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TITLE: The Brief Case: *Mycoplasma hominis* Extragenital Abscess

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CASE

A 55-year-old woman with an unexplored 1-month history of rectal bleeding presented to the emergency department with abdominal pain, chills, and fever. The patient received rituximab, a monoclonal antibody targeting the CD20 antigen expressed on B cells, for rheumatoid arthritis. An abdominal computed tomography (CT) scan highlighted a right peri-rectal collection and focal sigmoiditis with few diverticula (Fig. 1). Owing to sepsis, the patient received piperacillin-tazobactam in association with gentamicin and underwent an early laparoscopy to drain the collection a few hours after initiation of antimicrobial therapy. Gram staining of the peri-rectal collection revealed numerous polymorphonuclear leukocytes with no visible microorganisms. Surgical samples and blood cultures remained sterile even after five days of incubation. The patient presented no improvement in her clinical condition. Persistent fever and recurring chills along with high levels of inflammatory blood markers resulted in a treatment change to vancomycin, cefepime, and metronidazole. A CT scan on day 10 showed a stable rectal abscess. On day 14, antibiotics were switched for meropenem, amikacin, and fluconazole due to the persistent fever. Other sets of blood cultures remained negative. The fever persisted with no explanation other than the rectal abscess.

On day 20, the patient underwent exploratory laparotomy and a low Hartmann's resection of the rectum. The pathological examination of the resected specimen led to a diagnosis of perforated rectal endometriosis. Gram staining of the peri-rectal collection again showed numerous polymorphonuclear leukocytes and no visible microorganisms. However, four days of incubation on blood agar at 35°C under 5% CO_2 resulted in the

formation of pinpoint-sized colonies resembling water droplets (Fig. 2A). These colonies could not be identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) ("no peaks found"). A Gram-stain performed on the colonies showed no bacteria. These results led us to suspect *Mycoplasma hominis,* and the colonies were transferred to differential agar medium (A7) for further analysis.

M. hominis identification was confirmed after 48 hours of incubation on A7 agar. In addition, after a total of six days of incubation on blood agar, MALDI-TOF MS (Bruker Daltonics, Wissembourg, France) identified the larger colonies as *M. hominis* with a score of 1.97 (database MBT IVD, Library 9.0).

The commercial MYCOFAST Revolution colorimetric assay (ELITechGroup, Puteaux, France) was used to determine antibiotic susceptibility, using the Clinical Laboratory Standards Institute (CLSI) breakpoints (1). The isolate was susceptible to both tetracycline (MIC ≤ 1 mg/L) and clindamycin (MIC ≤ 0.25 mg/L), but resistant to erythromycin/azithromycin (MIC > 16 mg/L), levofloxacin (MIC > 4 mg/L), and moxifloxacin (MIC > 2 mg/L) (Fig. 2B). The patient was treated with doxycycline for three weeks with a favorable outcome.

DISCUSSION

Mycoplasma belongs to the class *Mollicutes*, which is characterized by the absence of a cell wall. *M. hominis* is a commensal bacterium colonizing the urogenital tract and is a causative agent for urogenital infections, such as pelvic inflammatory disease or chorioamnionitis. Although extragenital infections are less frequent, *M. hominis* has been reported to cause mediastinitis (2), abscesses, and bacteremia, particularly in postoperative patients and immunocompromised patients (3, 4).

Immunosuppression is the main risk factor for developing *M. hominis* extragenital infections (3). Approximately 50% of patients with *M. hominis* extragenital infections had an impaired cell-mediated immune system or hypogammaglobulinemia (3). In our case, the patient had received rituximab, which decreases B cell number for more than six months after administration and may also decrease immunoglobulin levels. Solid organ transplant recipients are particularly at risk of developing extragenital *M. hominis* infections (4). *M. hominis* has also been identified in postoperative infections (1). Hyperammonemia syndrome, an encephalopathy due to high plasma ammonium levels, has also been linked to *Mycoplasma* and *Ureaplasma* following lung, kidney, or hematopoietic stem cell transplantation (5). Notably, ammonium is produced from arginine by one of the major energy-producing pathways of *M. hominis*. Overall, *M. hominis* infection should be evoked in immunocompromised patients with extragenital abscesses, particularly when numerous leukocytes are present with no visible microorganisms.

