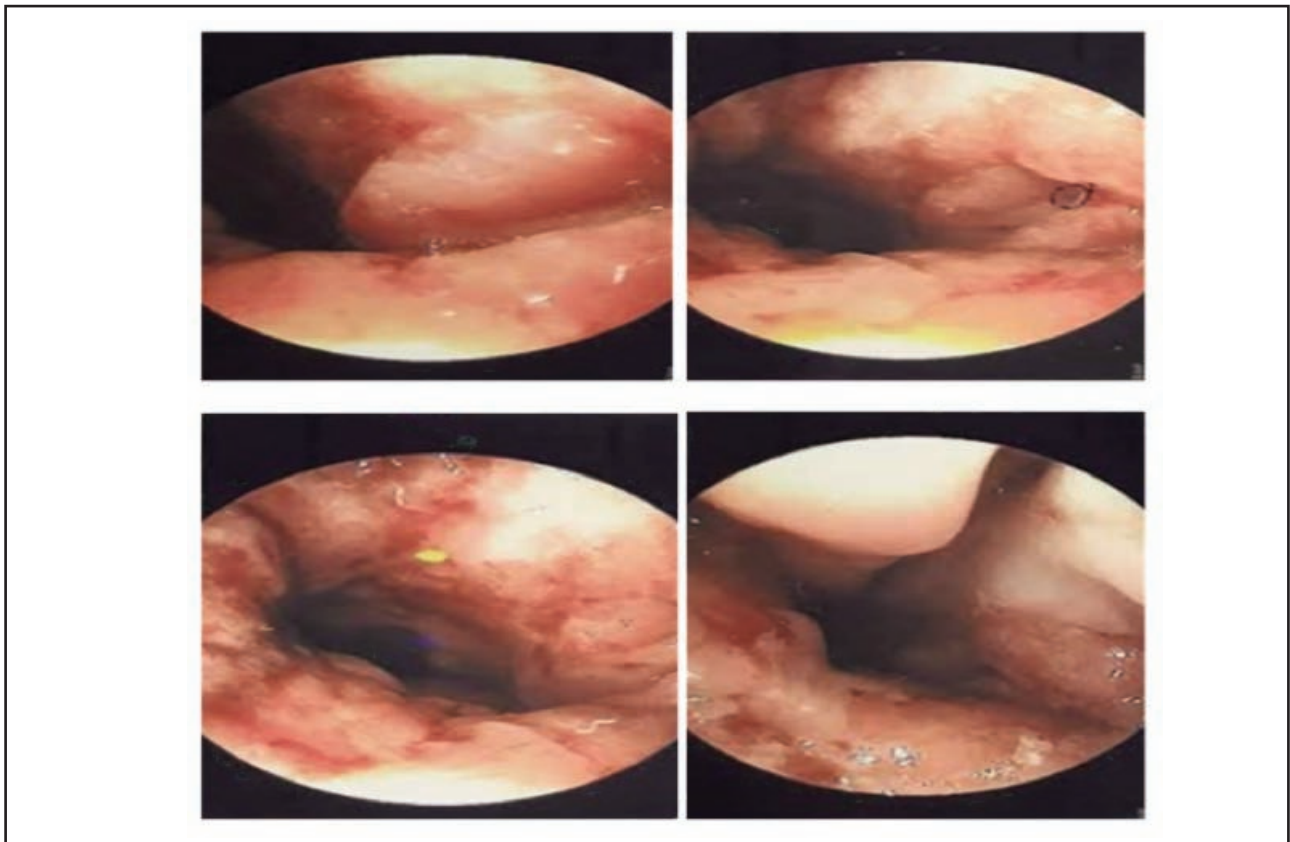


Figure 1: Colonoscopy showed active pouchitis with mucosa characterized by diffuse redness, severe edema with erosions and ulcers

