Brief Communication

Assessing the Need for Routine Screening for *Mycoplasma genitalium* in the Low-risk Female Population: A Prevalence and Co-infection Study on Women from Croatia

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Abstract

Background: There is an ongoing debate regarding possible cost and benefits, but also harm of universal screening for the emerging sexually transmitted pathogen *Mycoplasma genitalium*. **Methods:** From the initial pool of 8665 samples that were tested, a subset of *Chlamydia trachomatis*-positive and randomly selected *C. trachomatis*-negative cervical swabs were further interrogated for *M. genitalium* by real-time polymerase chain reaction, using a 224 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase gene. **Results:** *M. genitalium* was detected in 4.8% of *C. trachomatis*-positive samples and none of *C. trachomatis*-negative samples. Accordingly, a significant association was shown between *M. genitalium* and *C. trachomatis* ($P < 0.01$), but also between *M. genitalium* and *Mycoplasma hominis* infection ($P < 0.01$). **Conclusions:** Based on the results, routine screening is recommended only for women with one or more identified risk factors. Moreover, younger age does not represent an appropriate inclusion/exclusion criterion for *M. genitalium* testing in the low-risk female population.

Keywords: *Cervical swabs, Chlamydia trachomatis, Mycoplasma genitalium, Mycoplasma hominis, screening, sexually transmitted infections*

Introduction

Mycoplasma genitalium represents an emerging cause of sexually transmitted infections (STIs).[1] It is considered as an independent risk factor for cervicitis in women, but its role in pelvic inflammatory disease, spontaneous abortion and infertility has not been ascertained until recently.[2,3] The prevalence of *M. genitalium* in women ranges from <1% to 42%,[4,5] depending on whether we consider low-risk population (i.e., attendees of general practitioners and public health services) or high-risk population (i.e., sexually transmitted disease clinics attendees or those with specific symptoms).

There is an ongoing debate regarding possible cost and benefits, but also harm of universal screening for *M. genitalium* among low-risk individuals. Similar to many other countries, *M. genitalium* infection is not routinely screened for in Croatia and the data of its prevalence in the country are scarce – especially for the female population. Only one study regarding *M. genitalium* prevalence in Croatia was published thus far, conducted in men attending fertility clinic.[5]

The aim of our study was therefore to determine the prevalence of *M. genitalium* in cervical swabs admitted to the public health laboratory, as well as to detect co-infection patterns of *M. genitalium* with *Chlamydia trachomatis* and other STIs in order to assess the necessity of implementing *M. genitalium* screening in the low-risk female population.

Methods

Swabs were taken from women with a low-risk for STIs, i.e., asymptomatic attendees of primary care gynecologist searching for screening and prenatal care. An unlinked anonymized method to test routinely collected and stored cervical swabs was used. The samples were collected at primary care and private gynecology offices in the Zagreb region, Croatia, and referred to the public health laboratory for routine *C. trachomatis* and genital *Mycoplasma* testing. Uniformity of samples collection was maintained

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by following the standard protocol and using collection kit comprised of dacron swab and MicroTest™M4RT® transport medium (Remel Inc., Lenexa, USA). From the total pool of 8,665 samples collected from March 2014 to February 2015, 146 *C. trachomatis* positive and 168 randomly selected *C. trachomatis* negative samples were used in the study. Results for routinely tested genital *Mycoplasma* (*Mycoplasma hominis* and *Ureaplasma* spp.) were recorded prior to *M. genitalium* testing. The study was approved by the Ethics Committee of the institute where the research took place.

For *C. trachomatis* detection real-time polymerase chain reaction (PCR) was performed using Cobas® Taqman® CT v2.0 test on Cobas® Taqman® Analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Identification of *M. hominis* and *Ureaplasma* spp. was performed by *Mycoplasma* IST 2 kit (bioMérieux SA, Lyon, France). *M. genitalium* was detected by real-time PCR LightMix® kit *Mycoplasma genitalium* test (TIB MOLBIOL, GmbH, Berlin, Germany) on LightCycler 480 II Instrument (Roche Diagnostics GmbH, Mannheim, Germany), using a 224 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase gene. Difference between groups was assessed using Fisher's exact test and strength of association using univariate logistic regression when appropriate. Statistical analysis was performed using STATA/SE ver 11.2 (StataCorp LP, TX, USA).

Results

During 1 year period, in women who were using services of the public health laboratory, *C. trachomatis* prevalence of 1.9% (165/8665), *M. hominis* prevalence of 3.2% (277/8665), and *Ureaplasma* spp. prevalence of 33.3% (2885/8665) were observed. A total of 314 cervical swabs were further selected according to the *C. trachomatis* status (146 *C. trachomatis* positive and 168 *C. trachomatis* negative) and interrogated for *M. genitalium*. The additional analysis of selected samples on routinely tested genital mycoplasmas (*M. hominis* and *U. urealyticum*) revealed that 54 of 314 samples were *M. hominis* positive, and 181 of 314 were *Ureaplasma* spp. positive [Table 1]. *C. trachomatis* positive samples more commonly harbored routinely tested genital *Mycoplasma* in comparison to *C. trachomatis* negative samples (101/146; 69.2% vs. 72/168; 42.9% Fisher's exact $P < 0.001$).

