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Mycoplasma genitalium and sexual behaviour: A cross-sectional study of students in Trondheim, Norway

Graduate Thesis in Medicine

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Trondheim, June 2016

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Abstract

Background. Screening of *Mycoplasma genitalium* is not recommended in Norway. Our objective was to investigate the prevalence of *M. genitalium* and associated sexual habits among students in Trondheim, Norway.

Methods. In total 1392 samples from students, 777 from women and 615 from men, were tested for *Chlamydia trachomatis*, *Mycoplasma genitailum* and *Neisseria gonorrhoeae* by PCR. Men delivered urine samples, and women both urine samples and vaginal swabs. All participants completed questionnaires.

Results. The prevalence of *M. genitalium*, *C. trachomatis* and *N. gonorrhoeae* were 1,9%, 5,1%, and 0,1%, respectively. Both *M. genitalium* and *C. trachomatis* were associated with recruitment site (P=0,021 and P < 0,001) and number of sex partners over the last six months (P=0,041 and P=0,010). *M. genitalium* infection was in addition associated with gender (P=0,003) and *C. trachomatis* infection with age (P < 0,001)**.**

Conclusions. We observed a difference in prevalence according to recruitment site that support selective screening in certain subpopulations. Number of sexual partners last six months could be used to select individuals for screening. Further research is needed to assess if selective screening prevent urogenital and reproductive complications.

Mycoplasma genitalium (*M. genitalium*) is a sexually transmitted pathogen. It is known to cause non-gonococcal urethritis in men (1-3), and urethritis and cervicitis in women (4-8). A meta-analysis published last year assessing *M. genitalium* and female reproductive tract disease concluded that *M. genitalium* infection was associated with an increased risk of cervicitis, PID, preterm birth, spontaneous abortion and female infertility (9).

Symptoms of *M. genitalium* infection in women involve vaginal discharge and dysuria, while in men, dysuria and urethral discharge. Like *Chlamydia trachomatis* (*C. trachomatis*), infection with *M. genitalium* is frequently asymptomatic. Studies have shown asymptomatic infection in 33-77% of infected women and in 27-39% of infected men (2, 10-12).

The detected prevalence of *M. genitalium* varies from 0,3% in an asymptomatic, non-selected population to 19,2% in sexually transmitted infection (STI) clinic attendees (13, 14). In Norway, studies have shown a *M. genitalium* prevalence of 4% among women and 3,7% among men attending a STI clinic, while a lower prevalence (2%) has been detected in samples from primary care, and in a non-clinical setting among students (1,1%) (7, 12, 15, 16). A recent study in Norway included 4665 samples received by the laboratory for testing of *C. trachomatis* from both primary care and specialists, and here a prevalence of 3,6% for *M. genitalium* was found (17).

In Norway, *M. genitalium* screening is not recommended in asymptomatic individuals (18). This does not correspond with the recommendations for testing in the 2016 European guidelines of *M. genitalium* infection (19). Studies have shown that young age, African ethnicity, anal intercourse, number of sex partners, douching, and smoking are positively associated with *M. genitalium* infection (6, 20-22).

Treatment of *C. trachomatis* and *M. genitalium* infection differs. First choice of treatment of *C. trachomatis* infection is doxycycline, while *M. genitalium* infection is treated with azithromyzin 500 mg on day 1 followed by 250 mg the next 4 days. Research has shown widespread macrolide resistance in *M. genitalium* strains, which complicates the treatment further (23). The Norwegian Communicable diseases control act includes *C. trachomatis* while *M. genitalium* is not mentioned. Consequently treatment of *M. genitalium* infection is not for free.

The aim of this study was to investigate the prevalence of *M. genitalium* among students in Trondheim, Norway, and to look for associations between sexual habits and *M. genitalium* infection. Preferably the results can help to identify individuals that should be tested for *M. genitalium* regardless of symptoms.

Material and methods

Sampling

Norwegian University of Science and Technology (NTNU) has 33 000 students in Trondheim. The students' origin is from all over Norway. Our study population was recruited among students in two ways. 1) Since February 2014 students attending STI testing days at campus were asked to participate in our study. STI testing at campus is a collaboration between medical students at the Faculty of Medicine, NTNU, and St. Olavs University Hospital, Trondheim. In total, six testing days were arranged from February 2014 to February 2016, alternating between the two main campuses of NTNU in the city. 2) Students attending the weekly Student Health Center for sexual health were from January 2015 to February 2016 randomly recruited by a medical student to join the study. Information was given orally and printed, and participants gave their written consent.

