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Mycoplasma genitalium prevalence and macrolide resistance-associated mutations and coinfection with *Chlamydia trachomatis* in Southern Jutland, Denmark

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This study aims to investigate prevalence of *Mycoplasma genitalium* and macrolide resistance-associated mutations and coinfection with other sexually transmitted bacteria in Southern Jutland, Denmark, where this information is very limited. Urinary samples from patients suspected of sexually transmitted bacterial infections collected at primary healthcare facilities in Southern Jutland are routinely tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. 601 of these samples were analysed with SpeeDx MG+23S reagents, which can detect *M. genitalium* and macrolide resistance-mediating mutations in the 23S rRNA gene. Moreover, 147 *C. trachomatis* positive urinary samples from routine test were also analysed with the PCR assay to detect *M. genitalium*. 72 out of 601 samples were detected positive for *C. trachomatis* (12%), five samples (0.83%) positive for *N. gonorrhoeae* and 25 samples positive for *M. genitalium* (4.2%). 14 of the 25 *M. genitalium* samples were detected having 23S rRNA gene mutations associated with macrolide resistance (56%). 25 of 147 *C. trachomatis* positive samples were tested positive for *M. genitalium* (17%) and two of them were positive for *M. genitalium* and *N. gonorrhoeae* (1.4%). The high prevalence of *M. genitalium* and macrolide resistance-associated mutation and the coinfection with *C. trachomatis* in the region suggesting that *M. genitalium* testing should be included in routine sexually transmitted infection screening.

Key words: *Mycoplasma genitalium*; macrolide resistance mutations; coinfection; *Chlamydia trachomatis*.

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INTRODUCTION

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the most common sexually transmitted bacterial infections globally [1, 2]. *Mycoplasma genitalium* is an emerging sexually transmitted infection with symptoms similar to those for *C. trachomatis* and *N. gonorrhoeae* [3]. The consequences of *M. genitalium* infection are similar to those for chlamydial infection, including non-gonococcal urethritis in male subjects and associated with urethritis and pelvic inflammatory disease, infertility, endometritis, ectopic pregnancy and preterm birth [4-13].

M. genitalium is usually not recommended among organisms, such as *C. trachomatis* and *N. gonorrhoeae*, for routine sexually transmitted infections (STI) screening in many countries [6, 12]. When general practitioners in our region suspect a patient of sexually transmitted bacterial infections, they will order tests for *C. trachomatis* and *N. gonorrhoeae*, but not for *M. genitalium*. This is mainly due to lack of information about prevalence of *M. genitalium* infection in the region and its coinfection with other sexually transmitted bacteria, and partly due to the test not being widely available in primary diagnostic laboratories in Denmark.

A meta-analysis shows that prevalence of *M. genitalium* is 1.3% in countries with higher levels of development and 3.9% in countries with lower levels of development [14]. A multicentre clinical study cohort in the United States shows that prevalence of *M. genitalium* was 16.1% for females and

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17.2% for males [15]. The prevalence of *M. genitalium* varies from country to country and from region to region. A recent study by Unemo et al. [16] shows that the prevalence of *M. genitalium* in Denmark, Norway and Sweden was 9%, 4.9% and 9.8%, respectively. However, the study in Denmark was carried out in Bispebjerg University hospital, which has the biggest STI clinic in Denmark, in the capital city, Copenhagen. The prevalence of *M. genitalium* in the remote regions in Denmark, such as Southern Jutland, is unknown. A Swedish study shows that prevalence of *M. genitalium* in the Skåne Region of Southern Sweden was 17% for male and 11.9% for females [17], which are higher than that in Unemo's study.

Macrolide azithromycin is the recommended first-line treatment for *M. genitalium* infection [18]. Emerging *M. genitalium* resistance to macrolide azithromycin is linked to mutations in the 23S rRNA gene. The macrolide-resistant rate *M. genitalium* has recently dramatically increased worldwide [19] and varies between geographical regions, for example, 18% in Stockholm, Sweden [20], 41% in London, UK [21], 41.4% in Copenhagen, Denmark [16], 47.3% in Canada [22], 52.6% in Dresden, Germany [23], 63.6% in Queensland, Australia [24] and 72% in Auckland, New Zealand [25].

Additionally, the information about coinfection of *M. genitalium* and other sexually transmitted bacteria is very limited. A study from California, USA shows that coinfection of *M. genitalium* and *C. trachomatis* is 3.1% in females and 9.7% in males [26]. A Croatian study shows that coinfection of *M. genitalium* and *C. trachomatis* is 4.8% in low-risk females [27]. Therefore, there is a great need to investigate the coinfection of *M. genitalium* and other sexually transmitted bacteria, such as *C. trachomatis* or *N. gonorrhoeae*.

