Prevalence of *M. genitalium* and *U. urealyticum* in urine tested for *C. trachomatis*

BACKGROUND *Mycoplasma genitalium* and *Ureaplasma urealyticum* cause sexually transmitted infections. While *M. genitalium* is an established aetiological agent, *U. urealyticum* is still controversial as a pathogen. Testing for these microbes is not yet widely available in Norway, and knowledge of their prevalence is limited. In this study we have investigated the prevalence of *M. genitalium* and *U. urealyticum* in a heterogeneous population from Vestfold and Telemark.

MATERIAL AND METHOD Urine samples (n = 4 665) received by the laboratory for testing for *Chlamydia trachomatis* in the period from February 2011 to January 2012 were subsequently tested for *M. genitalium* and *U. urealyticum*. Samples were analysed using an in-house PCR protocol.

RESULTS The prevalence of *C. trachomatis*, *M. genitalium* and *U. urealyticum* was 11.9 %, 3.6 % and 17.9 % respectively. *M. genitalium* was found most frequently in women aged 20–24 years (5.1 %), while the proportion of samples positive for *U. urealyticum* was greatest in persons aged 15–24 years (22.8 %).

INTERPRETATION *M. genitalium* was highly prevalent in urine samples submitted for *C. trachomatis* testing. *M. genitalium* testing was requested for only a minority of the samples analysed, suggesting limited knowledge of this microbe. *U. urealyticum* was the most predominant microbe in the study, which may indicate that it is largely non-pathogenic.

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MAIN MESSAGE

M. genitalium and _U. urealyticum_ are common sexually transmitted microbes

M. genitalium causes genital infections more often than _U. urealyticum_, and its prevalence appears to be increasing

Due to a lack of availability of diagnostic tests and limited awareness, many disease cases probably remain undiagnosed and untreated

The rapid development of antimicrobial resistance in _M. genitalium_ complicates recommendations for testing and treatment

Mycoplasma genitalium and *Ureaplasma urealyticum* are transmitted through sexual contact and can cause urogenital infections. While *M. genitalium* is established as an aetiological agent of genital infections (1), *U. urealyticum* is still controversial as a pathogen (2, 3). Testing for these microbes is not widespread in Norway and there is no surveillance of their prevalence.

In 2013, the guidelines for antibiotic treatment of chlamydia infection were amended. Doxycycline is now first-line therapy for chlamydia, as azithromycin use has been shown to contribute to antimicrobial resistance (4). As a result of this change, only 20–40 % of *M. genitalium* infections are now cleared via treatment of urethritis, cervicitis and co-infection with chlamydia. The prevalence of *M. genitalium* may thus increase (5, 6).

In Europe, the two most common microbes causing urethritis are *Chlamydia trachomatis* and *M. genitalium*; these are detected in 22 % and 7–13 % of patients with urethritis respectively (7, 8).

In men, *M. genitalium* most often causes urethritis. The microbe is also found in the urethra of men with epididymitis and in tissue from patients with chronic prostatitis (1). The greater complexity of the female urogenital system means that the clinical picture too can be more complex in women. Nevertheless, cervicitis appears to be the most common manifestation of infection (9, 10). Cervicitis is often defined differently across clinical trials, which makes it difficult to draw unambiguous conclusions (1, 11, 12). Cervicitis is a common antecedent of upper genital tract infections (13, 14). In common with *C.trachomatis*, *M. genitalium* is associated with reactive arthritis in both women and men (1, 11).

U. urealyticum is a common microbe in both the female and male urogenital tracts, and is probably less virulent than *M. genitalium* (2, 15). *U. urealyticum* is found more frequently in men with urethritis than in non-infected men, and is assumed to be an aetiological agent in urethritis (3, 4, 16).

Knowledge of the prevalence of *M. genitalium* and *U. urealyticum* is important for ensuring a good and targeted testing regime, correct treatment and reduction of infection. In this study we have investigated the prevalence of these microbes in samples submitted for *C. trachomatis* testing.