Men delivered a first void urine sample and women were asked to deliver both a urine sample and a self-taken vaginal swab (flocked swab in 2 ml Universal Transport Medium, UTM™ (Copan Italia S.P.A., Brescia, Italy). Medical students demonstrated the sampling method for the participants, and instructions with pictures and informative text were available at the toilets. The participants filled out a questionnaire asking for age, gender, genital symptoms, and sexual habits during the last six months. The specific genital symptoms in the questionnaire included itching, warts, sore, blisters, and abdominal pain in addition to symptoms of urethritis and cervicitis (discharge, dysuria). Questions of sexual habits included gender of partners, number of partners, type of sex, and condom use according to type of sex.

Tests

All samples were tested by PCR at the Department of Medical Microbiology, St. Olavs Hospital, Trondheim, Norway. DNA was extracted from 1 ml urine and 200µl transport medium from swabs using NucliSENS® easyMAG® (bioMérieux SA, Marcy l'Etoile, France). After November 2014 the FTD Urethritis basic kit (Fast-track diagnostics Ltd, Esch-sur-Alzette, Luxembourg) for the simultaneous detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *M. genitalium* was used. Both methods included an internal control to detect inhibition.

Follow up

Positive tests from sites were followed up by the STI clinic at St. Olavs Hospital and the Student Health Center respectively. Treatment of *M. genitalium* infection was free for the participants.

Ethics

The study was approved by The Regional Ethical Committee of Middle Norway (REK Midt-Norge, nr 2013-753 and 2014-1728).

Statistics

Data were analyzed using SPSS 23.0 for Mac. 105 students delivered more than one sample during the study period. For the estimation of prevalence we included both samples from the same student if they occurred with a three-month or longer interval. In the tests of association we included both samples if they occurred with six-month interval because we asked for behavior the last six months in the questionnaire. The second test was consistently excluded. Associations between *M. genitalium* infection and recruitment sites, demographics and sexual habits were analyzed using the Chi-square test or, Fisher's exact test. The use of multivariate logistic regression models was discussed but not performed due to the low number of *M. genitalium* cases.

Results

A total of 1277 students agreed to participate in the study and delivered 1392 specimens for testing. During the study period 94 individuals participated two times, nine three times, and one four times. 1065 specimens were delivered from campus, and 327 specimens from the Student Health Center. The characteristics of the study population in total is shown in Table 1.

Table 1 Study population characteristics according to recruitment site and gender. N(%)