The aim of the study is to investigate the prevalence of *M. genitalium* and its macrolide resistance-associated mutations, and its coinfection with *C. trachomatis* or *N. gonorrhoeae* in the region with new multiplex quantitative PCR MG+23S reagents (SpeeDx) [29]. The study population is the patients, who were suspected by themselves of having sexually transmitted bacterial infections and visited their general practitioners in Southern Jutland, Denmark.

MATERIALS AND METHODS

Study design

Department of Clinical Microbiology, Hospital of Southern Jutland in Soenderborg, Denmark is the primary diagnostic laboratory for the hospital itself, other care institutions and general practitioners in the area. Urinary samples from patients suspected of sexually transmitted bacterial infections collected by general practitioners in Southern Jutland are routinely sent to the department for *C. trachomatis* and *N. gonorrhoeae* (CT/NG) testing. Collection of urine was performed in Cobas PCR Urine Sample kit (Roche Diagnostics GmbH, Mannheim, Germany). The CT/NG testing was performed using qPCR in closed system – *In vitro Diagnostic*, Cobas® 4800 CT/NG Amplification/Detection Kit at COBAS z480 (Roche Diagnostics) in a routine clinical setting.

601 of the routinely analysed urinary samples were randomized collected by laboratory technicians from 1 March to 25 August 2018. Samples were analysed with new multiplex quantitative PCR MG+23S reagents (SpeeDx) on the COBAS z480 (Roche Diagnostics) for detection of *M. genitalium* and macrolide resistance-mediating mutations in the 23S rRNA gene. The information about the patients' age and gender is listed in Table 1.

147 *C. trachomatis* positive urinary samples (80 female, 67 male, 5 *C. trachomatis*/*N. gonorrhoeae* positive), which were tested with the cobas® 4800 CT/NG Test in 2017, were further analysed with MG+23S reagents to detect *M. genitalium* and macrolide resistance-mediating mutations, to provide information on coinfection and macrolide resistance.

Laboratory diagnostics of *M. genitalium*, *C. trachomatis* and *N. gonorrhoeae*

Cobas® CT/NG is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. Results from the analyses are presented as positive, negative or invalid for *C. trachomatis* and *N. gonorrhoeae,* respectively.

The MG+23S reagents (SpeeDx) were employed for detecting *M. genitalium* and the macrolide resistance-determining region (MRDR) of the 23S rRNA gene (five mutations A2058C, A2058G, A2058T, A2059C and A2059G) and were performed with the COBAS z480 (Roche Diagnostics). Data analysis reporting the presence or absence of *M. genitalium*, 23S rRNA mutation and internal control was performed using the supplied analysis software (SpeeDx).

RESULTS

Table 1 shows that out of 601 samples, 72 samples were detected positive for *C. trachomatis* (12%), 5 (0.83%) positive for *N. gonorrhoeae* and 25 positive for *M. genitalium* (4.2%). 14 of 25 *M. genitalium* positive samples (56%) were detected having 23S rRNA gene mutations associated with macrolide resistance (Table 2). 70.8% of *C. trachomatis* and 52% of *M. genitalium* positive patients are in the age of 13-25 years old (Table 1).

There is only one *M. genitalium*/*C. trachomatis* coinfection and one *C. trachomatis*/*N. gonorrhoeae* coinfection detected in the 601 samples (Table 2).

Out of 147 *C. trachomatis* positive samples, 25 samples were detected as *M. genitalium* positive and 16 of them were detected having 23S rRNA

Table 1. Prevalence of *Mycoplasma genitalium*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* age and sex group (601 randomized samples collected by laboratory technicians from 1.03.2018 to 25.08.2018)

	All patients	*C. trachomatis* positive	*M. genitalium* positive	*N. gonorrhoeae* positive
Age	13–75 years old (%)	15–58 years old	18–55 years old	20–41 years old
13–25 years old	269 (44.76)	51	13	1
26–39 years old	212 (35.27)	13	9	3
>40	120 (19.97)	8	4	1
Gender				
Male	432 (71.88)	56	22	4
Female	169 (28.12)	16	3	1
Total	601	72 (11.98%)	25 (4.16%)	5 (0.83%)

Table 2. Evaluation of the *M. genitalium* 23S assay for the detection of macrolide-resistant *M. genitalium* and coinfection of *M. genitalium*, *C. trachomatis* and *N. gonorrhoeae* in 601 urinary samples

Results group	Samples
Mg positive, 23S mutant not detected	11
Mg positive, 23S mutant detected	14 (56%)
Coinfection MG/CT	1 (55 years old male)
Coinfection MG/NG	0
Coinfection NG/CT	1 (17 years old female)