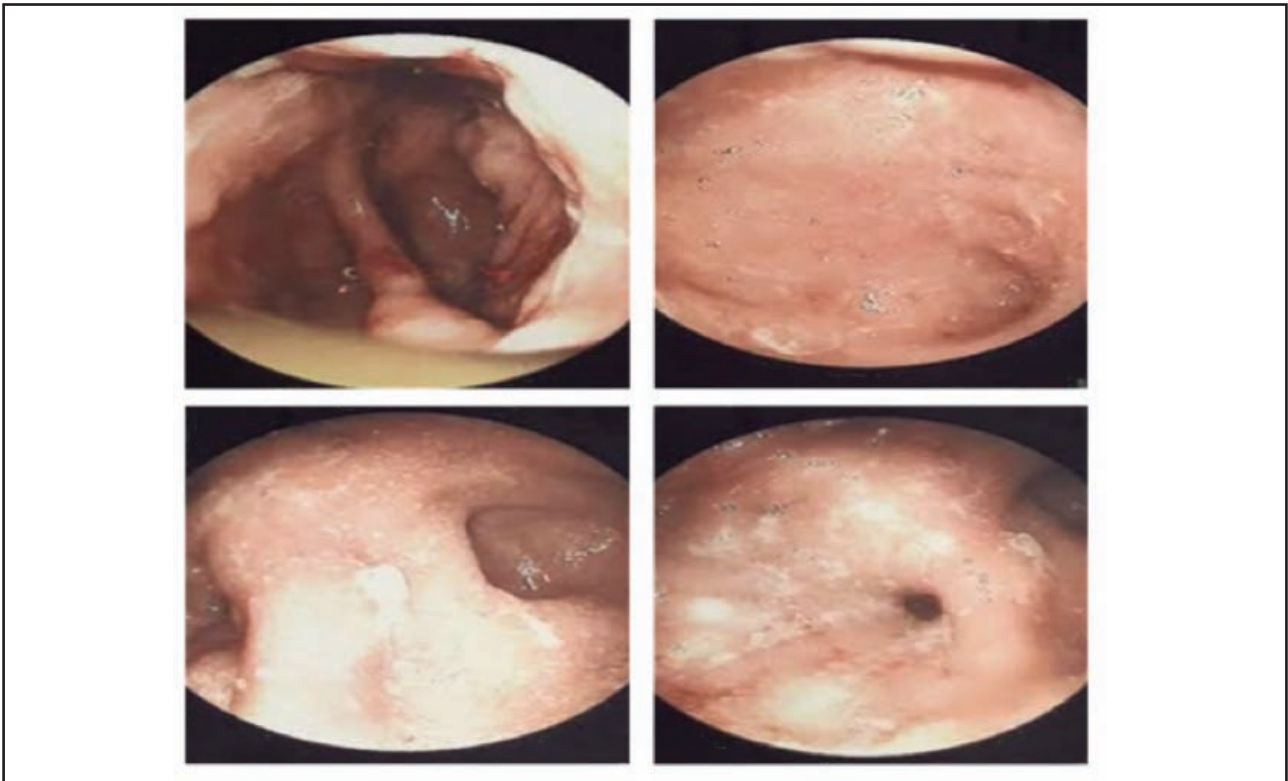


Figure 2: Coloscopy: Ileal pouch fistula

A colonic opacification has been prescribed and showed the pouch and the anastomosis (figure 3).