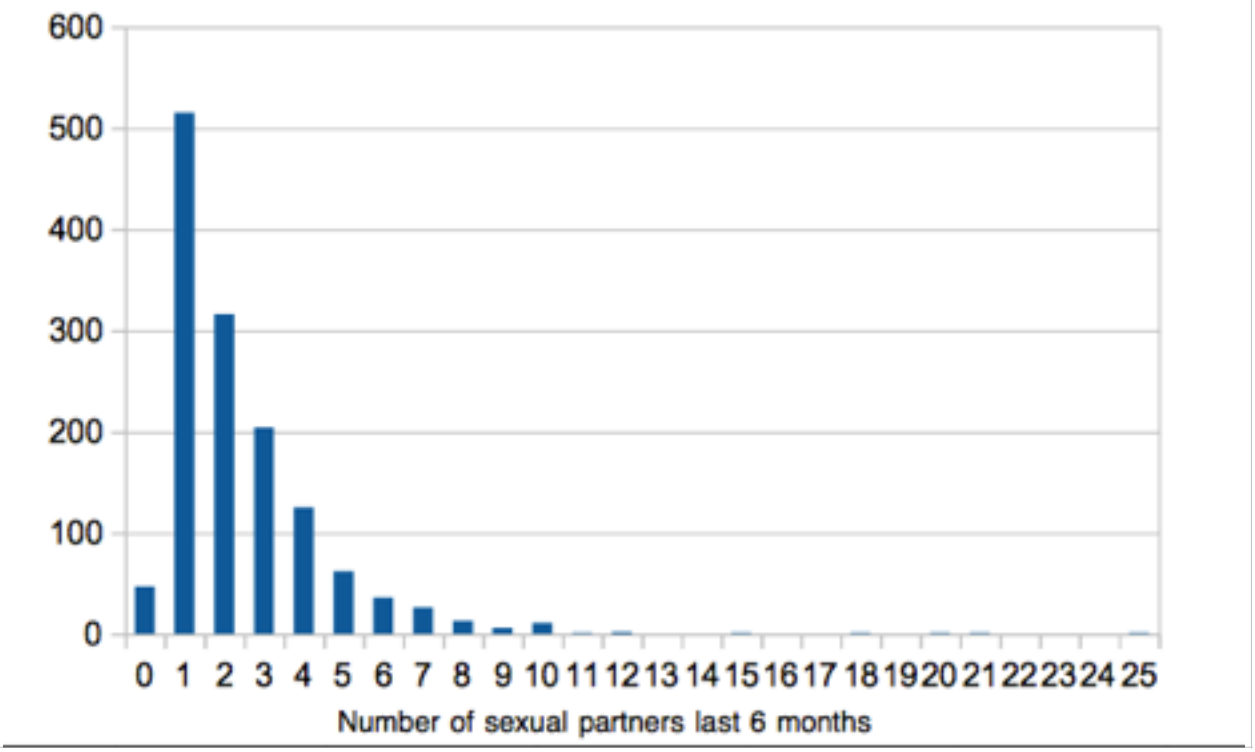
Characteristics	Total N=1392	Campus N=1065	Student Health Center N=327	Female N = 777	Male N= 616
Age Median (25 perc, 75 perc)	22 (21, 24)	22 (21, 24)	22 (20, 23)	22 (20, 23)	22 (21, 24)
< 20	90 (6,5)	72 (6,8)	18 (5,5)	68 (8,8)	22 (3,6)
20-21	518 (37,2)	379 (35,6)	139 (42,9)	304 (39,1)	214 (34,8)
22-23	431 (31,0)	330 (31,0)	101 (30,9)	230 (29,6)	201 (32,7)
24-25	238 (17,1)	186 (17,5)	52 (15,9)	125 (16,1)	113 (18,4)
> 25	115 (8,3)	98 (9,2)	17 (5,2)	50 (6,4)	65 (10,6)
Unknown	0				
Gender					
Female	777 (55,8)	587 (55,1)	190 (58,1)	-	-
Male	615 (44,2)	478 (44,9)	137 (41,9)		
Unknown	0				
Reported symptoms					
Yes	357 (25,8)	266 (25,1)	91 (28,1)	266 (25,1)	102 (16,6)
No	1025 (74,2)	792 (74,9)	233 (71,9)	792 (74,9)	511 (83,4)
Unknown	10				
Number of partners last 6 months median (P25, P75, P90),	2 (1, 3, 5)	2 (1, 3, 4)	3 (2, 4, 6)	2 (1, 3, 4)	2 (1, 4, 6)
0	47 (3,4)	47 (4,5)	0 (0,0)	18 (2,4)	0 (0,0)
1	515 (37,6)	447 (42,7)	68 (21,1)	318 (41,7)	68 (21,1)
2	316 (23,1)	237 (22,7)	79 (24,5)	187 (24,5)	79 (24,5)
3	204 (14,9)	140 (13,4)	64 (19,8)	111 (14,6)	64 (19,8)
> 3	287 (21,0)	175 (16,7)	112 (34,7)	128 16,8	112 (34,7)
Unknown	23				
Gender partner last 6 months					
Male	-	-	-	716 (92,5)	16 (2,6)
Female				26 (3,4)	562 (92,0)
Both				15 (1,9)	6 (1,0)
None				17 (2,2)	27 (4,4)
Condom use vaginal intercourse last 6 months					
Never	457 (35,4)	348 (35,8)	109 (34,1)	265 (36,3)	109 (34,1)
Seldom	445 (34,5)	308 (31,7)	137 (42,8)	251 (34,3)	137 (42,8)
Often	285 (22,1)	223 (23,0)	62 (19,4)	154 (21,1)	62 (19,4)
Always	104 (8,1)	92 (9,5)	12 (3,8)	61 (8,3)	12 (3,8)
Unknown	101				

Table 2 shows number and characteristics of reported symptoms, and Figure 1 shows the distribution of number of sexual partners last six months.

