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The first reported case of pelvic inflammatory disease caused by *Actinobaculum massiliense*

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ABSTRACT

We report the first case of pelvic inflammatory disease (PID) caused by *Actinobaculum massiliense*. A 53-year-old woman attended the emergency department with symptoms compatible with a PID episode, finally resolved by intramuscular antibiotic treatment. *Actinobaculum* sp. was isolated by culture, and *A. massiliense* was confirmed by matrix assisted laser desorption time-of-flight mass spectrometry and 16S rRNA gene sequencing. Only a few cases of *A. massiliense* infections have been reported, and the pathogenesis of infections by these bacteria is poorly understood. The introduction of new diagnostic methods into hospital routines will improve the detection of new and little-studied pathogens.

1. Case report

A 53-year-old woman attended our emergency department for pain in the hypogastrium that was refractory to anti-inflammatories. She reported nausea, frequent urination, malodorous leucorrhoea, and sensations of fever (with no thermometer readings), while her bowel movements were normal. She had a history of interstitial cystitis, irritable bowel syndrome, hypothyroidism, caesarean section and appendectomy. She had two pregnancies that ran a normal course, with vaginal delivery in the first and caesarean section in the second. She had experienced menopause at the age of 45 years. Examination revealed a good general condition with normal vital signs but abdominal pain to palpation of the hypogastrium, with no signs of peritoneal irritation. Hemogram and urine sediment test results were normal. The patient was then referred to the gynecology emergency department for assessment. She reported no risky sexual intercourse and no recent intervention or gynecological examination. Speculoscopy evidenced non-specific leucorrhoea. In bimanual vaginal examination, cervical mobilization caused pain. On transvaginal ultrasound, the uterus was in anteversion with an atrophic endometrial line and a small (15 mm) calcified myoma on the anterior aspect, while the adnexa were normal and the pouch of Douglas contained free fluid. Pelvic inflammatory disease (PID) was suspected, and endocervical and vaginal exudates were taken for microbiological study as previously described [1,2]. Antibiotic treatment was prescribed with intramuscular monodosis of 250 mg ceftriaxone as well as a 14-day course of 100 mg doxycycline every 12 h and 500 mg metronidazole every 8 h, after which the patient attended a follow-up session in the gynecology consulting room.

No *Candida* spp., *Trichomonas vaginalis,* or *Gardnerella vaginalis* were detected in vaginal exudates (Becton-Dickinson Diagnostics, Sparks, MD, USA), and PCR results were negative for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* (BD Max, Becton-Dickinson Diagnostics). After 48-h incubation in CO_2 on blood agar (BD Columbia Agar 5% Sheep Blood, Becton Dickinson, Franklin Lakes, NY), *Actinobaculum massiliense* colonies in pure culture were identified by matrix-assisted laser desorption time-of-flight mass

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spectrometry (MALDI-TOF MS) (Bruker Biotyper, Billerica, MA, USA, score of 2.296), (Fig. 1) and confirmed by 16S rRNA gene sequencing at the National Microbiology Center (CNM, Majadahonda, Madrid, Spain) using oligonucleotides 5′- AGAGTTTGATCCTGGCTCAG-3′ and 5′-ACGGCTACCTTGTTACGACTT-3′ as previously described by Weisburg et al. [3]. The 1427 bp sequence generated (Accession number MH645801.1) was compared with the National Center for Biotechnology Information (NCBI) GenBank database, obtaining high homology (99%) with *A. massiliense* strain FC3 (Accession number LN870313.1). Antibiotic susceptibility testing was performed using an Etest (Liofilchem, Roseto degli Abruzzi, Italy) (MIC value in mg/L) on Brucella agar supplemented with hemin (5 μg/mL), vitamin K1 (1 μg/mL), and laked sheep blood (5% v/v), (Becton-Dickinson, BD, Franklin Lakes, NJ, USA) at 36 °C ± 1 °C under anaerobic conditions for 48 h. Resistance was assessed by applying the 2018 EUCAST breakpoints for gram-positive anaerobic bacteria. Results are exhibited in Table 1. The isolate was susceptible to amoxicillin-clavulanate, imipenem, penicillin, and vancomycin but resistant to clindamycin and metronidazole. At the follow-up, the patient was asymptomatic and her clinical, microbiological, and gynecological examination results were normal.

