Manuscript title: “Isolation of *Haemophilus influenzae* and *Haemophilus parainfluenzae* in urethral exudates from men with acute urethritis: A descriptive study of 52 cases”

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ABSTRACT

Objectives: To describe the clinical characteristics and therapeutic outcomes from male patients diagnosed of *Haemophilus spp.* urethritis.

Methods: A chart review of patients who presented to our Hospital from January 2013 to December 2014 with symptoms of acute urethritis in which *Haemophilus spp.* was isolated in their urethral samples was performed.

Results: *Haemophilus spp.* was isolated in 52 out of 413 urethral samples (12.6%) received in our laboratory from patients with symptoms of acute urethritis during the study period. Seven cases corresponded to *H. influenzae* and 45 cases to *H. parainfluenzae*. The most common clinical presentation was mucopurulent urethral discharge (71%). Eight per cent were HIV-infected patients, and 60% were men who have sex with men. *Haemophilus spp.* was isolated as a single pathogen in 6.8% (28 of 413) of cases. Seventeen per cent of *Haemophilus spp.* were beta-lactamase producers. All patients reported having practiced unprotected insertive oral sex the month before consultation, and 5 of them denied having had another sexual contact apart from this exposure. In all cases in which follow-up was available, empirical treatment achieved a complete clinical resolution.

Conclusions: *Haemophilus spp.* was considered a pathogen in at least the 6.8% of the patients from the evaluated area. It affected men regardless their sexual orientation or HIV status. Unprotected oral sex could play a role in its transmission. The limitations of the study (small sample size, lack of a representative control group) do not allow to prove the true pathogenic role of *Haemophilus spp.* in acute urethritis.

INTRODUCTION

Acute urethritis is one of the most common presentations of sexually transmitted diseases (STD) among men. An increased incidence along with a change in the profile of the involved pathogens has been recently described.[1-3] Among non-gonococcal urethritis (NGU), *Chlamydia trachomatis* is the most common identified agent, accounting for 30%- 50% of the cases.[1] Other pathogens associated with NGU include *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Trichomonas vaginalis*, *Haemophilus* species, *Streptococcus* species, *Gardnerella vaginalis* and viruses, such as adenoviruses and herpes virus.[1,4]

An increasing number of cases of *Haemophilus spp.* other than *Haemophilus ducreyi* causing genital tract infections, such as urethritis, cervicitis and vaginitis have been reported.[5-7] Both *Haemophilus influenzae* and *Haemophilus parainfluenzae* are colonizers of the upper respiratory tract and are part of the normal oral microflora. Sexual transmission of both *H. influenzae* and *H. parainfluenzae* has been described. Transmission of oropharyngeal commensal flora to the urethra via insertive oral sex is recognized as a mode of transmission of several pathogens including *Haemophilus spp.*[1]

We conducted a retrospective study to describe the clinical characteristics and therapeutic outcomes of male patients diagnosed with *Haemophilus spp.* urethritis during 2013 and 2014 in Hospital del Mar, Barcelona.

METHODS

Study Population. All patients who presented to our Hospital from January 2013 to December 2014 reporting less than one month of symptoms of urethritis in which *Haemophilus spp.* was isolated from their urethral samples were included in the study. Symptoms suggestive of urethritis included urethral discomfort, dysuria and/or mucopurulent or clear discharge. Clinical, laboratory, demographic and behavioral data were obtained by reviewing medical charts.

Laboratory methods. Specimens were obtained during consultation from patients in whom it had been at least 2 hours from last void. If urethral discharge was identified, samples were taken by inserting a sterile Dacron swab approximately 2 cm into the urethra. If there was no evident discharge, leukocyte esterase dipstick was performed on first- stream urine after which urine was delivered for processing. Gram-staining of urethral smears was performed in all cases. Detection of microorganisms in urethral exudates or urine was performed by four methods: multiplex polymerase chain reaction (PCR) for detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Trichomonas vaginalis*, PCR for detection of herpes simplex virus (if clinical suspicion), detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* by the Mycoplasma IST2 system (BioMérieux) and cultures on 5% sheep blood agar, chocolate agar PolyViteX, chocolate agar PolyViteX VCAT3, Gardnerella selective agar and SGC2 Sabouraud gentamicin-chloramphenicol agar (BioMérieux). Identification and characterization of *Haemophilus spp.* was performed by the API20E system (API system, BioMérieux). The beta-lactamase activities were detected by the chromogenic cephalosporin method.