Material and method

Material

In the period from February 2011 to January 2012, 5 611 urine samples were tested for *C. trachomatis*. Samples from children under the age of 15 years, those in which the DNA was not intact, and control/follow-up samples were excluded. In all, 4 665 samples were included in the study and tested for *M. genitalium* and *U. urealyticum*. Of these, 484 had already undergone routine testing for the latter microbes, and were thus tested twice. Screening samples and samples from

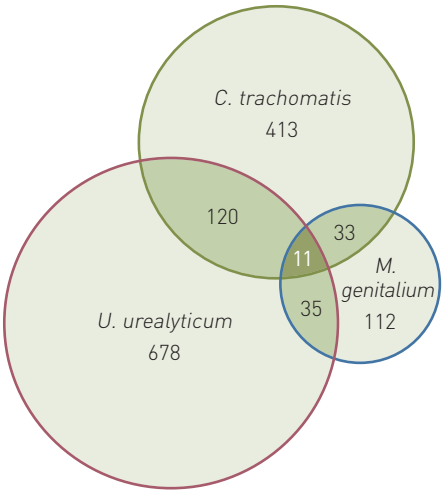

Figure 1 *Venn diagram showing the number of positive test results and co-infections for* Chlamydia trachomatis, Mycoplasma genitalium *and* Ureaplasma urealyticum *(n = 1 369)*

patients with symptoms of chlamydia were submitted from both the primary and specialist health service, mainly by gynaecologists and urologists in and outside hospitals.

Personal data and sample information were obtained from the laboratory information system Mlx (Miclis AS) and were anonymised. Clinical information was often incomplete and was therefore not included in the data. The study was judged by the Regional Ethics Committee to fall outside the scope of the Health Research Act and was therefore exempt from approval requirements.

Laboratory method

DNA was isolated from 200 µL of sample material with the MagNa Pure LC/Total Nucleic Acid kit (Roche Diagnostics Norway). An in-house multiplex PCR was performed using the LightCycler 480 (Roche).

The method for qualitative detection of *M. genitalium* is based on the target gene «*gap*» (glyceraldehyde-3-phosphate dehydrogenase) (17). For *U. urealyticum* the target gene was «*ureaplasma multiple banded antigen*». Taqman probes and primers were synthesised by Eurogentec and supplied by BioNordika/Medprobe.

Statistical analyses

Microsoft Excel 2007 (Microsoft, Redmond, WA) was used to calculate the sampling distribution, proportion of positive results and average age with standard deviation. Differences in prevalence between men and women were examined with an unpaired t-test. Confidence intervals were calculated with the online McCallum Layton calculator (18), and the R-3.1.1 calculator for Windows was used to determine significance and to prepare a Venn diagram (19).

Results

Subject characteristics

The average age of the entire sample was 26.1 years (SD 9.6 years), with an average age for women of 24.2 years (SD 8.5 years) and for men of 28.7 years (SD 10.4 years). Age and sex distribution are shown in Table 1.

Prevalence

Of the 4 665 samples tested previously for *C. trachomatis*, 29 % (1 369/4 665) were positive for one or more of the three sexually transmitted microbes. Of these, 11.9 % were positive for *C. trachomatis* (555/4 665), 3.6 % (169/4 665) for *M. genitalium* and 17.6 % (822/4 665) for *U. urealyticum*. Prevalence specified by age and sex is presented in Table 1.

The average age of patients who tested positive for *C. trachomatis* was 23.6 years (SD 6.9 years), for *M. genitalium* 25.4 years (SD 8.4 years) and for *U. urealyticum* 23.2 years (SD 8.0 years). In all three cases, the women who tested positive were significantly younger than the men ($p < 0.01$).

Analysis of *M. genitalium* and *U. urealyticum* was requested for 10 % of the 4 665 samples. Of the samples that were positive for *M. genitalium*, 89 % (151/169) were first identified in connection with this study. For *U. urealyticum*, this was the case for 93 % (763/822).

Of the samples that tested positive for *C. trachomatis*, 5.9 % (33/555) were also positive for *M. genitalium*, while 21.6 % (120/555) were positive for *U. urealyticum*. Of samples positive for *M. genitalium*, 20.7 % (35/169) were also positive for *U. urealyticum*. Eleven patients were positive for all three microbes. Figure 1 shows the distribution (in per cent) of the three agents in positive samples.