2. Discussion

Actinobaculum is a bacterial genus created in 1997 [4] by reclassifying *Actinobaculum suis*, which is the most common cause of urinary tract infection (UTI) in pigs [5]. Three other species (*Actinobaculum schaalii*, *A. massiliense* and *Actinobaculum urinale*) were later included, associated with infections in humans [6]. *A. massiliense* is characterized as a facultative anaerobe or anaerobe but is also aerotolerant and adapts to growth under CO_2 atmosphere-supplemented aerobic conditions. It is a gram-positive rod that does not form spores and lacks movement. *A. massiliense* was first isolated in 2001 from the urine of an elderly woman with recurrent episodes of cystitis [7]. The reference strain was deposited in the Institute Pasteur's Collection (Paris, France) under reference CIP107404 (=CCUG47753 = DSM19118). This original strain was lost, as reported by Yassin et al. and the deposited strain was found to be *A. schaalii* [8]. In 2015, a new strain of *A. massiliense* isolated from the urine of a 12-year-old patient with acute cystitis was classified as strain FC3 and deposited in the Collection de Souches de l'Unité des Rickettsies with the reference CSUR P1982 (=DSM100580); this strain has been formally proposed as a neotype strain of *A. massiliense* [9]. The *A. massiliense* FC3 strain was fully sequenced, and it was possible to verify a large number of genes and putative virulence markers acquired by lateral transfer from *A. schaalii* [10].

Fig. 1. Image of colonies of *Actinobaculum massiliense* in blood agar culture medium after 48-h incubation in the presence of ampicillin and metronidazole E-test.

Table 1
Antibiotic susceptibility profile of *Actinobaculum massiliense* according to the EUCAST breakpoints.

Antibiotic	MIC[a] (in μg/ml)	Clinical category
Clindamycin	>256	R
Ampicillin	0.19	S
Amoxicillin/clavulanic acid	0.016	S
Penicillin	0.047	S
Imipenem	0.012	S
Metronidazole	>256	R
Moxifloxacin	1	
Vancomycin	0.19	S
Piperacillin-Tazobactam	1	S
Ceftriaxone	0.016	
Tetracycline	0.094	

[a] MIC (minimum inhibitory concentration).

PID is an acute or subacute infection of the upper genital tract in women, affecting the uterus, fallopian tubes, ovaries, or even adjacent organs. It can cause endometritis, salpingitis, oophoritis, peritonitis, perihepatitis, or tubo-ovarian abscesses. Known risk factors for PID include: age under 25 years, multiple sexual partners, non-utilization of barrier methods, previous history of PID, presence of other sexually transmitted diseases or bacterial vaginosis, insertion of intrauterine device (up to 3 months post-insertion), or the application of other invasive, diagnostic, or therapeutic procedures in the uterus [11].

Two phases of the disease can be distinguished. The first is characterized by inflammation of pelvic soft tissues, with the involvement of facultative aerobic microorganisms, while intra-abdominal abscesses may form in the second phase, with the involvement of anaerobic microorganisms. The majority of PID cases (up to 85%) are caused by sexually transmitted pathogens, mainly *N. gonorrhoeae* or *C. trachomatis*, and a smaller number by enteric (e.g., *Escherichia coli*, *Bacteroides fragilis*, group B streptococci, or *Campylobacter* spp.) or respiratory (e.g., *Haemophilus influenzae*, *Streptococcus pneumoniae*, group A streptococci, or *Staphylococcus aureus*) microorganisms that have colonized the lower genital tract. Cytomegalovirus, *M. hominis*, *U. urealyticum*, and *M. genitalium* have also been associated with some cases. Regardless of the initial causal agent, the approach to treatment should consider PID as a mixed polymicrobial infection (facultative and anaerobic) [12].

Very few cases of *A. massiliense* have been described to date, almost always associated with UTI [6,7,9] and even producing bacteremia in one patient [6]. Only one case has been isolated in a different location, associated with a superficial skin infection [13]. Some authors have proposed that *A. massiliense* may form part of the commensal flora of the urethra or perineum, as in the case of the genetically similar *A. schaalii* [7,9], and would become a potential pathogen if it ascended to the urinary tract, although its ability to adhere to the uroepithelium and its invasive properties remain unclear [14]. Accordingly, the present case may possibly result from colonization by *A. massiliense* of the perineal area and its ascent to the lower genital tract. However, the pathogenesis of

infections by these bacteria is poorly understood and requires further investigation.

In conclusion, this is the first report of PID caused by *A. massiliense*. The bacterium was identified after ruling out other common pathogens by culture. Positive identification of *A. massiliense* was performed by MALDI-TOF and 16S RNA gene sequencing. The incidence of infections by these types of little-known bacteria may be underestimated, and the frequently positive response of patients to generic treatments can prevent the further identification of numerous pathogens. Improvements in diagnostic methods can be expected to enhance the detection of little-studied pathogens in the near future.

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None.

Ethical statement

The study protocol was carried out in accordance with the Declaration of Helsinki. This was a non-interventional study with no additional investigation to routine procedures. Biological material was only used for standard infection diagnostics following physicians' prescriptions. No additional sampling or modification of the routine sampling protocol was performed. Data analyses were carried out using an anonymous database. For these reasons, ethics committee approval was considered unnecessary according to national guidelines. The Clinical Microbiology Clinical Management Unit of the University Hospital Virgen de las Nieves of Granada (Spain) granted permission to access and use the data.

Conflicts of interest

None.

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