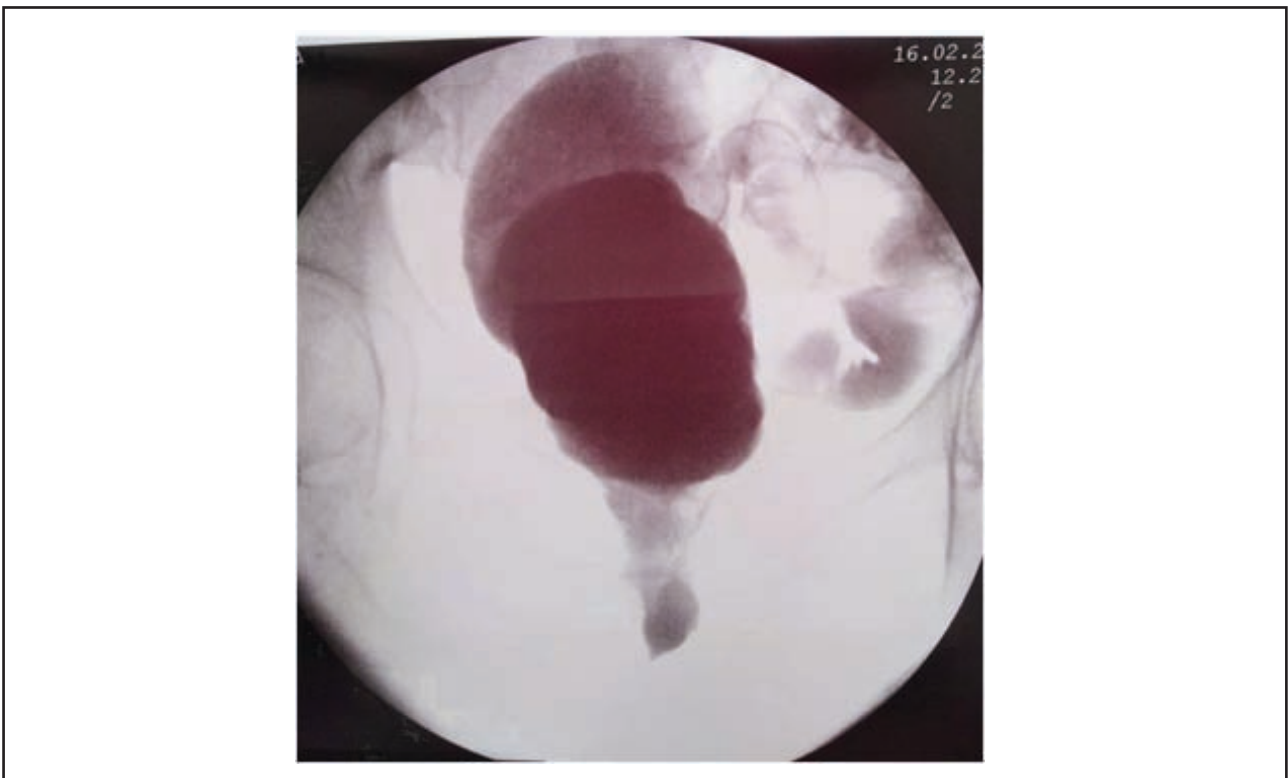


Figure 3: Colonic opacification showed the pouch and the anastomosis

During surgery, the stenosis was manually dilated. Brownish-colored pus came out and was extracted by inserting a Nelaton catheter. The sample was immediately sent to the Microbiology Department and the pouch was cleaned with saline. The patient was treated with cefotaxime, 1g three times a day, and metronidazole, 500mg three times a day, while awaiting bacteriological results.

The pus was inoculated in 5% sheep blood agar, ordinary agar, chocolate agar with Polyvitex (bioMérieux, France), Drigalski agar, and heart-brain broth. As an anaerobic etiology can be present in such cases, the sample was simultaneously processed for anaerobic culture in a 5% horse blood agar. A fetid odor was noticed during the seeding of the culture media. Cultures were incubated under anaerobic conditions with Genbox (bioMérieux, Marcy L'Etoile, France) for a total of 5 days. After 18–24 h of aerobic incubation on 5% sheep blood agar and Drigalski agar, growth of *Morganella morganii* was found using the GN gallery of VITEK-2 system (bioMérieux, France). After 72h of anaerobic incubation, very small greyish colonies grew on chocolate agar and on blood agar incubated in anaerobic conditions with the presence of a zone of beta-hemolysis. The bacteria was non motile in direct microscopic examination of wet mount preparation. Gram staining of the isolate revealed short, slightly staining Gram-negative rods. The rods were bile-esculin medium positive, oxidase-negative, catalase-negative and resistant to vancomycin.

The isolate was confirmed as *P. disiens* by automated ANC cards in VITEK-2 system (bioMérieux, France). The report of both aerobic and anaerobic infection was conveyed to the clinicians. Susceptibility results were interpreted using EUCAST breakpoints. *P. disiens* was susceptible to cefotaxim, ticarcillin, piperacillin-tazobactam, cefalexin, cefepim, amoxicillin-clavulanic acid, ceftazidim, ampicillin, ertapenem, imipenem, ciprofloxacin. The E-test strips for metronidazole susceptibility of *P. disiens* was performed (bioMérieux, Marcy L'Etoile, France) on brucella agar plates supplemented with 5% horse blood, under anaerobic conditions for 48h and showed that this latter was susceptible (<0,016 mg/L).

M. morganii was susceptible to ticarcillin, combination piperacillin and tazobactam, cefotaxime, ceftazidime, ceftipime, aztreonam, ertapenem, gentamicin, amikacin, norfloxacin, ciprofloxacin, and fosfomycin. It was resistant to nalidixic acid, combination trimethoprim/sulfamethoxazol.

