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Differential association of ureaplasma species with non-gonococcal urethritis in heterosexual men

Raphael O Ondondo^{1,2,3}, William L H Whittington², Sabina G Astete², and Patricia A Totten^{1,2}

¹Department of Global Health, Pathobiology Interdisciplinary Program, University of Washington, Seattle, Washington, USA

²Department of Medicine, Division of Infectious Diseases, University of Washington, Seattle, Washington, USA

³Kenya Medical Research Institute, Center for Microbiology Research, Research Care and Training Program, Kisumu, Kenya

Abstract

Objective—To assess the role of *Ureaplasma urealyticum* and *Ureaplasma parvum* in patients with non-gonococcal urethritis (NGU) using specimens from a previously reported study of NGU.

Methods—Species-specific PCR assays for *U urealyticum* and *U parvum* were used to detect these organisms in specimens from men enrolled in a case-control study based in a Seattle STD clinic in order to evaluate their association with NGU. Urethritis was defined by clinical examination and the presence of inflammation on Gram stained smear. Controls had normal examination findings and no evidence of inflammation on Gram stain smear or by the leucocyte esterase test.

Results—*U urealyticum* was detected in 26% (31/119) of cases and 16% (19/117) of controls, resulting in an association with NGU (adjusted odds ratio (aOR)=2.3, 95% CI 1.04 to 4.9) after adjusting for age, race, history of prior urethritis and other NGU pathogens (*Chlamydia trachomatis*, *Mycoplasma genitalium*). The association of *U urealyticum* and NGU was strongest in white men <28 years of age (OR=5.4, 95% CI 1.3 to 22.2). *U parvum* was detected in 14% (17/119) cases and 31% (36/117 controls) and thus was negatively associated with NGU (aOR=0.4, 95% CI 0.2 to 0.8). The prevalence of *U urealyticum* (16%) in controls was higher than that of *C trachomatis* (3.4%) or *M genitalium* (4.3%, $p<0.05$, each comparison).

Correspondence to Dr Patricia A Totten, Division of Infectious Diseases, Department of Medicine, University of Washington, Harborview Medical Center, Box 359779, 325 9th Ave, Seattle, WA 98104, USA; patotten@u.washington.edu.

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Competing interests None.

Ethics approval This study was conducted with the approval of the University of Washington.

Contributors Study concept and design: WHLW and PAT; acquisition of data: WHLW, PAT, SGA and ROO; analysis and interpretation of data: ROO, WHLW, SGA and PAT; drafting of manuscript, ROO, WHLW, and PAT; statistical analysis: ROO, WHLW and PAT.

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Conclusions—Unlike *U parvum*, *U urealyticum* was associated with urethritis. The strong effect in younger white men and high rates in controls may suggest variability in virulence among *U urealyticum* strains or in host innate or acquired immunity.

INTRODUCTION

Urethritis, characterised by urethral inflammation, urethral discharge and dysuria is one of the most common sexually transmitted syndromes in men. This syndrome was associated with *Neisseria gonorrhoeae* in the 1800s, with *Chlamydia trachomatis* in the 1970s¹ and with *Mycoplasma genitalium* in the 1990s.²³ Less frequently, this syndrome has also been associated with *Trichomonas vaginalis*⁴ and herpes simplex viruses.⁵ However, the aetiology of 20–50% of cases remains unknown. Among organisms hypothesised to cause urethritis, ureaplasmas are perhaps the most studied and the most controversial.

Initially designated ‘T-strain’ mycoplasmas, ureaplasmas were first identified in urethral specimens in 1954 by their characteristic tiny colonies and the absence of a cell wall.⁶ The subsequent discovery that these bacteria hydrolysed urea led to their designation as a new genus, *Ureaplasma*, comprising only one species, *Ureaplasma urealyticum*.⁷ Findings of studies assessing the association of ureaplasmas with non-gonococcal urethritis (NGU) have been contradictory in that some have reported an association of these bacteria with nonchlamydial NGU,⁸ while others have not,^{9–12} leading to speculation that *Ureaplasma* strains differ in virulence and that these differences might be linked to biovar (of which there are two) or serovar (of which there are 14). Indeed, the ureaplasmas have now been designated as two distinct species, *Ureaplasma parvum* (formally biovar 1) and *U urealyticum* (formally biovar 2) based on whole-cell DNA homology, genome size, serotype grouping, sequence homology and divergence among urease and other selected genes.¹³

To date, four published studies have generated conflicting results on the association of these two species with NGU.^{14–17} However, these studies have differed in their definition of cases and controls, PCR assays used and the use of multivariate analyses to control for confounding. In this study, we used specimens collected during our previous study,⁹ species-specific PCR assays and multivariate analyses to evaluate the role of *U urealyticum* and *U parvum* in NGU.

