Prevalence of *Chlamydia trachomatis*, *Ureaplasma* spp., *Mycoplasma genitalium* and *Mycoplasma hominis* among outpatients in central Greece: absence of tetracycline resistance gene tet(M) over a 4-year period study

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Abstract

A total of 301 men and women attending local urologists and gynaecologists in the state of Thessaly, central Greece, were tested for *Chlamydia trachomatis, Ureaplasma* spp., *Mycoplasma genitalium* and *Mycoplasma hominis* DNA. Investigation of the *tet*(M) gene, which confers tetracycline resistance in these genera, was also performed. Low incidence of *C. trachomatis* and *Mycoplasma* spp. as well as high prevalence of *Ureaplasma* spp., especially among women, were found. The *tet*(M) gene was absent in all cases, notably in a region where doxycycline administration remains the first therapeutic option unless special medical conditions direct otherwise.

Keywords: Fertility, Fitz-Hugh-Curtis, PCR, pregnancy, uropathogen

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Introduction

Chlamydia trachomatis, Ureaplasma spp. and *Mycoplasma* spp. (C-U-M) are associated with urethritis, prostatitis, bacterial vaginosis, cervicitis and pelvic inflammatory disease and are considered to be sexually transmitted pathogens [1–3] that might affect human fertility. Administration of doxycycline (100 mg by mouth every 12 hours for 7 to 14 days, depending on the site of infection) is a standard pharmaceutical treatment for patients found to be positive for C-U-M [4]. However, doxycycline efficacy might be compromised by the *tet*(M) determinant, a ribosome protection gene which is usually carried on Tn1545-like conjugative chromosomal elements [5] and causes tetracycline resistance in these genera [6].

In May 2015 we diagnosed in a 16-year-old female patient Fitz-Hugh–Curtis syndrome that was attributed to *Ureaplasma urealyticum* [7], which is nevertheless considered to constitute normal urogenital flora [8]. We thus decided to report on the occurrence of C-U-M in central Greece because to date a relatively large-scale study in Greek outpatients has not been reported.

Patients and Methods

From February 2012 until November 2015 a total of 301 men and women attending local urologists and gynaecologists in the state of Thessaly (which has more than 700 000 inhabitants), Greece, were tested. The Biogonidiaki Center of Infertility Investigation and Genetic Research, Volos, Greece (which serves the broad region of the state of Thessaly), performed C-U-M DNA investigation in semen and cervical samples. Individuals who underwent C-U-M DNA testing presented either with genital signs and symptoms of infection (urethritis, prostatitis, cervicitis, leukocytes in semen analysis, Papanicolaou test etc.) or with a history of infertility (at least 12 months of unprotected sexual intercourse without pregnancy). Many individuals underwent prenatal screening for C-U-M. Duplicate samples were excluded, and all participants were enrolled onto the study during their initial presentation. All samples were investigated for C-U-M DNA using the Amplisens (Bratislava, Slovak Republic) DNA-Sorb-AM Nucleic Acid Extraction Kit as well as Amplisens *C. trachomatis/Ureaplasma/Mycoplasma genitalium*–MULTI-PRIME-FRT and Amplisens *Mycoplasma hominis*–FRT diagnostic CE-IVD PCR kits. The presence of a *tet*(M) determinant was investigated using previously described primers and amplification conditions [9].

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Results

A total of two participants (0.66%) were found to be positive for *C. trachomatis*, 76 (25.25%) were positive for *Ureaplasma* spp. and 11 (3.65%) were positive for *M. hominis*, while no participant was found to be positive for *M. genitalium*. A total of 212 participants (70.43%) were negative for C-U-M DNA. Among the 171 men tested, one (0.59%) was found to be positive for *C. trachomatis*, 23 (13.45%) for *Ureaplasma* spp. and six (3.51%) for *M. hominis*, while 141 (82.46%) were negative for C-U-M DNA. Similarly, among the 130 women tested, one (0.77%) was found to be positive for *C. trachomatis*, 53 (41.18%) for *Ureaplasma* spp. and five (3.85%) for *M. hominis*, while 71 (54.62%) were negative for C-U-M DNA. It is worth mentioning that *Ureaplasma* spp. was found to occur at a higher rate in women than men (41.18% vs. 13.45%); most men (95, 55.56%) undergoing C-U-M testing presented with a history of infertility, and most women (87, 66.92%) presented for prenatal screening (Table 1). The *tet*(M) gene was not detected in either case.