Antimicrobial chemotherapy with cefotaxim and metronidazole continued until discharge, 15 days later. Postoperative recovery was uneventful, and the patient remained afebrile and asymptomatic.

DISCUSSION

Anaerobic Gram-negative bacilli mostly belong to the human microbiota in the oral cavity (*Fusobacterium*,

Prevotella, *Porphyromonas*). They are present in many types of infections mostly of polymicrobial origin (oral and dental infections, respiratory infections, intra-abdominal infections...). The species of *Prevotella* genus are predominant bacteria of the oral cavity, and are involved in all types of oral infections. They can however be responsible for various other kinds of infections (genitourinary, skin and soft tissue infection) (7). Many cases of infections due to *Prevotella* have been reported that were mostly due to *Prevotella* alone such as skin and soft tissue infections (*P. loescheii*) (8), aortitis (*P. intermedia*) (9), intra-abdominal abscesses after gastrectomy (10) and a bloodstream infection in an elderly heart failure patient (11). Other infections were described as mixed infections such as pyonephrosis due to *P. disiens* and *Escherichia coli* co-infection (12).

Although the involvement of anaerobic bacteria has rarely been isolated in pouchitis, the pathophysiology suggests, depending on the microbiota of the digestive system, that they could be involved in the disease (13). The majority of bacteria that have been described as involved in pouchitis were *Escherichia coli*, *Klebsiella*, other coliforms, *Pseudomonas*, and *Morganella* in isolation or in combination (14).

Indeed, the great majority of anaerobic infections are mixed infections, involving aerobic and anaerobic bacteria that act synergistically. Suppurations occur in the neighbourhood of a normal microbiota. Intestinal infections are generally related to damage to the intestinal barrier: surgical interventions, traumas, neoplasms. The pathogenic power of *Prevotella sp.* seems to be linked to their enzymatic equipment such as proteases and fibrinolysins (16).

The aspect of pus is very suggestive: an abundant pus with a smelly odor. The culture must be carried out quickly. Their isolation and their identification by the conventional methods requires from a few days to a few weeks. The isolation of the anaerobic bacteria remains tricky. They develop slowly, require enriched media, and live in symbiosis among themselves and with the associated aerobic bacteria. The colonies of *Prevotella* are small, from 1 to 3 mm in diameter, gray, bright, and sometimes brown or black at the center (16). The Petri dishes are incubated for 48 hours under anaerobic conditions, read a first time then incubated a second time under anaerobic conditions for 5 more days. Besides the isolation on Petri dishes, it is necessary to inoculate an anaerobic broth (Rosenow, thioglycolate, Schadler, *Brucella*) (16). The *Prevotella* species are immobile in the direct microscopic examination of wet mount preparation, catalase-negative, oxidase-negative, urease-negative, they ferment glucose and are resistant to vancomycin and kanamycin at 1000µg. *P. disiens* is variable esculin-bile. Strains of *Prevotella* are naturally highly

sensitive to penicillin, but approximately 60-75% (7, 16) of the strains produce an acquired β -lactamase that inactivates oral aminopenicillins, 1st generation cephalosporin, 2rd generation cephalosporin and 3rd generation cephalosporin. Activity is restored when combined with a β -lactamase inhibitor. No strain is resistant to carbapenems. Resistance to clindamycin and metronidazole is very rare. Finally, moxifloxacin and tigecycline are active *in vitro* (7). *Prevotella* is naturally resistant to aztreonam, aminosides, trimethoprim, sulfonamides, quinolones, fosfomycin, fusidic acid, and glycopeptides (16).

CONCLUSION

The treatment of anaerobic bacterial infections is complicated because of their long culture, the poly-microbial nature of the culture and the increasing resistance to

antibiotics of such bacteria (6). In accordance with the recommendations of the College of Infectious and Tropical Diseases (17), the probabilistic antibiotic therapy depends on the anatomical situation and takes account of the aerobic Microbiota associated. In abdomino-peritoneal infections, the spectrum of probabilistic antibiotic therapy must include anaerobes, enterobacteria and enterococci and it generally combines metronidazole and a 3rd generation cephalosporin (17).

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Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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