METHODS

Study population

The study’s case–control population has been previously described⁹ and specimens from the same participants were evaluated in this project. Specimens were available from 119 of the 121 cases and from all of the 117 controls. Heterosexual men aged 16–49 who attended the Public Health, Seattle & King County STD clinic in Seattle Washington, from December 1998 to August 1999 were enrolled. Cases were defined by the presence of urethral discharge on examination and urethral inflammation (≥ 5 polymorphonuclear leucocytes (PMNs)/1000× field on Gram stain smear). Controls were men without complaints of urethral discharge or dysuria, urethral discharge on examination or evidence of urethral inflammation (<5 PMNs/1000× field on Gram stain or negative leucocyte esterase test).

Men diagnosed with primary genital herpes or HIV were excluded as were those with sex partners diagnosed with gonorrhoea, chlamydial infection or trichomoniasis. Specimen collection routines were as previously described.⁹ The study protocol was approved by the University of Washington human subjects committee.

***U urealyticum* and *U parvum* PCR assays**

The *U urealyticum* and *U parvum* PCR assays have been previously described.¹⁸ Positive reactions were defined by the presence of a 474 bp (*U urealyticum*) or 469 bp (*U parvum*) band reacting with the *U urealyticum* and *U parvum* specific probes, respectively, on Southern blots. The *U urealyticum* assay was positive for all *U urealyticum* serovars (2, 4, 5 and 7–13; tested on ATCC strains 27814, 27816, 27817, 27819, 27618, 33175, 33699, 33695, 33696 and 33698, respectively) and negative for all *U parvum* serovars (1, 3, 6 and 14; tested on ATCC strains 27813, 27815, 27818 and 33697, respectively). Similarly, the *U parvum* assay was positive for all *U parvum* serovars and negative for all *U urealyticum* serovars. Both PCR assays were negative for *M genitalium* strain G37 and a clinical isolate of *Mycoplasma hominis*. The analytical sensitivity of each of these assays, determined by serial twofold serial dilutions of measured quantities of DNA from *U urealyticum* serovar 9 and *U parvum* serovar 3, was 10 genome equivalents. These two strains were used as positive and negative controls, as appropriate, in all *U urealyticum* and *U parvum* PCR assays.

The *U urealyticum* and *U parvum* PCR assays were performed on archived specimens maintained at –80°C until testing, using our published procedure,¹⁸ except that the amount of processed specimen used in the PCR assay was 10 µl rather than 5 µl and represented 30 µl of the original patient urine specimen.

Statistical analysis

Analyses were performed using the Statistical Program for the Social Sciences (SPSS, version 17). Dichotomous variables were compared between cases and controls using χ^2 tests. Continuous variables were compared using parametric or non-parametric tests as appropriate. Logistic regression was used to control for variables considered to be important in urethritis based on published literature, hypothesised relationships and results of the univariate analyses.

RESULTS

Study structure and performance of PCR assays

The results of the species-specific PCR assays were compared with the culture results of undifferentiated ureaplasmas obtained in our original study (figure 1).⁹ Of the 126 culture-negative men, four (3.2%) were positive for *U urealyticum*, two (1.6%) were positive for *U parvum* and 120 (95%) were negative by both species-specific assays. Of the 110 culture-positive men, 75 (68%) were positive by one or more of the species-specific assays performed on the urine specimens. To assess the urea-plasma species in the 35 culture-positive/PCR-negative men, *U urealyticum* and *U parvum* PCR assays were performed on the urethral swab specimens from these men, thereby identifying *U urealyticum*, *U parvum*,

or both species in 16 additional urea-plasma-positive men. Among the 19 men initially culture positive and subsequently PCR negative for both *U parvum* and *U urealyticum* from all specimens, eight were cases (6.7% of the 117 cases) and 11 (9.4% of the 119 controls) were controls. The combined results of urethral and urine species-specific PCR assays are used in subsequent analyses.

Characteristics of men by organism detected

By PCR, *U urealyticum* only, *U parvum* only, or both organisms were detected in 46 (19.5%), 47 (19.9%) and four (1.7%) of the 236 participants, respectively (table 1). Men infected with *U urealyticum* alone were more likely to have a current diagnosis of urethritis ($p=0.02$) and less likely to identify themselves as white than were men infected with *U parvum* alone ($p<0.05$, table 1). By organism identified, men did not differ by number of reported lifetime sex partners or by a history of having received fellatio during the past 60 days (table 1).