Discussion

To the best of our knowledge, this study is the most comprehensive survey of *M. genitalium* and *U. urealyticum* in Norway. The prevalence of *C. trachomatis* has been well mapped and provides information on the population being tested. This forms a basis for evaluating and discussing the prevalence of the other sexually transmitted microbes.

M. genitalium was relatively common in this study population, with a prevalence of 3.6 % compared to 11.9 % for *C. trachomatis*. A study from Molde in 2010 showed a significantly lower prevalence of *M. genitalium* (2 %, n = 950, p = 0.01) (20). This may be due to methodological differences or to differences in the two populations studied.

Our observations are consistent with figures published from Central Norway from 2012, where *M. genitalium* was detected in 3.9 % of women and 3.2 % of men in a sample of 1 114 persons (21). In both studies the prevalence was highest in women aged 20–24 years: 5.1 % in Vestfold/Telemark and 6.5 % in Central Norway. However, prevalence may be affected by testing patterns for *C. trachomatis*. For example, figures from the Norwegian Institute of Public Health indicate that women are more likely to undergo routine testing for *C. trachomatis*, whereas men are more often tested as part of contact tracing or on the basis of symptoms (22).

Several factors suggest that this study has probably underestimated the prevalence of *M. genitalium*. There is debate regarding the type of sample most suitable for detecting *M. genitalium* in women. In a few studies where both urine samples and swabs from the vagina/cervix were tested from the same patients, the positivity rate was higher for the swabs (12, 23). Prevalence may thus have been underestimated in our population, in which only urine samples were examined

M. genitalium is more difficult to detect than *C. trachomatis* as the bacterial load of the former is often low. The method used to extract bacterial DNA is therefore critical (24, 25). The importance of choosing an appropriate method is illustrated by a study that compared three different methods for extraction of *C. trachomatis* DNA and found that the proportion of positive findings ranged from 4.3 % to 7.7 % (26). No association has been observed between bacterial load and disease. Indeed, a small number of bacteria can also cause disease, and detection of weak positive samples is therefore important (24). Emphasising this issue has resulted in methodological improvements in our laboratory.

The fact that only 10 % of tests in this study were ordered primarily for *M. genita-*

Table 1 Number of positive test results for *C. trachomatis*, *M. genitalium*, *U. urealyticum* (percentages in parentheses) with confidence intervals (CI)

Prevalence	Number	(%)	*C. trachomatis*	± 95 % CI	*M. genitalium*	± 95 % CI	*U. urealyticum*	± 95 % CI
Total	4 665		555 (11.9)	(11.0–12.8)	169 (3.6)	(3.1–4.1)	822 (17.6)	(16.5–18.7)
Women	2 664	(57)	278 (10.4)	(9.2–11.6)	91 (3.4)	(2.7–4.1)	539 (20.2)	(18.7–21.7)
Men	2 001	(43)	277 (13.8)	(12.3–15.3)	78 (3.9)	(3.1–4.8)	283 (14.1)	(12.6–15.6)
Women, age (yrs)								
15–19	971	(36)	126 (13.0)	(10.9–15.1)	31 (3.2)	(2.1–4.3)	252 (26.0)	(23.2–28.8)
20–24	828	(31)	109 (13.2)	(10.9–15.5)	42 (5.1)	(3.6–6.6)	182 (22.0)	(19.2–24.8)
25–29	332	(12)	25 (7.5)	(4.7–10.3)	10 (3.0)	(1.2–4.8)	42 (12.7)	(9.1–16.3)
30–39	348	(13)	11 (3.2)	(1.4–5.1)	5 (1.4)	(0.2–2.6)	37 (10.6)	(7.4–13.8)
40–49	144	(5)	6 (4.2)	(0.9–7.5)	2 (1.4)	(0–3.3)	20 (13.9)	(8.3–19.6)
≥ 50	41	(2)	1 (2.4)	(0–7.1)	1 (2.4)	(0–7.1)	6 (14.6)	(3.8–25.4)
Men. age (yrs)								
15–19	308	(15)	40 (13.0)	(9.2–16.8)	11 (3.6)	(1.5–5.7)	69 (22.4)	(17.7–27.1)
20–24	595	(30)	108 (18.2)	(15.1–21.3)	19 (3.2)	(1.8–4.6)	114 (19.2)	(12.0–26.4)
25–29	395	(20)	65 (16.5)	(12.8–20.2)	17 (4.3)	(2.3–6.3)	44 (11.1)	(1.8–20.4)
30–39	393	(20)	46 (11.7)	(8.5–14.9)	20 (5.1)	(2.9–7.3)	35 (8.9)	(0–18.3)
40–49	207	(10)	15 (7.2)	(3.7–10.7)	8 (3.9)	(1.3–6.5)	12 (5.8)	(0–19.0)
≥ 50	103	(5)	3 (2.9)	(0–6.1)	3 (2.9)	(0–6.1)	9 (8.7)	(0–27.0)