M. genitalium was detected in seven of 314 cervical swabs tested (2.2%, 95% confidence interval = 0.9%–4.5%). However, all positive samples for *M. genitalium* were also positive for *C. trachomatis* [Table 1]. Furthermore, five *M. genitalium* positive samples were detected in cervical swabs with initially proved triple infection (*C. trachomatis*, *M. hominis* and *Ureaplasma* spp.) and two *M. genitalium* positive specimens were detected in cervical swabs with initially proved dual infection. Eleven percent of those infected with *M. hominis*, 4.8% of those infected with *C. trachomatis* and 3.3% of those infected with *Ureaplasma* spp. were co-infected with *M. genitalium*. All selected samples negative to other sexually transmitted bacteria were also negative to the *M. genitalium.* Significant association was shown between *M. genitalium* and two bacterial STIs: *C. trachomatis* and *M. hominis* infection. There was no association between detection of *M. genitalium* and *Ureaplasma* spp. [Table 1]. The mean age of investigated women was 30.9 ± 9.9 years (age range: 1–69); women infected with tested bacteria (with the exception of those infected with *M. genitalium*) were significantly younger when compared to the uninfected women [Table 2].

Discussion

Such low prevalence of *M. genitalium* on selected sample of low-risk female population tested for *C. trachomatis* is comparable to other published studies also conducted on cervical swabs tested for *C. trachomatis*.[6] However, it seems that the true prevalence of *M. genitalium* in Croatian low-risk female population is substantially lower, since our study has found *M. genitalium* only in *C. trachomatis* positive samples. This presumptive, very low prevalence of *M. genitalium* infection in low-risk population of women in Croatia is concordant with reported prevalence of 0.8% in French women attending routine screening,[7] but also with previous report of unusually low prevalence of *M. genitalium* detected in Croatian infertile men and their asymptomatic controls (1.4% and 0%, respectively).[5] Present study also revealed that 4.8% of women with *C. trachomatis* and 11% of women with *M. hominis* also had *M. genitalium* infection, which is lower

Table 1: Univariate associations between *Mycoplasma genitalium* infection and other bacterial sexually transmitted infections

STI*	Number of samples with result		OR (95% CI)	*P*‡
	***M. genitalium* positive/other STI* positive**	***M. genitalium* positive/other STI* negative**		
C. trachomatis	7/146	0/168	NA	0.004
M. hominis	6/54	1/260	32.4 (3.8-275)	<0.001
Ureaplasma spp.	6/181	1/133	4.5 (0.5-38.1)	0.245
Total	7/247	0/67	NA†	0.353

**C. trachonatis*/*M. hominis*/*Ureaplasma* spp. infection. ‡Fisher's exact test. NA=Not applicable, *M. genitalium=Mycoplasma genitalium*, STI=Sexually transmitted infection, *C. trachomatis=Chlamydia trachomatis*, *M. hominis=Mycoplasma hominis*, OR=Odds ratio, CI=Confidence interval

Table 2: Mean age of patients with and without detected sexually transmitted infections

STIs	Mean age (year)		*P**
	Positive patients	Negative patients	
C. trachomatis	26.9±6.4	34.3±11.1	<0.001
M. hominis	27.3±9.3	31.6±9.9	0.001
Ureaplasma spp.	28.6±8.7	33.9±10.7	<0.001
M. genitalium	25.0±6.4	31.0±9.9	0.120

*Mann-Whitney test. *M. genitalium=Mycoplasma genitalium*, STIs=Sexually transmitted infections, *C. trachomatis=Chlamydia trachomatis*, *M. hominis=Mycoplasma hominis*

when compared to 9% of *C. trachomatis-M. genitalium* co-infected women that underwent population-based screening in London[8] and 11% in a screening study conducted in Norway.[9]

The recommended treatment of uncomplicated *C. trachomatis* and *M. genitalium* infection is the same, but unlike *C. trachomatis* that does not show any homotypic resistance,[10] *M. genitalium* has a high potential for developing resistance.[1] This is the reason why the treatment of cervicitis or nongonoccocal urethritis should be based upon specific diagnostic testing, and a control PCR should be pursued 4–5 weeks after treatment.[11] Moreover, since the therapy for *C. trachomatis* may not be effective for *M. genitalium*, the latter pathogen may represent a "Trojan horse" and hamper successful treatment, which is why it is significant to screen for co-infection.

At the moment, the decision to screen or not to screen is usually based on the discussion between health providers and patients (taking into account personal risk factors), especially aiming to test symptomatic women if molecular methods are available.[1] Although recent meta-analysis has shown that testing high-risk symptomatic women on *M. genitalium* is warranted,[3] our study suggest that in low-risk population it would be reasonable to implement *M. genitalium* screening only for those with proven risk factor such as *C. trachomatis* and/or *M. hominis* infection. Of course, acquiring precise insights into local *M. genitalium* epidemiology and tracking antimicrobial resistance development may represent a rationale to undertake screening endeavors regardless of the low prevalence of infection.

Conclusions

Other risk factors (i.e., multiple sexual partners, bacterial vaginosis, being symptomatic, smoking, prior miscarriage, black ethnicity, social class, marital status) including younger age are also associated with *M. genitalium* infection in the literature.[12] Still, although this study demonstrated that women infected with *M. genitalium* were younger than woman without the infection, this was not statistically significant– hence younger age would not be an appropriate inclusion/exclusion criterion for *M. genitalium* screening in the low-risk population. In any case, further studies are needed to confirm or reject results of our investigation, especially those trying to elucidate the relationship between younger age and *M. genitalium* infection.

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Conflicts of interest

There are no conflicts of interest.

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