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Burden of *Mycoplasma genitalium* and Bacterial Coinfections in a Population-Based Sample in New Mexico

Anne Hammer, PhD,†‡ Patti E. Gravitt, PhD,§ Rachael Adcock, PhD,¶ Nicole Patterson, BS,‖ Jack Cuzick, PhD,¶ and Cosette M. Wheeler, PhD,‖***
on behalf of the New Mexico HPV Pap Registry Steering Committee

Abstract: In this population-based US study, the overall prevalence of *Mycoplasma genitalium* was 1.95% (95% confidence interval [CI], 1.62%–2.34%), declining from 6.12% (95% CI, 4.72%–7.92%) in women aged 21 to 24 years to 0.48% (95% CI, 0.25%–0.94%) in women aged 40 to 64 years. The prevalence of coinfections with *Chlamydia trachomatis* and *Trichomonas vaginalis* was low.

Sexually transmitted infections (STI) are a significant burden of disease in the United States, with an estimated 19.7 million incident infections in 2008.[1] Although most STIs are asymptomatic or cause transient inflammation, some STIs, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, may be associated with significant reproductive harms.[2,3]

Accumulating evidence suggests that *Mycoplasma genitalium*, a common cause of male urethritis,[4] may be associated with cervicitis, endometritis, pelvic inflammatory disease, and possibly tubal factor infertility in women.[4–8] Although screening for *C. trachomatis* may effectively prevent pelvic inflammatory disease (PID) and subsequent infertility,[9] the benefit of screening high-risk women for *M. genitalium* remains unclear and controversial.[8] This may be because of limited knowledge on the potential reproductive harms associated with an asymptomatic infection, and because the burden of *M. genitalium* infections at the population level in the United States remains unclear. We therefore aimed to describe the overall and age-specific prevalence of *M. genitalium*, and the proportion of cases coinfected with other bacterial STIs in a population-based sample.

METHODS

We conducted a population-based, cross-sectional study using liquid-based cytology (LBC) residuals, with selections performed through collaboration with the New Mexico HPV Pap Registry (NMHPVPR).

From the *Department of Obstetrics and Gynecology, Gødstrup Hospital, Herning; †Department of Clinical Medicine, Aarhus University, Aarhus N; ‡Department of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus, Denmark; §Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD; ¶Wolfson Institute of Preventive Medicine, Queen Mary University of London, Charterhouse Square, London, United Kingdom; and Departments of ‖Pathology and **Obstetrics and Gynecology, University of New Mexico Health Sciences Center, MSC02-1670 House of Prevention Epidemiology, Albuquerque, NM

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A.H. and P.E.G. contributed equally to this work.

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Correspondence: Cosette M. Wheeler, PhD, Departments of Pathology and Obstetrics & Gynecology, University of New Mexico Health Sciences Center, MSC02-1670 House of Prevention Epidemiology, Albuquerque, NM 87131. E-mail: cwheeler@salud.unm.edu.

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Samples were obtained from 3 volunteering that who perform cervical screening tests on approximately 70% of the women receiving cervical cancer screening statewide (80% screening coverage[10]). After routine clinical testing, discarded LBC residuals were deidentified and subsequently stored at 4°C until testing. Using the NMHPVPR data resource, an age- and cervical cytology–stratified random sample of residual LBC specimens was selected for STI analysis (Table 1). This study was deemed exempt from approval by the University of New Mexico Human Research Review Committee.

The study population consisted of women who attended cervical cancer screening in New Mexico from August 1, 2013, to July 31, 2014. The sampling design was set up with the intention of oversampling younger women and women with abnormal cervical cytology to allow for the future estimation of cervical intraepithelial neoplasia risk associated with prevalent STIs (Table 1). Women were eligible for final analysis if they were aged 21 to 64 years and had valid test results for *C. trachomatis*, *M. genitalium*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and human papillomavirus (HPV).

From each LBC sample, we removed 1 mL and placed in an Aptima specimen transport kit (Hologic, cat. no. 301154c) according to the in vitro diagnostics product insert. Samples subsequently underwent testing at our University of New Mexico research laboratory for *C. trachomatis* and *N. gonorrhoeae* using the US Food and Drug Administration (FDA)–approved Aptima Combo2 assay (Hologic), for *T. vaginalis* using the FDA-approved Aptima *T. vaginalis* assay (Hologic), and for HPV using the FDA-approved Aptima HPV assay (Hologic). Testing for *M. genitalium* was performed using a prototype of the Aptima *M. genitalium* assay (Hologic), which received FDA approval January 23, 2019. All STI analyses were performed on the Panther platform, a fully automated system, following the manufacturer's instructions. Results were recorded as positive, negative, invalid, or equivocal (*C. trachomatis* only). Invalid test results were excluded from final analysis as missing (n = 297). Equivocal results on *C. trachomatis* (n = 1) were considered positive.