Association of *U urealyticum* and *U parvum* with urethritis

Men with urethritis (29%) were less likely to identify themselves as white than were controls (68%) and were more likely to report a history of prior urethritis (68% vs 38%, $p<0.01$, each comparison). In addition, men with urethritis were somewhat older than men with without urethritis (mean 29.9 vs 28.2 years, $p=0.06$).

Thirty-one (26.1%) of 119 men with urethritis were positive for *U urealyticum* compared with 19 (16.2%) of 117 men without urethritis ($p=0.08$, OR=1.8, (95% CI 0.96 to 3.4), table 2). The prevalence of *U urealyticum* (16.2%) in controls was higher than that of *C trachomatis* (3.4%) or *M genitalium* (4.3%, $p<0.05$, each comparison). *U urealyticum* positive men in the control group were similar in age, number of sex partners and history of prior urethritis to *U urealyticum* negative controls (data not shown). When controlling for age, racial identity, history of previous urethritis, and established causes of urethritis (*M genitalium* and *C trachomatis*), the association of *Ureaplasma urealyticum* with urethritis was strengthened somewhat (adjusted odds ratio (aOR)=2.3, 95% CI 1.04 to 4.9). The relationship between *U urealyticum* and urethritis was most pronounced among younger (age ≤ 28 years) men who identified themselves as white (33% (6/18) cases and 8.5% (4/47) of controls (OR=5.4 95%, CI 1.3 to 22.2)). Among younger and older men with other racial identifiers, and among older white men, infection with *U urealyticum* was not significantly associated with urethritis (OR for each group ranged from 0.63 to 1.67).

In contrast to the positive association of *U urealyticum* with NGU, *U parvum* was negatively associated with this syndrome, as this organism was detected in 14.3% cases and 29.1% of controls ($p<0.01$, OR=0.4, (95% CI 0.2 to 0.8), table 2). This negative association remained after controlling for age, racial identity and established causes of urethritis (*M genitalium* and *C trachomatis*), (aOR=0.4, 95% CI 0.2 to 0.8). The negative association of *U parvum* with NGU was not significantly different among men stratified by racial identity and age (data not shown).

DISCUSSION

In this case-control study of men with and without NGU, we detected an association of *U urealyticum* with NGU (aOR=2.3, 95% CI 1.04 to 4.90) among heterosexual men attending an STD clinic. In contrast, *U parvum* was negatively associated with NGU (aOR=0.4, 95% CI 0.2 to 0.8). These findings are in contrast to our previous assessment of the qualitative (table 2) or quantitative detection of undifferentiated ureaplasmas by culture, in which no association with NGU was detected.⁹ Our findings of a strong association of *U urealyticum* with NGU among young, white men (OR=5.4, 95% CI 1.3 to 22.2), and the frequent detection of *U urealyticum* in control subjects raise interesting questions about possible differences in pathogenicity between strains and the effect of host factors, including immunity, on development of signs and symptoms of urea-plasma-associated NGU.

Since their initial detection in the 1970s, the association of ureaplasmas with NGU has been controversial; in some studies, these organisms were associated with NGU, in others there was no association. In 1973, McCormack and colleagues¹⁹ found that the detection of undifferentiated ureaplasmas in urine specimens from asymptomatic men in local fraternities was linearly associated with number of lifetime sexual partners, consistent with sexual transmission of a non-pathogenic organism. In a subsequent study of Caucasian men aged <36 years reporting to an STD clinic in Seattle, ureaplasmas were isolated significantly more often and in higher numbers (10^3 colour-changing units) from men complaining of symptoms and signs of nonchlamydial NGU than from men without these findings.⁸ Subsequent studies of men recruited from various STD clinics did not show an association of ureaplasmas with acute NGU, although the association of ureaplasmas with chronic NGU was reported in one study.²⁰