The diagnosis of invasive *M. hominis* infections is challenging. As *M. hominis* lacks a cell wall, it cannot be detected by Gram staining. DNA fluorochrome staining (acridine orange or Hoechst 33258) may be used to detect *Mycoplasma* in body fluids, but these stains are not specific. Without clinical suspicion of *M. hominis* infection, specific tests for *Mycoplasma* detection are not routinely performed. Our case shows the serendipitous diagnosis of *M. hominis* through the observation of pinpoint-sized colonies, which can grow on blood and chocolate agar after two to seven days of incubation (2). The small size of these colonies (0.2 mm in diameter) renders them difficult to detect without careful inspection under reflected light. This knowledge could prove useful to clinical microbiologists. In our case, no bacterial growth was detected from the first surgical samples. Even if *M. hominis* can grow on blood or chocolate agar, this is not always reliable. *M. hominis* translucent colonies may have been overlooked before discarding the agar media because they can be easily mistaken for water droplets. Prolonged incubation is necessary to allow *M. hominis* colonies to develop.

M. hominis should be suspected when Gram staining fails to detect microorganisms from pinpoint-sized colonies, warranting subculture onto mycoplasma media. If mycoplasma media is unavailable locally, another alternative workflow would be to perform an acridine orange stain on the colonies to prove the presence of microorganisms and send the isolate to a reference laboratory for identification by MALDI-TOF MS or 16S rRNA sequencing. There are several types of mycoplasma media, including SP4 agar supplemented with arginine, Hayflick agar, and A7 agar, with penicillin G generally added for selectivity. Agar plates should be incubated under 5–10% CO_2 or under anaerobic conditions at 35°C for a minimum of five days. A

stereomicroscope can aid in the visualization of the colonies, identifiable by their typical "fried egg" appearance. The biochemical profile helps to differentiate *M. hominis* from *Ureaplasma* spp.: *M. hominis* utilizes arginine (alkaline shift from orange to deep-red, Fig. 2B), contrary to *Ureaplasma* spp. which utilizes urea. Other *Mycoplasma* species are also able to utilize arginine, but *M. hominis* is the only human pathogenic species of *Mycoplasma* able to grow on blood or chocolate agar.

For a definite identification at the species level, MALDI-TOF MS can be used. *M. hominis* is present in both Bruker MALDI Biotyper and Vitek MS databases. In total, the Bruker MBT 8326 IVD database contains 12 species of *Mycoplasma*, and the Vitek MS V3.2 database contains 14 species of *Mycoplasma*. However, the main issue is the low biomass of the *Mycoplasma* colonies, especially after incubation on blood or chocolate agar instead of specific mycoplasma media. In our case, MALDI-TOF MS identification failed after four days of incubation on blood agar but was successful after six days of incubation on blood agar or two days on A7 agar, with a score of 1.97. Pereyre *et al.* suggested that accurate species-level identification was achievable for *Mycoplasma* with a score ≥ 1.70 for Bruker MALDI-TOF MS, instead of the classical threshold of ≥ 2.00 (6).

Molecular methods provide an alternative for diagnosing extragenital *M. hominis* infections, not only for identifying suspect colonies but also directly from clinical samples. Several in-house real-time PCR assays have been developed for *M. hominis* detection, targeting either 16S rRNA, *gap*, or *yidC* genes. However, minor sequence variations were reported in 16 rRNA or *gap* genes, which may lower clinical sensitivity (7). Real-time PCR targeting the *yidC* gene was estimated to have a limit of detection of

seven copies/µL, making it more sensitive than culturing urogenital samples (7). Direct 16S rRNA sequencing from clinical samples has been successfully used to diagnose extragenital *M. hominis* infections (2). Extending this to next-generation sequencing or shotgun metagenomic sequencing is promising for diagnosing pathogens in abscesses.

M. hominis is naturally resistant to all antibiotics targeting cell wall synthesis (β-lactams, glycopeptides, fosfomycin), erythromycin/azithromycin, sulfamides, and rifampicin. Without a definitive diagnosis, it is unlikely that patients suffering from *M. hominis* extragenital infections will receive effective antimicrobial therapy. For example, patients with intra-abdominal abscesses or mediastinitis typically receive a broad-spectrum β-lactam, associated with an antibiotic covering Gram-positive bacteria (glycopeptide or oxazolidinone). These antibiotics are not active on *M. hominis*. Inadequate antimicrobial therapy can lead to poor outcomes, including complications and iterative readmissions with prolonged hospital stays (4). *M. hominis* is potentially susceptible to tetracyclines, clindamycin, and fluoroquinolones (levofloxacin or moxifloxacin). However, acquired resistance has been reported. High-level resistance to tetracyclines is carried by the *tet(M)* gene, and isolates resistant to fluoroquinolones harbor mutations in the *gyrA*, *parC,* or *parE* genes (8). In France, the resistance rate was 15% for tetracyclines, 3% for levofloxacin, and 2% for moxifloxacin (8). Therefore, antimicrobial susceptibility testing must be performed to select adequate antimicrobial therapy.