Table 2 Number and characterisitc of reported genital symptoms among participants with symptoms.

	N (%)
Number of symptoms, N= 353	
1 symptom	244 (69,1)
> 2 symptoms	109 (30,9)
Type of genital complaints, N= 496	
Discharge	173 (34,9)
Dysuria	58 (11,7)
Rash	18 (3,6)
Itch	105 (21,2)
Sore, blisters	15 (3,0)
Wart	21 (4,2)
Abdominal pain	53 (10,7)
Other complaints	53 (10,7)

Figure 1 Number of sexual partners last 6 months.



We excluded three specimens from patients who had > 2 tests during a three-month period from the estimation of prevalence. Out of 1389 specimens, 26 tested positive for *M. genitalium* (1,9%), 71 for *C. trachomatis* (5,1%) and one for *N. gonorrhoeae* (0,1%). Among the *C. trachomatis* positive specimens, two also tested positive for *M. genitalium* and one for *N. gonorrhoeae*. The prevalence of the three microorganisms according to recruitment location and gender is shown in Table 3.

Table 3 Prevalence of microorganisms according to recruitment site and gender, N (%).

Microorganism	Total N=1389	Campus N=1064	Student Health Center N=325	Female N=776	Male N=613
M. genitalium	26 (1,9)	15 (1,4)	11 (3,4)	22 (2,8)	4 (0,7)
C. trachomatis	71 (5,1)	39 (3,7)	32 (9,8)	42 (5,4)	29 (4,7)
N. gonorrhoeae	1 (0,1)	0 (0,0)	1 (0,3)	1 (0,1)	0 (0,0)

Association of positive tests for *M. genitalium* and *C. trachomatis* to recruitment site, demographics and sexual habits in the total study population are shown in Table 4. 32 specimens were excluded from patients who had > 2 tests during a six-month period. Location of recruitment and the number of sexual partners during the last six months were significantly associated with both organisms. Gender was significantly associated with *M. genitalium* infection and age with *C. trachomatis* infection. Symptoms and condom use were not significantly associated with either of the microbes.

Table 4 Associations between population characteristics and *M. genitalium* and *C. trachomatis* infection.

	M. genitalium			***C. trachomatis***		
Characteristic	**Neg N=1334**	**Pos N=26**	**p value**	**Neg N=1291**	**Pos N=69**	**p value**
Location						
Campus	1028	15 (1,4%)	0,021	1005	38 (3,6%)	< 0,001
Student health centre	306	11 (3,5%)		286	31 (9,8%)	
Gender						
Female	742	22 (2,9%)	0,003	723	41 (5,4%)	0,577
Male	592	4 (0,7%)		568	28 (4,7%)	
Age						
< 20	87	1 (1,1%)	0,359	74	14(15,9%)	< 0,001
20-21	493	10 (2,0%)		476	27 (5,4%)	
22-23	411	12 (2,8%)		405	18 (4,3%)	
24-25	230	3 (1,3%)		226	7 (3,0%)	
> 25	113	0 (0,0%)		110	3 (2,7%)	
Reported symptoms						
Yes	349	8 (2,2%)	0,477	332	25 (7,0%)	0,064
No	1008	17 (1,7%)		979	46 (4,5%)	
Number of partners last 6 months						
None	47	0 (0,0%)	0,041	47	0 (0,0%)	0,010
1	506	4 (0,8%)		500	10 (2,0%)	
2	299	7 (2,3%)		289	17 (5,6%)	
3	190	8 (4,0%)		186	12 (6,1%)	
> 3	269	7 (2,5%)		247	29 (10,5%)	
Unknown: 23						
Condom use vaginal intercourse last 6 months						
Never	437	7 (1,6%)	0,112	417	27 (6,1%)	0,053
Seldom	426	7 (1,6%)		406	27 (6,2%)	
Often	269	10 (3,6%)		267	12 (4,3%)	
Always	103	0 (0,0%)		103	0 (0,0%)	
Unknown: 101						

Discussion

In this study we estimated the prevalence of *M. genitalium* and *C. trachomatis* among students that were positive to STI testing in Trondheim, and identified behavioral risk factors for infection. The prevalence of infection was 1,9% for *M. genitalium* and 5,1% for *C. trachomatis*. Both infections were associated with recruitment location and the number of sexual partners over the last six months. *M. genitalium* infection was in addition associated with gender and *C. trachomatis* infection with age.