The discovery that the ureaplasmas were composed of two distinct species, designated *U urealyticum* and *U parvum*, allowed the subsequent assessment of their possible differential association with NGU. However, the results of these studies have been contradictory. For example, Povlsen *et al*¹⁷ detected *U urealyticum* in 22 (18%) of 125 cases (defined by 4 PMNs/HPF) and 18 (9%) of 205 controls (<4 PMNs/1000× field, $p<0.05$, our calculations) recruited from a venereal disease clinic in Sweden. Similarly, Deguchi *et al*¹⁶ identified *U urealyticum* among 50 (16%) of 317 men with signs and symptoms of NGU and inflammation defined by 5PMNs/1000× field compared with 11 (8%) of 141 men with no signs or symptoms ($p=0.025$), all recruited from men attending a hospital urology clinic in Japan. Bradshaw *et al*¹⁵ reported no association of *U urealyticum* with urethritis in their study of men reporting to the Melbourne Sexual Health Centre in Australia: *U urealyticum* was detected in 43 (13%) of 329 cases and in 54 (18%) of 307 controls, with an aOR of 0.7 (95% CI 0.5 to 1.1) after controlling for possible confounders. However, in contrast to the above studies, cases and controls were defined by urethral symptoms and measures of urethral inflammation were not required. Differences in the populations studied, criteria used to define NGU cases and controls and the PCR tests used for detection of these two species may explain, in part, the differential associations of *U urealyticum* with NGU among these studies.

Several other studies have suggested a role for *U urealyticum* (biovar 2) in the aetiology of NGU. Yoshida *et al*¹⁴ showed an association of non-mycoplasmal non-chlamydial NGU with greater quantities of *U urealyticum* ($p<0.005$), but not *U parvum* ($p=0.556$), detected in urine. Further, Yokoi *et al*²¹ showed that *U urealyticum* was associated with post-gonococcal urethritis (OR=3.64, 95% CI 1.24 to 10.63) by multivariate analysis. Finally, Taylor-Robinson *et al*²² reported that urethritis was induced in two men who inoculated themselves intraurethrally with *U urealyticum* (biovar 2). Further studies are needed to assess the role of different strains and innate and acquired host immunity on the induction of urethritis in appropriate animal models.

The negative association of *U parvum* with NGU in our study is consistent with three previous studies, in which *U parvum* was detected more often in controls than in cases (21% vs 14%),¹⁷ (16% vs 8%),¹⁶ (14% vs 7%),¹⁵ and may explain the frequent failure to find an association of undifferentiated ureaplasmas with NGU when these two species are summed. In our previous analysis of the population analysed in our current study,⁹ undifferentiated ureaplasmas were not associated with NGU, either by qualitative culture, quantitative culture or by genus-specific PCR assays, a finding consistent with the higher prevalence of *U parvum* in controls, which would have masked the association of *U urealyticum* with NGU when these two species were considered together.

Using urine specimens archived from our previous study,⁹ we detected *U urealyticum* or *U parvum* by PCR among 75 (68%) of 110 culture-positive men and in six (4.8%) of 126 culture negative men. The ureaplasma species infecting an additional 16 men was determined by analysing urethral swab specimens from culture-positive/urine PCR-negative men, allowing the identification of a ureaplasma species in 83% of culture-positive men. However, our inability to test all urethral swab specimens by the species-specific PCR assays is a limitation of the study. Further, the inability to detect ureaplasma species in all culture-positive specimens may indicate that specimens with low quantities of ureaplasma DNA have been missed in the PCR assays, a conclusion that is not surprising considering that PCR and culture were analysed on 30 μ l and 500 μ l of specimen, respectively. Alternatively, freezing and archiving the specimens and the presence of inhibitors, not detected in our PCR assays may have slightly reduced the sensitivity of our PCR assays. A similar sensitivity and specificity of PCR relative to culture (68% and 99%) was reported by Povlsen *et al*²³ using urethral specimens from men. These studies suggest that further improvements of the sensitivity of species-specific PCR assays are needed. Future studies should include a comparison of the different species-specific PCR assays relative to each other and to culture.

In our study, *U urealyticum* was strongly associated with NGU in younger white men (OR=5.4, 95% CI 1.3 to 22.2). Although this association has wide CIs and requires further study, it is consistent with the results of Bowie *et al*⁸ who detected an association of undifferentiated ureaplasmas among young white men with first episode NGU. It is tempting to speculate that signs and symptoms of NGU may have been reduced among older men who have been exposed to ureaplasmas during previous episodes of NGU. Interestingly, Povlsen *et al*¹⁷ found that *U urealyticum* was more often associated with young age than *U parvum* (OR=3.99, 95% CI 1.54 to 10.33) among urea-plasma-positive men, reflecting

differences in risk factors associated with infection of these two species and perhaps explaining the masking of the association of ureaplasmas among older men and those with a history of urethritis.