We calculated weighted population prevalence estimates of *M. genitalium*, including 95% confidence intervals (CIs), overall and by age (21–24, 25–30, 31–34, 35–39, and 40–64 years; Table 1). Estimates were calculated by weighting back to 84,686 women with an available LBC residual. Weighted logistic models were fit to test for trend in prevalence with increasing age. The sampling fractions and weights (inverse of the sampling fractions) were adjusted to reflect missing data. In addition, we calculated the prevalence of pairwise coinfections between *M. genitalium* and *C. trachomatis* and between *M. genitalium* and *T. vaginalis*. Analyses were conducted in Stata version 13 (StataCorp. College Station, TX) using the Survey (SVY) commands.

RESULTS

A total of 84,686 residual LBC samples from New Mexico laboratories were available for testing, from which 7867 (9.3%) were originally selected for STI analysis using stratified random sampling (Table 1). From these, we excluded samples from women outside of the age range for routine cervical screening <21 (n = 297) and >64 years (n = 305) and samples with incomplete STI panel results (missing sample aliquots [n = 111], invalid test runs with one or more STI panel [n = 217]), leaving 7057 with complete test results in the final analysis.

The overall weighted population prevalence of *M. genitalium* was 1.95% (95% CI, 1.62%–2.34%), with significant differences by age group. The prevalence was highest in women aged 21 to 24 years (6.12%; 95% CI, 4.72%–7.92%) and declined steadily as women aged, to 0.48% (95% CI, 0.25%–0.94%) in women aged 40 to 64 years ($P_{trend} < 0.0001$; Table 2). Overall, the prevalence of coinfections was low (0.25% for *M. genitalium* and *C. trachomatis* and 0.29% for *M. genitalium* and *T. vaginalis*). Highest rates of *M. genitalium* and *C. trachomatis* coinfection were in women aged 21 to 24 years (1.26%; 95% CI, 0.70%–2.27%) and declined significantly with increasing age ($P_{trend} = 0.003$; Table 2). Similarly, highest rates of *M. genitalium* and *T. vaginalis* coinfection were in women

TABLE 1. Sampling Fractions, Intended and Adjusted for Missing Values

	Intended Sampling Design					Actual Sampling Design*				
		Intended								
Cytology	Age, y (15 Strata)	Sample	Population	Sampling Fraction, %	Sampling Weight	Age, y (13 Strata)	Actual Sample	Adjusted Population†	Sampling Fraction, %	Sampling Weight
Normal	15–20	113	1529	7.39	13.53					
	21–24	683	7589	9.00	11.11	21–24	653	7589	8.60	11.62
	25–30	1110	12472	8.90	11.24	25–30	1,071	12472	8.59	11.65
	31–34	334	8350	4.00	25.00	31–34	318	8350	3.81	26.26
	35–39	356	8476	4.20	23.81	35–39	347	8476	4.09	24.43
	40–44	333	8538	3.90	25.64	40–44	323	8538	3.78	26.43
	45–49	322	8474	3.80	26.32	45–49	308	8474	3.63	27.51
	50–54	356	9368	3.80	26.31	50–54	343	9368	3.66	27.31
	55–59	329	8436	3.90	25.64	55–59	316	8436	3.75	26.70
	60–64	267	6846	3.90	25.64	60–64	251	6846	3.67	27.27
	65+	201	5289	3.80	26.31					
Total normal		4404	85,367				3930	78,549		
ASCUS	All	2312	4624	50.00	2	21–64	2,124	4396	48.32	2.07
LSIL	All	732	1464	50.00	2	21–64	635	1348	47.11	2.12
ASC-H	All	223	223	100.00	1	21–64	199	209	95.22	1.05
HSIL	All	196	196	100.00	1	21–64	170	184	92.39	1.08
Total abnormal		3463	6507				3128	6137		
Total		7867	91,874				7058	84,686		

The table summarizes details on the intended and actual sampling design fractions and resulting weights used in the analysis to estimate population-level statistics. Actual sampling design weights in the last column were used.

*Actual sampling design adjusts for missing data.

†Population estimates as supplied by the NMHPVPR adjusted to reflect routine screening age.

