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Short communication

Emergence of genital infections due to *Haemophilus pittmaniae* and *Haemophilus sputorum*

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ABSTRACT

Background: We report for the first time the association of *Haemophilus pittmaniae* and *Haemophilus sputorum* with urethritis in men who have sex with men and who engage in unprotected intercourse.
Patients and methods: A search for genital pathogens was conducted using urethral exudate cultures and PCR tests for *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma* spp., and *Ureaplasma* spp. Recovered microorganisms were identified by MALDI-TOF mass spectrometry, and their susceptibility was evaluated by diffusion gradient test.
Results: H. pittmaniae and *H. sputorum* were isolated. They both proved susceptible to ampicillin, cefixime, and trimethoprim/sulfamethoxazole. *H. pittmaniae* was also susceptible to tetracycline and *H. sputorum* to moxifloxacin.
Conclusion: The increased frequency of potentially resistant *Haemophilus* spp. isolates in genital exudates highlights the need for greater surveillance of these microorganisms and for their consideration in the differential diagnosis of genital system infections.

1. Introduction

Urethritis is one of the most frequent sexually transmitted infections (STIs) in males. It is often accompanied by urethral meatus discharge (purulent or not), dysuria, and an itching or burning sensation in the urethra [1]. It is classified as gonococcal (GU) or non-gonococcal (NGU) [2] urethritis according to the presence or not of *Neisseria gonorrhoeae*, the most widely implicated microorganism [3]. The main microorganisms responsible for NGU are *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Ureaplasmas*, and herpes simplex virus type 1 and 2 [4,5]; however, it is estimated that the etiology is unknown in up to 40% of NGUs [6] when PCR is the sole diagnostic method. Haemophilus influenzae and Haemophilus parainfluenzae, *which* colonize the healthy upper respiratory tract, have been reported as rare agents responsible for NGU [2,7], especially among men who have sex with men (MSM) [6]. It is known that various pathogens, including Haemophilus spp., can be transmitted to the urethra during oral sex [3]. Within the *Haemophilus* genus, two new species have been described in recent years [8]: *Haemophilus pittmaniae* and *Haemophilus sputorum*. *Haemophilus pittmaniae,* which is not genetically related to *H. parainfluenzae,* was reported as a new species in 2005 and forms part of the healthy oral microbiota. It has been isolated in various body fluids from patients, but few clinical cases have been reported [9]. *H. sputorum* has occasionally been implicated in human infections, being isolated in blood cultures from patients with cystic fibrosis and in periodontal exudate from patients with periodontal disease [10]. However, neither *H. pittmaniae* nor *H. sputorum* has previously been found responsible for urethritis in males. This study presents the first reported case of urethritis by *H. pittmaniae* and *H. sputorum*.

2. Case presentation

2.1. Case 1

A 25-year-old male attended the hospital STI clinic for a one-week history of mild urethritis with no purulent secretion. Clinical examination revealed no other signs or symptoms and the absence

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of fever. The patient reported having unprotected insertive oral and anal sex with a new asymptomatic male partner three weeks before symptom onset. Nine months earlier, he had tested negative for hepatitis C virus (HCV), syphilis, and HIV. Urethral samples and anal and oral swabs were taken for routine STI screening and serology. Urethral, rectal, and pharyngeal exudates were each seeded on blood agar (Becton-Dickinson, CIUDAD Spain), chocolate agar (Becton-Dickinson), and Martin-Lewis agar (Becton-Dickinson) media for *N. gonorrhoeae* detection in CO_2-rich atmosphere for 48 hours. All samples underwent PCR testing for *C. trachomatis, N. gonorrhoeae, T. vaginalis, M. genitalium, M. hominis*, and *U. urealyticum* detection. Anal culture, PCR, syphilis, and HIV tests were all negative, but *H. parainfluenzae* grew on the urethral culture. The patient was prescribed 100 mg of oral doxycycline twice daily for 7 days but returned to the clinic at one month with no improvement. Another urethral exudate was collected, and PCR tests were all negative, but the cultured sample was positive for *H. pittmaniae*, as confirmed by 16S rRNA gene sequencing at the National Microbiology Center (CNM Majadahonda, Madrid, Spain). E-test results (MIC Test Strip Liofilchem®, Roseto degli Abruzzi, Italy) were interpreted in accordance with EUCAST criteria (2021), obtaining the following MIC values: 0.75 μg/mL for ampicillin, 0.032 μg/mL for cefixime, 0.5 μg/mL for trimethoprim/sulfamethoxazole, 3 μg/mL for azithromycin, 0.19 μg/mL for moxifloxacin, and 0.38 μg/mL for tetracycline. The patient was then prescribed 100 mg of minocycline every 12 hours for 12 days, reporting clinical improvement after five days of treatment.