We found a low prevalence of *M. genitalium* (1,9%) in the total study population. This is similar to earlier published studies in Norway outside STI clinics (15, 16). Jensen et al. found a lower prevalence of 1,1% among students in northern Norway (16). This could be due to different sampling strategies. We asked of participation after the students had agreed to test themselves, while Jensen et. al invited the students to participate in the study first. Consequently our study population is more selected, and does not represent the general student population in Trondheim.

The prevalence of both *M. genitalium* and *C. trachomatis* were significantly higher among students attending the Student Health Center than those tested at campus, 3,5% vs 1,4% and 9,8% vs 3,7%, respectively. These findings correspond well with the characteristics of the two groups. A higher proportion of the Student Health Center attendees were younger, female, had symptoms and had > 3 sexual partners. All had sexual partners during the last six months and fewer always used condoms compared to campus attendees. The differences between the recruitment sites reflect that the prevalence varies according to which population you study.

As expected, the number of sexual partners in the last six months was associated with both *M. genitalium* and *C. trachomatis* infection. *M. genitalium* has previously been found to be associated with increasing number of partners last year (24-27). Andersen et al. found an association between *M. genitalium* and number of partners the last six months (24). The European guidelines of 2016 recommend testing of individuals with more than five lifetime partners and that never have been tested before, or of individuals with more than three sexual partners the last year (19). Our findings support the importance to inquire for number of sexual partners as a tool to select individuals for screening.

The use of condom was not significantly associated with the prevalence of either of the microbes. For *C. trachomatis* there is a tendency of an increasing infection rate by less frequent condom use (Table 4). A similar tendency is lacking for *M. genitalium*, possibly due to the low number of cases. Besides, recall bias and social expectations could have given an over-reporting of the condom use. Furthermore, we found no infections among individuals who always uses condom, supporting its preventive effect. The lack of association with symptoms and infection in this study was expected due to the broad range of symptoms the participants were asked to report.

It was easier to recruit female students than male. In our study population 55,8% was females and 44,2% males. Other studies show a similar tendency (28). A slightly higher proportion of male participants tested at campus suggest that a more outreaching strategy could help in recruiting young males for STI testing.

Unexpectedly, only four men were *M. genitalium* positive compared to 22 females. Gesink et al. reported female gender as a p1414redictor of *M. genitalium*-positivity (28). The opposite was reported by Salado-Rasmussen et al., they found a greater proportion of *M. genitalium*-positivity among men (29). In their study the positive rate was highest among the 25-30 year old men, and among the 20-25 year old women. Our population includes only a small proportion of individuals over 25 years, this could explain the gender difference. In addition our group of men were more likely to be tested at campus and fewer had symptoms compared to the women. However they also had a larger number of sexual partners and a smaller proportion that always used condom. The few number of *M. genitalium* cases made it not advisable to use multivariate logistic regression to investigate the difference further.

Some limitations to our study need to be addressed. First, the study population was a selection of mainly 20-25 year old, well informed students that were positive to testing for STIs. *M. genitalium* infection is associated with this age group (17), and thus, the prevalence in this study is not generalizable, although the study could applicate to similar groups. Second, a number of individuals had more than one sample included in the study. These were assumed to be independent, thus we did not account for a possible dependency.

Conclusion

Overall, we found a low prevalence of *M. genitalium* among students in Trondheim. The higher prevalence among students tested at the Student Health Center show that selective screening could be appropriate in certain subpopulations. Our study confirmed that the number of sexual partner is a risk factor for *M. genitalium* infection, and this fact could be used to select individuals for screening. Further research is needed to assess if selective screening prevents urogenital and reproductive complications.

Acknowledgments

We wish to thank all the medical students who contributed to carry out the testing days at campus, the employees at the Student Health Center and St. Olavs University Hospitals STI clinic and the Department of Medical Microbiology, Øyvind Mikkelsen and Turid Follestad for their assistance and support throughout this project. This study was supported by financial contribution from the Norwegian University of Science and Technology and St. Olavs University Hospital.

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