RESULTS

Haemophilus spp. was isolated in 52 out of 413 urethral samples (12.6%) received in our laboratory from patients with symptoms of acute urethritis during the study period. Seven cases (13%) corresponded to *H. influenzae* and 45 cases (87%) to *H. parainfluenzae*. In total, 6.8% (28 of 413) of cases had *Haemophilus spp.* identified as a single pathogen: *H. influenzae* in 5 cases and *H. parainfluenzae* in 23 cases. Clinical and microbiological data of both groups are summarized in Table 1.

Characteristics of the study population

The mean age of the patients included was 31.8 years (range 19-62). Forty per cent of patients reported to be exclusively heterosexual, and the other 60% to have sex with men (MSM). Four cases (8%) were HIV-infected patients. Nine patients (17.3%) reported a previous STD.

Clinical presentation and sexual exposure

In both groups, the most common clinical presentation was mucopurulent urethral discharge (71.2% and 64.3% of patients respectively). Patients were asked about their sexual exposures the month before consultation. All cases reported having practiced unprotected insertive oral sex. Five out of 28 patients with *Haemophilus spp.* isolated as a single pathogen reported this exposure as their only sexual practice in the preceding month (data not shown in Table 1). Less frequent sexual exposures were insertive anal and vaginal intercourse.

Co-infection with other microorganisms

In 24 out of 52 cases (46%), *Haemophilus spp.* was isolated with another pathogen: 10 cases (42%) with *Neisseria gonorrhoeae*, 8 cases (33%) with *Chlamydia trachomatis*, 3 cases (13%) with *Candida albicans*, 2 cases (8%) with *Gardnerella vaginalis* and 1 case (4%) with *Ureaplasma urealyticum*.

Resistance mechanism

Nine cases (17%) of *Haemophilus spp.* were beta-lactamase producing; all of them corresponded to strains of *Haemophilus parainfluenzae*.

Prescribed treatment and outcomes

In 48 cases the prescribed treatment was ceftriaxone 250 or 500mg I.M. associated with a single dose of azithromycin 1gr orally or doxycycline 100mg b.i.d. for 7 days. In the other 4 patients, the treatment consisted of a single dose of azithromycin 1gr (1 case), ciprofloxacin 500mg b.i.d. for 5 days (1 case) or doxycycline 100mg b.i.d. for 7 days (2 cases). Forty-six per cent (24 cases) of the patients returned to the clinic 1 week later, and none had persistent symptoms. Twenty-eight cases were lost for follow-up.

DISCUSSION

In the present study, *Haemophilus spp.* was isolated in 12.6% of the evaluated samples. In concordance with previous studies, *Haemophilus parainfluenzae* was much more frequent than *Haemophilus influenzae* (87% vs 13%).[5,6]

It is difficult to prove the pathogenic role of microorganisms that can be isolated as colonizers under healthy conditions. In our series, *Haemophilus spp.* was detected together with other microorganisms in 24 out of 52 cases (46%). Those cases could correspond to a dual infection, although the pathogenicity of *Haemophilus spp.* remains uncertain as it could be merely a colonizer. In the remaining 28 cases, *Haemophilus spp.* was isolated as a single pathogen. Taking into account that only symptomatic patients were included and that other agents involved in urethritis were excluded, *Haemophilus spp.* could be considered a pathogen in at least 6.8% of the patients. Rates of isolation vary in the literature, from 1.2% to 10%.[5-7].

Urethritis by *Haemophilus spp.* seems to affect men regardless their sexual orientation or HIV status. In our study, it was isolated from both heterosexual males and MSM, with a slight predominance in the subgroup of MSM. Previous reports have described cases in both MSM,[8] and heterosexual males in relation to prostitution.[6]

Indistinctive clinical symptoms make it difficult to differentiate urethritis due to *Haemophilus spp.* from other causes of urethritis, and symptoms can vary from purulent urethral discharge to mucous clear discharge, with or without meatitis. Some cases may complain only of dysuria or urethral discomfort. In our series, the most common clinical presentation was mucopurulent urethral discharge.

Patients were asked about their sexual exposures the month before consultation. In our series, all cases reported having practiced unprotected insertive oral sex, and 5 of them denied having had another sexual contact apart from this exposure. These two facts suggest that unprotected oral sex could be the main route of transmission of this pathogen, although vaginal or anal intercourse cannot be completely excluded.