TABLE 2. Weighted Prevalence Estimates of *Mycoplasma genitalium* and *Chlamydia trachomatis* and *Trichomonas vaginalis* Coinfections Among Women Aged 21 to 64 Years Attending Routine Cervical Cancer Screening in New Mexico

Age, y	n	Mycoplasma genitalium Prevalence, % (95% CI)	Coinfection Prevalence, % (95% CI)	
			Chlamydia trachomatis	*Trichomonas vaginalis*
All	84,686	1.95 (1.62–2.34)	0.25 (0.15–0.43)	0.29 (0.18–0.47)
21–24	8765	6.12 (4.72–7.92)	1.26 (0.70–2.27)	0.78 (0.37–1.66)
25–30	13,916	3.65 (2.78–4.77)	0.12 (0.03–0.49)	0.66 (0.35–1.23)
31–34	9069	2.72 (1.50–4.87)	0.59 (0.15–2.28)	0.05 (0.01–0.18)
35–39	9180	1.61 (0.78–3.32)	0.05 (0.01–0.18)	0.27 (0.04–1.87)
40–64	43,756	0.48 (0.25–0.94)	0.06 (0.01–0.43)	0.13 (0.03–0.48)

aged 21 to 24 years (0.78%; 95% CI, 0.37%–1.66%), with declining rates as age increased ($P_{trend} = 0.012$).

DISCUSSION

In this population-based study, we assessed the weighted population prevalence of *M. genitalium* among women aged 21 to 64 years, attending routine cervical cancer screening in New Mexico. We found an overall *M. genitalium* prevalence of 1.95%, with highest rates among women aged 21 to 24 years (6.12%). The majority of *M. genitalium* infections across all ages occurred as single infections.

The observed prevalence of *M. genitalium* was more than 2-fold higher than the prevalence reported in a previous population-based US study in which asymptomatic individuals aged 18 to 27 years were tested (2.0% vs. 0.8%),[11] although the latter test used different tests and sample types, which may have affected their test sensitivity relative to more recently validated tests. The prevalence in our study was much lower than prevalence rates based on individuals with symptoms or seeking care at STD clinics (2.0% vs. up to 26%).[12–14] The high *M. genitalium* prevalence in the present study, particularly among the youngest women, is concerning because these estimates may reflect a significant number of asymptomatic women. If these infections remain undetected and untreated, these women may not only be at risk for future disease themselves but may also provide a reservoir for further transmission.

Among women aged 21 to 24 years, the prevalence of *M. genitalium* was 2-fold higher than the prevalence of *C. trachomatis* reported in the 2007–2012 National Health and Nutrition Examination Survey (6.1% vs. 2.9%),[15] but similar to the *C. trachomatis* prevalence in the present study (6.12% vs. 6.22%). Whereas efforts are made to reduce the burden of *C. trachomatis* and associated disease by screening individuals,[16] no efforts are currently in place to reduce the burden of *M. genitalium*, despite that screening for STI may be efficacious at prevalence rates greater than 3.1%.[17] Screening and treatment of *C. trachomatis* are associated with a lower risk of PID and infertility,[2,9,18] but screening for *M. genitalium* remains controversial, mainly because PID is more commonly attributed to *C. trachomatis* than *M. genitalium*.[8] However, these estimates were based on much lower prevalence rates. Well-designed prospective studies are needed to assess the potential impact of screening for *M. genitalium* on reproductive harms.[19]

The relatively low rate of coinfections between *M. genitalium* and *C. trachomatis* may be partially explained by treatment effects on *M. genitalium* in women with screen-detected *C. trachomatis* infections. This may also explain the reported increase in macrolide resistance for *M. genitalium* over time, up to 42% to 69% in the United States, with an increased risk of resistance among individuals with coinfections and with important differences by sex, race, and sexual orientation.[14,20–23] To reduce the burden of *M. genitalium* and risk of treatment failure, increased awareness of this emerging pathogen and correct diagnostics are critical. Routine testing for *M. genitalium* should be considered when women present with symptoms or clinical evidence of infection, particularly in case of treatment failure. Testing may also be considered before invasive procedures among women at high risk for being infected, such as women undergoing surgical abortion, as this would allow for adequate diagnostics and treatment, which subsequently may reduce risk for postoperative complications, including PID.[24] This may be critical, as the prophylaxis used in conjunction with surgical abortion (i.e., doxycycline)[25] does not confer antimicrobial eradication of *M. genitalium*.

Our sampling frame reflects women attending cervical screening in New Mexico. Although we believe that this provides a stronger population-based sample compared with clinic-based data, it cannot be taken as a true population-based sample. Prevalence rates may be underestimated, as women not attending cervical cancer screening may have a higher prevalence of STI because of lower access to care and, possibly, shared risk factors. On the other hand, prevalence rates may be overestimated, as women undergoing screening may have been screened because of symptoms. Finally, we cannot rule out that rates of *C. trachomatis* and *M. genitalium* coinfections may have been underestimated, as we have no knowledge on previous testing and treatment of *C. trachomatis*.

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