Table 3. Number of macrolide-resistant *M. genitalium* and characteristics of *M. genitalium* positive patients in 147 *C. trachomatis* positive urinary samples

Results group	Samples
Mg positive, 23S mutant not detected	9
Mg positive, 23S mutant detected	16 (64%)
	Number of MG/CT positive (%)
Age	
17–25 years old	20[1]/100 (20)
26–52 years old	5[1]/47 (10.6)
Gender	
Male	22/60 (36.7)
Female	3[2]/87 (3.4)

The results show that the coinfection of *M. genitalium* and *C. trachomatis* is 17%, which suggests that it is necessary to test *M. genitalium* infection when a patient is suspected with *C. trachomatis* infection.
[1]All patients are 23S mutant detected.
[2]One of them is 23S mutant detected.
[3]MG/CT/NG/positive: 2 (one of them is 23S mutant detected).

mutations, associated with macrolide resistance (Table 3).

Most of *M. genitalium*/*C. trachomatis* coinfection patients belong in the category of 17-25 years old males. There are only two *M. genitalium*, *C. trachomatis* and *N. gonorrhoeae* coinfections detected in 147 *C. trachomatis* positive samples (Table 3).

DISCUSSIONS

This is the first study on prevalence of *M. genitalium* and associated macrolide resistance in the region. A recent study by Unemo et al. from 2017 shows that the prevalence of *M. genitalium* in Copenhagen, Denmark was 9% [16], which the prevalence is double so high than that (4.16%) in our region. The reason for this difference is probably due to the study population difference. In Copenhagen, the capital city, the study population is the patients who visited the biggest STI clinic in Denmark. In our study, it is the patients, who were suspected by themselves for STI (for example, they have symptoms, new partner, infected partner) and visited their general practitioners in Southern Jutland, a remote region. We believe that it is very interesting finding and further investigation is definitely needed to elucidate the difference. Furthermore, our region is near northern Germany, where there is no report of prevalence of *M. genitalium* in the last decade. It is unclear whether there is any relation concerning prevalence of *M. genitalium* between these two regions.

Multidrug resistance in *M. genitalium* is currently a major concern in recent years. Numerous studies on *M. genitalium* resistance to macrolide azithromycin have been reported worldwide [18-25]. The rate of macrolide-resistant *M. genitalium* in our region is a little higher than that in Copenhagen (56% vs. 41.4%), and more macrolide-resistant *M. genitalium* in male is higher than female. This resistance is linked to mutations in the 23S rRNA gene. Unemo's study shows that the 23S rRNA gene mutation A2059G was predominant (53.5% of mutated samples) in Denmark and Norway (59.6%), while A2058G (50.0%) was slightly more common in Sweden [16].

In the three tested STI microorganisms, *C. trachomatis* has highest prevalence, *M. genitalium* second and *N. gonorrhoeae* third. A recent study shows that the prevalence of *M. genitalium* (9.6%)

is higher than *C. trachomatis* (7.1%) in patients attending youth clinics in the Region of Västra Götaland, Sweden [30]. There was only one *C. trachomatis*/*M. genitalium* coinfection and one *C. trachomatis*/*N. gonorrhoeae* coinfection in the 601 urinary samples. However, the coinfection of *M. genitalium*/*C. trachomatis*/ is 17% and *M. genitalium*/*C. trachomatis*/*N. gonorrhoeae* is 1.3% in the 147 *C. trachomatis* positive samples in our study, which is higher than other studies [26-28]. One out of the two *M. genitalium*/*C. trachomatis*/*N. gonorrhoeae* coinfection is macrolide resistant.

The data in the study also show that prevalence of *C. trachomatis* and *M. genitalium* is age related, the age of 13–25 years old >26–39 years old >40 years old, which may due to more sexual activity in younger people.

Lack of information about the prevalence of *M. genitalium*, its macrolide resistance and its coinfection to other STI organisms in the primary health care can influence the diagnosis and treatment of the organism infection. Furthermore, the test for *M. genitalium* is not widely available in primary diagnostic laboratories. In Denmark, most of the *M. genitalium* tests are carried out at Statens Serum Institut due to most of territorial hospitals do not have the test in their laboratories. Based on our results, *M. genitalium* test should be considered including in routine STI screening, especially for the patients in the age of 13–39 years old or the patients with *C. trachomatis* infection.

CONCLUSIONS

This study provides data regarding the prevalence of *M. genitalium* infection, its macrolide resistance and its coinfection to *C. trachomatis* and *N. gonorrhoeae* in the region. We suggest that *M. genitalium* testing should be included in routine STI screening. The high level of macrolide-resistant *M. genitalium* raises concern over future use of azithromycin for treatment of *M. genitalium* infection. Active surveillance on *M. genitalium* infection is recommended.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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