2.2. Case 2

A 22-year-old man was referred for testing to the hospital STI clinic by the emergency department, which he had visited five days earlier for a purulent exudate from a shallow ulcer on the dorsal foreskin. He had been suspected of syphilis, a sample of exudate had been taken for serology and culture, and he had been administered with benzathine penicillin G (2.4 million IU). He had no history of STI and was not receiving any medication. Physical examination at the STI clinic showed that the ulcer was almost completely healed and confirmed the absence of lymphadenopathy, urethral secretions, inflammation, and mucocutaneous lesion in the oropharynx and rectum. He reported no other systemic abnormalities. He had no steady partners but had engaged in anal and oral sex with five casual partners during the previous six months, including one session of unprotected insertive sex in the previous two weeks. Throat and urethral samples were obtained for culture and molecular study, and an ulcer swab was taken to test for *T. pallidum*, HSV-1, HSV-2, Varicella-zoster virus, and *C. trachomatis*. The urethral sample culture was positive for *H. sputorum*, with a negative PCR result. *H. sputorum* was confirmed by 16S rRNA gene sequencing at the National Microbiology Center (CNM Majadahonda, Madrid, Spain). E-test results (MIC Test Strip Liofilchem®, Roseto degli Abruzzi, Italy) were interpreted using EUCAST criteria (2021), obtaining the following MIC values: 0.38 μg/mL for ampicillin, < 0.016 μg/mL for cefixime, 0.38 μg/mL for trimethoprim/sulfamethoxazole, 0.5 μg/mL for azithromycin, 0.047 μg/mL for moxifloxacin, and 12 μg/mL for tetracycline. The throat sample culture was negative, but the PCR test was positive for *N. gonorrhoeae.* Syphilis and HIV test results were negative. He was intramuscularly injected with 500 mg of ceftriaxone and was symptom-free at four days. The patient reported complete abstention from sex during his treatment and the following month. PCR tests and cultures were repeated at six weeks post-treatment and were all negative; he remained free of symptoms.

3. Discussion

Our hospital laboratory had not previously detected *H. pittmaniae* or *H. sputorum* in cultured urethral exudates. We started screening samples from the hospital STI approximately one year ago, including those of the present cases. The morphology of these two microorganisms does not differ from that of other species in the *Haemophilus* genus [11] except for their smaller colony size, which reaches a diameter of 0.5–1.5 mm after 24 hours on chocolate agar medium [10]. Both species were correctly identified by MALDI-TOF, which would be the first-line method for routine identification of these isolates. Their phenotypic profile was characterized by high susceptibility to the antibiotics usually prescribed to treat species of this genus. Nevertheless, systematic laboratory studies are required to confirm this susceptibility pattern, given evidence of a possible rise in *Haemophilus* spp. isolates that are resistant to multiple antibiotics (unreported data).

Haemophilus spp. can occasionally be sexually transmitted and cause acute NGU in males. However, elucidation of their etiological role is challenging because they colonize healthy individuals, being reported in up to 9% of asymptomatic males [6]. Furthermore, they are frequently associated with microorganisms more often implicated in urethritis, such as *N. gonorrhoeae*, C. trachomatis, or *M. genitalium* [2]. However, *Haemophilus spp*. can be readily transmitted during some types of sexual activity, an increased bacterial load may lead to disease onset, and they may be associated with co-infections, supporting their pathogenic relevance. In a previous Spanish study, *Haemophilus spp*., mainly *H. parainfluenzae*, were isolated as the sole microorganisms in 6.8% of urethritis cases [2]. The type of sexual behavior reported by the present patients may explain these observations. The emergence of these microorganisms as urethritis-causing agents underscores the need for more complete diagnostic procedures and microbiological analyses, including enriched cultures, PCR tests for the usual STI agents and, when possible, typing [7].

In conclusion, it is difficult to establish the etiological role of *H. pittmaniae* and *H. sputorum* as responsible for urethritis because of their possible colonization in asymptomatic individuals. Nevertheless, these microorganisms should be considered in the diagnosis of genital system diseases in individuals with clinical symptoms who engage in risky sexual behaviors in the absence of other pathogenic microorganisms.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments.

Disclosure of interest

The authors declare that they have no competing interest.

Contribution of authors

Conception and design of the manuscript: AFA, BEL, ARC, JMNM, JGF.

Data collection: AFA, BEL.

Analysis and interpretation of the data: BEL. JMNM, JGF.

Writing, review, approval of the submitted manuscript: AFA, BEL, ARC, JMNM, JGF.

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