Empirical antibiotic therapy is offered in all cases at presentation, and includes ceftriaxone plus azithromycin or doxycycline. In all our cases in which follow-up was available, this empirical treatment achieved a complete clinical resolution. Beta-lactamase-positive strains represented the 17% of isolates in our study, lower than other Spanish series. Orden Martinez et al reported a 21.3% of beta-lactamase-producing strains of *Haemophilus spp.* in both genitourinary and respiratory samples,[9] and Orellana et al reported a 41.9% in urethral samples.[10] No cefotaxime-resistant strains

were found in both series.[9,10] This has to be taken into account in cases where ceftriaxone is not indicated as first-line treatment, as in severe penicillin allergy or avoidance of intramuscular route, where resistance can be an issue. Tinguely et al have reported recently two cases of gonococcal urethritis with the concomitant isolation of *Haemophilus parainfluenzae* non-susceptible to ampicillin, amoxicillin-clavulanate, cefotaxime, cefepime, meropenem, azithromycin, ciprofloxacin and tetracycline.[8] We do not perform routinely antibiogram on *Haemophilus spp.* strains from the genitourinary area, but it would be advisable to carefully follow cases with persistent symptoms and perform antibiogram on at least such cases.

Several limitations of this study have to be pointed out. The lack of a representative control group does not allow proving the true pathogenic role of *Haemophilus spp.* in urethritis. As it is a retrospective study, some clinical information retrieved from clinical records could be lacking. Biotypes have not been checked and it could be of interest in order to differentiate commensal from pathogenic strains. The small sample size and the high number of lost cases for follow-up do not allow making accurate conclusions about therapeutic outcomes.

To sum-up, we presented a series of 52 cases of urethritis where *Haemophilus spp.* was isolated, being the sole pathogen in 28 cases. It affected both heterosexual males and MSM, and mucopurulent urethral discharge was the most common clinical presentation. Unprotected oral sex could play a role in its transmission.

This information could be useful for clinical providers who treat men with urethritis. Detection of *Haemophilus spp.* at least in cases of non-specific urethritis which do not resolve with empirical treatment would be advisable. Further case-control studies are necessary to clarify the exact role of *Haemophilus spp.* in NGU and its mechanism of transmission.

Competing interests. None

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Contributors. GD and GME collected and analyzed the patient data. JG provided the laboratory data. JVG and AS were involved in the study design. RMP revised the manuscript before submission. All authors contributed to the preparation of the manuscript.

TABLE

Table 1. Clinical and microbiological data of the study population

Data	<i>Haemophilus spp.</i> (n=52)	<i>Haemophilus spp. as a single pathogen</i> (n=28)
	Number of cases (%) or mean (range)	Number of cases (%) or mean (range)
Age, years	31.8 (19-62)	33.7 (20-62)
Sexual orientation		
MSM	31 (59.6)	15 (53.6)
Heterosexual	21 (40.4)	13 (46.4)
Known HIV-infection		
Total	4 (8)	3 (10.7)
<i>H. influenzae</i>	2	1
<i>H. parainfluenzae</i>	2	2
Previous STD		
Total	9 (17.3)	5 (17.9)
Urethritis by <i>N. gonorrhoeae</i>	4	2
Urethritis by <i>M. genitalium</i>	1	1
Syphilis	3	1
Lymphogranuloma venereum	1	1
Clinical presentation		
Mucopurulent urethral discharge	37 (71.2)	18 (64.3)
Clear urethral discharge	13 (25)	9 (32.1)
Dysuria without evident discharge	2 (3.8)	1 (3.6)
Sexual exposures		
Protected oral sex	0 (0)	0 (0)
Unprotected oral sex	52 (100)	28 (100)
Protected vaginal sex	3 (5.8)	2 (7,1)
Unprotected vaginal sex	16 (30,8)	9 (32,1)
Protected anal sex	6 (11,5)	2 (7,1)
Unprotected anal sex	23 (44,2)	11 (39,3)
Beta-lactamase-positive strains		
Total	9 (17.3)	5 (17.9)
<i>H. influenzae</i>	0	0
<i>H. parainfluenzae</i>	9	5
Prescribed treatment		
Ceftriaxone + azithromycin/ doxycycline	48 (92.3)	28 (100)
Azithromycin 1g	1 (1.9)	0 (0)
Ciprofloxacin 500mg/12h for 5 days	1 (1.9)	0 (0)
Doxycycline 100mg/12h for 7days	2 (3,8)	0 (0)
Outcomes		
Symptoms resolved	24 (46,2)	14 (50)
Persistent symptoms	0 (0)	0 (0)
Lost follow-up	28 (53,8)	14 (50)

MSM, men who have sex with men; HIV, human immunodeficiency virus; STD, sexually transmitted diseases.

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