It is puzzling that the prevalence of *U urealyticum* in controls in our study (16%) was higher than that of *C trachomatis* or *M genitalium* (3–4%). This finding may indicate that *U urealyticum* strains (or serotypes) differ in virulence factors that promote the induction, inflammation and symptoms. Future studies focusing on the immunobiology of *Ureaplasma* spp are needed to assess the differential pathogenicity of *U parvum* and *U urealyticum* strains, the effect of host immunity on signs and symptoms of NGU, the clinical relevance of *U urealyticum* detection and the appropriate antibiotic regimen.

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Key messages

- ▶ In his clinic-based case–control study, we used PCR assays to differentiate and assess the association of the newly recognised ureaplasma species with NGU.
- ▶ *U urealyticum* was positively associated with NGU (aOR=2.3, 95% CI 1.04 to 4.90) and *U parvum* was negatively associated with this syndrome (aOR=0.4, 95% CI 0.2 to 0.8).
- ▶ The higher than expected prevalence of *U urealyticum* in controls as well as its association with young age and white race deserve further study.

Figure 1.

Undifferentiated *Ureaplasma* spp (formally designated *Ureaplasma urealyticum*) culture and *U urealyticum* and *Ureaplasma parvum* PCR results reported as indicated by case–control study phase (original or current study). *Specimens were not available from two case participants for evaluation by species-specific PCR assays in this study. Both participants were negative for *U urealyticum* by culture in the original study. **Available urine specimens from all participants were tested by species-specific PCR assays. In addition, these PCR assays were performed on urethral swab specimens from men whose urine specimens were ureaplasma culture positive and species-specific PCR negative, as indicated in the text.

Table 1Demographic, behavioural and clinical characteristics of men infected with *U urealyticum* and *U parvum**

	<i>U urealyticum</i> (n = 46)	<i>U parvum</i> (n = 47)
Urethritis	30/46 (65.2)*	16/47 (34.0)
Age, years; median (range)	27.5 (17–42) [†]	26 (17–49)
Race—self-identified as white	16/46 (34.8)*	26/47 (55.3)
Lifetime sex partners, median (range)	30.5 (10–200)	40 (10–500)
Received oral sex (past 60 days)	27/45 (60.0)	34/46 (73.9)
Prior urethritis	26/46 (56.5)*	21/47 (44.7)
Other agents identified		
<i>Chlamydia trachomatis</i>	6 (13.0)	5 (10.6)
<i>Mycoplasma genitalium</i>	5 (10.9)	5 (10.6)

Unless otherwise indicated, data are presented as no./total (%) of patients with characteristic. Men positive for both (N=4) *U urealyticum* and *U parvum* are excluded.

* p<0.05 for difference in frequency of characteristic between *U urealyticum* and *U parvum* positive men;

[†] 0.1 <p>0.05 for difference in frequency of characteristic between *U urealyticum* and *U parvum* positive men.

Table 2

Identification of organisms detected in men with or without non-gonococcal urethritis

Agent	Cases (n = 119)	Controls (n = 117)	OR (95% CI)	aOR (95% CI)*
Prior study ⁹				
<i>Chlamydia trachomatis</i>	36 (30.3)	4 (3.4)	12.2 (4.7 to 35.8)	NA
<i>Mycoplasma genitalium</i>	27 (22.7)	5 (4.3)	6.4 (2.4 to 17.4)	6.5 (2.1 to 19.9)
<i>Ureaplasma</i> spp by culture [†]	51 (42.9)	59 (50.4)	0.9 (0.4 to 1.2)	NA
Current study—species-specific PCR				
<i>U. urealyticum</i>	31 (26.1)	19 (16.2)	1.8 (0.96 to 3.4)	2.3 (1.04 to 4.9)
<i>U. parvum</i>	17 (14.3)	34 (29.1)	0.4 (0.2 to 0.8)	0.4 (0.2 to 0.8)

Unless otherwise indicated, data are presented as no./total (%) of patients with characteristic.

* Estimates for *U. urealyticum* adjusted for age, racial identity, history of previous urethritis, and detection of *C. trachomatis* and *M. genitalium*, all characteristics that differed among cases and controls ($p < 0.1$) in our original report. Estimates for *U. parvum* adjusted for age, racial identity and detection of *C. trachomatis* and *M. genitalium*.

[†] Ureaplasma culture results, which do not differentiate the two ureaplasma species, were obtained from a previous study⁹ The detection of the newly recognised ureaplasma species, *U. urealyticum* and *U. parvum*, by PCR was performed in the current study.

NA, Not applicable.