lium and *U. urealyticum* may indicate that there is little emphasis on this issue by those ordering the tests. This is underlined by the fact that in 89 % of the samples positive for *M. genitalium,* analysis of this microbe had not been requested; the results were therefore not reported back to those who ordered the tests.

Undiscovered infections lead to increased risk of transmission of infections within the population. As with *C. trachomatis*, studies have shown that *M. genitalium* can lead to significant sequelae for some patients. In a large study from the USA, Haggerty and colleagues showed that 15 % of women with clinically suspected pelvic inflammatory disease were infected with *M. genitalium*, and a causal link was established between the microbe and endometritis (27). A Swedish study from 2012 showed a strong association between *M. genitalium* infection and pelvic inflammatory disease, as did a recent meta-analysis of mycoplasma infection and female reproductive system diseases (28, 29).

Untreated pelvic inflammatory disease can have consequences such as ectopic pregnancy, chronic pelvic pain, recurrent pelvic inflammatory disease and infertility (14). *M. genitalium* has also been observed attaching to sperm *in vitro*; if large numbers of microbes act in this way, sperm motility may be reduced. Whether this affects male fertility is uncertain (1).

The course of untreated *M. genitalium* infection is little documented, and is difficult to study for ethical reasons as the microbe is regarded as pathogenic. Studies of patients with urethritis who were treated empirically with doxycycline show that persistent mycoplasma infections do occur (30). Some of the mechanisms behind the bacterium's ability to cause persistent infection were revealed in an *in vitro* study of endocervical cells (31).

Since the treatment of chlamydia infection is different from that of mycoplasma, diagnostics for *M. genitalium* will be essential (4, 6). The current study shows that co-infections do occur (Fig. 1) and that proven chlamydia infection does not rule out infection with *M. genitalium*. Treatment of mycoplasma infection is more complex than that of chlamydia as the former microbe has a high mutation rate and rapidly adapts to its surroundings (1). It thus has an extensive ability to develop antibiotic resistance, and the number of treatment options is decreasing. Today a 5-day course of azithromycin is recommended for treatment of *M. genitalium* infection (4).

Genital infections such as urethritis and pelvic inflammatory disease are treated empirically with doxycycline, but this cures only 22–45 % of mycoplasma infections (6). *M. genitalium* was detected in 41 % of men with urethritis who returned after experiencing therapeutic failure with doxycycline (30). Check-ups revealed that these patients subsequently tested negative for *M. genitalium* after treatment with azithromycin and 90 % were by then symptom free. There were similar findings of *M. genitalium* infection (44 %) in women with pelvic inflammatory disease who returned after therapeutic failure (27).

In a recent Norwegian study, macrolide resistance was demonstrated in 48 % of *M. genitalium* infections (32). This means that azithromycin does not cure all cases of mycoplasma infection either. Moxifloxacin is at present the only treatment option in cases of macrolide resistance. This antibiotic may have unpleasant side effects and is unlicensed in Norway (33). The rapid development of antimicrobial resistance could lead to a situation where it is difficult to provide adequate treatment. However, we can prevent the development and spread of resistant strains by checking therapeutic efficacy after 3–4 weeks and by including resistance

testing as part of the diagnostic process (6, 34).