Discussion

Michou *et al.* [10] reported different respective rates of C-U-M prevalence among 87 women presenting with a history of infertility (18.3% *C. trachomatis*, 16.1% *U. urealyticum* and 19.5% *M. hominis* in PCR-tested cervical samples); their overall C-U-M detection rate (44.8%) was higher than ours (29.86%). The respective discrepancies could be partially attributable to the different demographic groups studied as well as the fact that occurrence of *Ureaplasma parvum* was not tested. Also, Al-Sweih *et al.* [11] reported higher rates of *C. trachomatis* (21.59%) and *M. hominis* (17.46%) positivity in men among those studied in Kuwait, while Gdoura *et al.* [12] found *C. trachomatis* and *Mycoplasma* spp. to prevail, at rates of 43.3% and 18.3%, respectively, among Tunisian men. The prevalence of *Ureaplasma* spp. among male participants in our study (13.45%) was similar to the previously mentioned studies.

Of note, in a region where doxycycline remains the first therapeutic option against C-U-M, no sample was found to be positive for the *tet*(M) determinant. Also, in all C-U-M DNA–positive cases, administration of doxycycline (100 mg by mouth every 12 hours for 10 days) resulted in a negative repeated C-U-M DNA test as well as remission of clinical signs and symptoms of infection. A similar study in France demonstrated an as yet relatively low incidence for the *tet*(M) gene among *Ureaplasma* spp. (2.2%) but a higher rate of *tet*(M) occurrence (18.75%) among *M. hominis* [13]. Also, a study from the United States reported that 45 of 100 isolates of *Ureaplasma* spp. carry the *tet*(M) gene [14]. With respect to chlamydiae, tetracycline resistance is rare, and only a few reports have identified the tetracycline resistance determinant *tetC* in the pig pathogen *Chlamydia suis* [15]. Of note, previously described *C. trachomatis* tetracycline-resistant clinical isolates [16] were eventually shown to be phenotypically tetracycline sensitive [17].

In conclusion, we report what is to our knowledge the first large-scale study reporting on C-U-M occurrence among outpatients in Greece. A technique of high specificity and sensitivity was used using commercial CE-IVD PCR kits because conventional microbiologic methods have been demonstrated to inadequately detect C-U-M [10,18]. Our study found a low incidence of *C. trachomatis* and *Mycoplasma* spp. in our region and a higher prevalence of *Ureaplasma* spp., especially among women. Unlike *C. trachomatis, Ureaplasma* and *Mycoplasma* genera are considered to constitute normal urogenital flora, although *Ureaplasma* spp. have been described as a risk factor for complications in pregnancy [8,19]. In addition, we recently reported the second incidence of Fitz-Hugh–Curtis syndrome worldwide that was attributed to *U. urealyticum* [7], while 10.11% of our PCR-confirmed C-U-M-positive cases presented with clinical manifestations (genital signs/symptoms) that showed remission after doxycycline administration. In this respect, physicians should be cautious when seeking microbiologic evaluation of cervicovaginal or semen samples, in order

TABLE 1. Accumulative data showing *Chlamydia trachomatis, Ureaplasma* spp., *Mycoplasma genitalium* and *Mycoplasma hominis* incidence relative to reason for outpatient presentation for C-U-M testing

Sample source	Characteristic	*C. trachomatis*	*Ureaplasma* spp.	*M. genitalium*	*M. hominis*	Negative	Total
Semen	Genital signs/symptoms	—	3	—	1	18	22
	Infertility history	1	11	—	5	78	95
	Prenatal screening	—	9	—	—	45	54
Cervix	Genital signs/symptoms	—	5	—	—	14	19
	Infertility history	—	8	—	1	15	24
	Prenatal screening	1	40	—	4	42	87
Total		2	76	—	11	212	301

C-U-M, *Chlamydia trachomatis, Ureaplasma* spp. and *Mycoplasma* spp.

to encourage C-U-M testing with PCR-based techniques. Especially for women, menstrual tissue versus cervical sample has been recently shown to increase the detection rate of C-U-M in combination with PCR [10].

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Conflict of Interest

None declared.

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