The disk diffusion method is not recommended for testing antimicrobial susceptibility as there is no correlation between inhibition diameter and MIC. The Clinical Laboratory Standards Institute (CLSI) describes the reference methods for antimicrobial susceptibility as agar dilution and broth microdilution (9). Agar gradient diffusion (E-test)

represents a potentially comparable method (10) but is not endorsed by the CLSI (9). Commercial antimicrobial susceptibility assays using *M. hominis* have also been reported to perform similarly to the reference methods (8). These commercial assays provide a simple method to screen several concentrations of antimicrobials in a microwell plate format.

Extragenital infections due to *M. hominis* are rare but not exceptional, particularly in immunocompromised patients, and are likely underdiagnosed. Clinical microbiologists should be aware of the appearance of *M. hominis* colonies on blood agar after prolonged incubation to ensure the appropriate testing for *M. hominis* in the case of culture-negative abscesses, particularly in immunocompromised individuals.

SELF-ASSESSMENT QUESTIONS

1. Which of the following condition is associated with *Mycoplasma hominis* extragenital infections?
   a. Neutropenia
   b. Hypogammaglobulinemia
   c. Obesity
   d. Diabetes
2. Which of the following statements concerning *Mycoplasma hominis* identification is correct?
   a. *M. hominis* is unable to grow on blood or chocolate agar under 5% CO_2.
   b. *M. hominis* is undetectable by Gram staining.
   c. *M. hominis* is absent from MALDI-TOF MS databases.
   d. *M. hominis* produce large colonies (> 1 mm) on mycoplasma media.
3. *Mycoplasma hominis* is naturally resistant to which class of antibiotics?
   a. Tetracyclines
   b. β-lactams
   c. Lincosamides
   d. Fluoroquinolones

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Figure Legends

Figure 1. Contrast-enhanced abdominopelvic CT scan. White arrow: peri-rectal abscess.

Figure 2. Microbial analysis images. (A) Pinpoint-sized colonies, later identified as *M. hominis*, on blood agar following four days of incubation under 5% CO_2 at 35°C. (B) Colorimetric antibiotic susceptibility results. MFX: moxifloxacin, E: erythromycin, CM: clindamycin, TE: tetracycline, LVX: levofloxacin.

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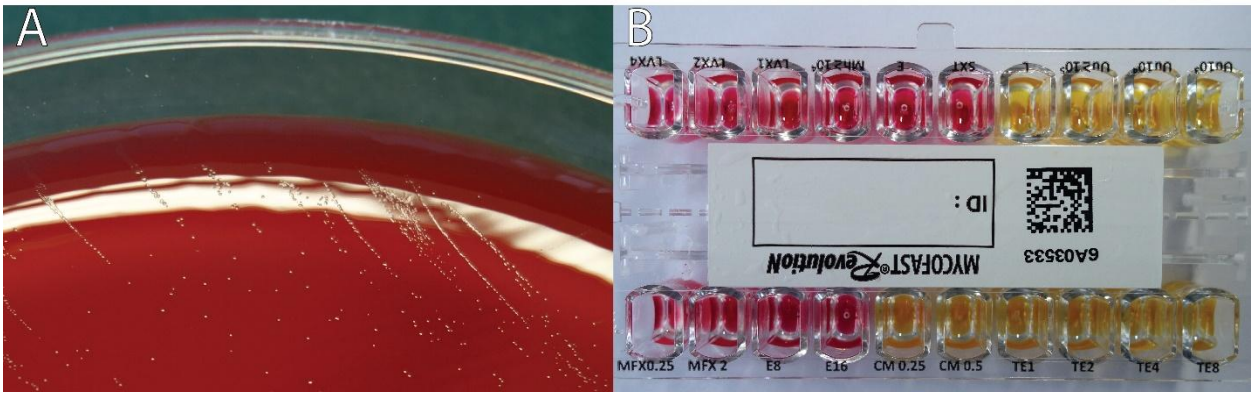

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