Of the microbes examined in this study, *U. urealyticum* was the most prevalent (17.6 %) and was most common in those aged 15–24 years. A study of students in Northern Norway yielded results similar to ours, although prevalence there was generally lower. *C. trachomatis* was found in 3.5 % of those tested, *M. genitalium* in 0.9 % and *U. urealyticum* in 8.7 % (15).

In clinical trials, the high prevalence of *U. urealyticum* in control groups can make results difficult to interpret (16). Nevertheless, a causal link between *U. urealyticum* and urethritis has been shown in young patients and those with few lifetime sexual partners. This may suggest that repeated exposure to the bacterium can lead to tolerance and asymptomatic infection without inflammation (2, 3). Conditions in the host and variable virulence thus appear to play a part in the microbe's pathogenicity. This forms a basis for testing patients with symptomatic urethritis for *U. urealyticum* if no other agents are detected.

In contrast to chlamydia, genital mycoplasma infections are not defined as infectious diseases of danger to public health in the Infectious Disease Control Act. *M. genitalium* does not cause merely a harmless «itch»; it can give rise to infections with serious consequences (1, 13, 29). Nevertheless, it is difficult to estimate how often the microbe migrates to the upper genital tract. The majority of cases of pelvic inflammatory disease appear to be asymptomatic and few patients seek treatment (13, 14, 35). In addition, samples are rarely taken from the upper genital tract due to its limited accessibility (12, 13), and study populations are therefore often small. Studies of pelvic inflammatory disease often rely on detection of pathogens in the cervix. Although detected indirectly, it is likely that these microbes can also cause pelvic inflammatory disease, in common with *C. trachomatis* (14, 29). From prospective studies, it is estimated that approximately 15 % of cases of untreated chlamydia develop into pelvic inflammatory disease, whereas the risk seems to be somewhat lower with mycoplasma infection (35).

The symptoms of mycoplasma and chlamydia infection cannot be distinguished, but *M. genitalium* seems to produce symptoms more often (7). We believe that *M. genitalium* should be included as a standard test when there are symptoms of urethritis and cervicitis, and in pelvic inflammatory disease in women (10, 13, 36). There may also be an indication for testing women prior to surgical abortion (35). Several studies show that *M. genitalium* is transferred to the partner in 55–70 % of cases (7, 9, 30), and partner therapy and possibly contact tracing should be considered.

Guidelines on antibiotic use in the primary healthcare service recommend the testing of patients with symptoms (4). Testing of asymptomatic individuals is, however, more difficult. In 40–60 % of cases, patients with *M. genitalium* do not have symptoms (10, 11, 25). Asymptomatic carriage is an epidemiological problem and without opportunistic screening, prevalence will increase. Laboratories in Norway unofficially report increasing numbers of positive samples. In a national retrospective study from Denmark (n = 31 600), a significant increase in *M. genitalium* was seen from 2006 (2.4 %) to 2010 (3.8 %). The increase is considered real, although part of it may be due to methodological improvements (37).

The spread of infection can be reduced by testing individuals upon change of sexual partner, where the individual in question has multiple sexual partners or a partner with a proven infection. Testing for *M. genitalium* in common with *C. trachomatis* may be a natural approach to this preventative work. Before initiating comprehensive screening, the potential consequences – both for society and for the individual – should be examined more closely. Any changes in testing strategy must take into account the increasing antimicrobial resistance and declining therapeutic options.

In Sweden, where doxycycline is conventionally used to treat chlamydia, studies show similar prevalence of *M. genitalium* and *C. trachomatis* (5, 28). When we see an increase both in Sweden and in Denmark, we can expect similar developments in Norway. Surveillance should therefore be established, and clearer guidelines for the testing and treatment of *M. genitalium* are required. Greater knowledge of the prevalence, pathogenesis and treatment of *M. genitalium* and *U. urealyticum* will be important going forward, and more studies should be initiated (38).

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