

Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* in asymptomatic women in Northern Greece

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Abstract

Aim: This study attends to determine the prevalence and the antimicrobial susceptibility of *Ureaplasma urealyticum* in asymptomatic women in Northern Greece.

Materials-Methods: A total of 347 cervical smears were divided into 5 groups according to age. Samples were cultured, *Ureaplasma urealyticum* isolation was confirmed by the detection of the urease gene and the antimicrobial susceptibility of the isolates to 9 commercially available antibiotics was determined.

Results: Fifty-six (16.13%) samples were found positive. Women >60 years old were rarely infected. Only 9 (16.07%) isolates were susceptible to all antimicrobials tested. Thirteen isolates were resistant and 34 intermediately resistant to ciprofloxacin, two resistant and 29 intermediately resistant to ofloxacin, three intermediately resistant to macrolides, while all were susceptible to tetracyclines.

Conclusions: In Northern Greece, 16.13% of asymptomatic women seem to be carriers of *Ureaplasma urealyticum* mostly in reproductive ages. Almost no resistance to macrolides and tetracyclines has been noticed, while most of the isolates present resistance or moderate sensitivity to quinolones. Hippokratia 2013; 17 (4): 319-321.

Keywords: *Ureaplasma urealyticum*, prevalence, women, asymptomatic, susceptibility, Northern Greece

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Introduction

Ureaplasma urealyticum belongs to the family Mycoplasmataceae, the smallest among free-living cellular microorganisms in nature. The bacterium is found in the urogenital system of many healthy people^{1,2}, while the infections caused may present various clinical features. Apart from symptomatic infections, subclinical ones or simple carriage may occur. The urogenital colonization depends on sex, age, sexual activity and hormonal status. It has been correlated to low socioeconomic levels, young ages, large number of sexual partners, African-American ethnicity and oral contraceptive use^{3,4}. The bacterium's pathogenic effects include obstetric, perinatal, urological and paediatric infections. Adults may manifest urogenital infections, such as non-gonococcal urethritis (NGU), chronic prostatitis and epididymitis in males, and vaginitis and cervicitis in females^{4,6}.

In 2002 *Ureaplasma urealyticum* was divided into two species, *U. parvum* (biotype 1) and *U. urealyticum* (biotype 2)⁷. *U. parvum* is more often detected in vaginal and cervical samples^{4,6,8-10}. B-lactam antibiotics cannot be used for *U. urealyticum* since they lack the cell wall structure. The antibiotics of choice are the ones interfering to protein synthesis (macrolides and tetracyclines) and to active nucleic acid composition (quinolones)¹¹. *U. urealyticum* resistance has been globally observed at all three categories of antibiotics, macrolides, tetracyclines and quinolones^{6,12-16}, which raises concerns and

increases the importance of ureaplasma surveillance. Similar findings have been reported in Greece^{17,18}, although *U. urealyticum* resistance is not well investigated at the moment.

Therefore, the present study investigates the prevalence of *U. urealyticum* in asymptomatic women in Northern Greece and to the susceptibility of the microorganism to various antimicrobial agents.

Materials and Methods

A total of 347 cervical smears were collected from asymptomatic women who visited Theagenion anticancer hospital of Thessaloniki for annual Papanikolaou test control as a part of the prevention screening programme.

The women selected were 20 to 70 years old, residents of Northern Greece, with no restriction as to race or nationality, reporting no genital system symptoms and no antimicrobial treatment during the previous month for any reason. The samples were divided into 5 age groups, 20-30, 31-40, 41-50, 51-60 and ≥61 years old, which included 42, 57, 109, 107 and 32 samples respectively.

The samples were obtained with cotton swab from the cervical transitional zone after the removal of excess vaginal and cervical secretions. The swab was immersed in the specific preservation and transport medium for mycoplasmas (Mycoplasma U-A Broth, Bioprep, Greece).

Small quantities of all broths were inoculated on A7

Agar plates (Mycotest Agar, Bioprep, Greece) and incubated for 48 hours in 37°C with 10% CO₂. *Ureaplasma* isolation was confirmed by plate microscopy.

Positive results obtained by traditional techniques were confirmed by molecular methods. After DNA extraction (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany), a Real-time PCR was designed for the detection of urease gene (*ureA*, *ureB*, part of *ureC*) in all the positive samples. The amplified urease gene consists of 429 bases. Primers were used for the detection according to Blanchard et al¹⁹ and Beeton et al⁶.

The strains of *U. urealyticum* spp. were then distinguished to *U. urealyticum* and *U. parvum* with Real-time Polymerase Chain Reaction (PCR), (Sacace Biotechnologies Srl, Italy) according to the manufacturer's instructions.

The phenotypical resistance control was carried out with a broadly used, standardized method which checks the sensitivity to 9 antibiotics in two concentrations (Mycoplasma IST 2, Biomerieux, France).

The method is based on the colour alteration as the pH increases due to the effect of the microorganism on a liquid culture medium. The antibiotics used were doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin and pristinamycin. Moderate sensitivity is indicated if the colour change is observed only in the lowest antibiotic concentration whereas resistance is reported when the change is observed in both antibiotic concentrations.

The statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Regarding the prevalence, *U. urealyticum* spp was detected in high concentration (>10⁴) in 56 (16.1%) out of 347 cultured samples. All positive samples were confirmed by Real-time PCR. The isolates were further classified into species: 54 of them belonged to *U. parvum* and 2 to *U. urealyticum*.

Of the 42 samples belonging to the 20-30 years age group, 8 (19.0%) were positive; of the 57 samples belonging to the 31-40 years age group, 12 (21.1%) were positive; of the 109 samples belonging to the 41-50 years age group, 21 (19.3%) were positive; of the 107 samples belonging to the 51-60 years age group, 14 (13.1%) were positive; while of the 32 samples belonging to the ≥61 years age group, only 1 (3.1%) was positive. The pair-wise statistical comparison among all age groups revealed statistically significant differences only for the ≥61 years old (compar-

ing to 20-30 years p=0.0379, to 31-40 years p=0.0175, to 41-50 years p=0.0263 and to the total p=0.0488). All other pair combinations presented p>0.05.

Regarding the susceptibility to antibiotics, only 9 (16.07%) of the 56 isolated strains were sensitive to all 9 antibiotics.

The examination of susceptibility to quinolones indicated that 13 (23.21%) strains were resistant and 34 (60.07%) were moderately sensitive to ciprofloxacin, while resistance to ofloxacin was observed in 2 (3.5%) strains and moderate sensitivity in 29 (51.79%).

Regarding macrolides susceptibility, 1 (1.7%) strain proved moderately sensitive to azithromycin, 1 (1.7%) moderately sensitive to clarithromycin and 1 (1.7%) to erythromycin.

All strains were sensitive to tetracyclines.

The age distribution of women with resistant and moderately sensitive strains is shown at Table 1.

Discussion

The present randomized sampling among the clinically healthy female population of N. Greece proved that 16.13% of these asymptomatic women were carriers of *Ureaplasma urealyticum* spp. in high concentrations, as healthy individuals who include the microorganism in their vaginal normal flora and/or as possible future patients. The existence of *Ureaplasma* spp has been generally reported in 40-80% of clinically healthy women in the USA⁴, but only 8.9% in a sample of very young women in Norway²⁰. Moreover, recent studies on non-asymptomatic women all over the world presented various results, such as 20.1% of infertile women in the USA²¹, 46.52% of women with genital infectious diseases in China²², 20.8 % of reproductive-age women with vulvovaginitis in Athens¹⁸.

The majority of *U. urealyticum* carriers have been found among women of reproductive age, fact which indicates the important role of hormone profile and sexual activity in the prevalence of the microorganism. The role of the hormonal status regarding the presence of *Ureaplasma* spp. in the female genital tract has already been documented since it has been detected in vaginal flora in 40% of sexually inactive, in 67% of sexually active women of reproductive age and in 25% of postmenopausal women²³. The intensity of sexual activity has also been already associated with the presence of *Ureaplasma* spp⁴.

Regarding species, 96.4% of the positive samples belong to *U. parvum* and only 3.6% to *U. urealyticum*, a

Table 1: Resistance of isolated strains of *U. urealyticum* to various quinolones and macrolides

Age Group (years)	Number of Isolates	Cipr. R	Cipr. MS	Ofi. R	Ofi. MS	Erythr. MS	Clar. MS	Azithr. MS
20-30	8	1	7		5			
31-40	12	3	8	1	8			
41-50	21	3	14		8	1		
51-60	14	5	5	1	7		1	1
≥61	1	1			1			
Total	56	13 23.21%	34 60.07%	2 3.5%	29 51.79%	1 1.7%	1 1.7%	1 1.7%

R: Resistant, MS: Moderately Sensitive, Cipr: Ciprofloxacin, Ofi: Ofloxacin, Erythr: Erythromycin, Clar: Clarithromycin, Azithr: Azithromycin.

common finding according to the international bibliography. In Australia the distinction of human ureaplasmas resulted in *U. parvum* 87% versus *U. urealyticum* 19%⁸. In Germany *U. parvum* biovar was found in 81% of the women examined, while 6% of them were co-infected with both biovars⁹. Similarly, in U. K. isolated ureaplasmas belonged to *U. parvum* and to *U. urealyticum* in a proportion of 80% and 20% respectively⁶.

Differences observed at the phenotypical drug resistance among groups of antimicrobials seem to be correlated with their use rates. Quinolones, following their continuously raising use for plenty of infections, present high percentages of resistance (3.5-60% depending on the antibiotic) in contrast to macrolides and tetracyclines towards which resistance was found to be very low or completely absent. Tetracyclines seem to be much more effective against *U. urealyticum* as the examined strains are all sensitive to them. In agreement to our results, Kechagia et al have found the resistance to the categories of antibiotic drugs that are prescribed as protocol treatment in Greece, 2008, as follows: tetracyclines 0-4.5%, macrolides 6.3-33.3% and quinolones 18-77.5%¹⁷. Baka et al have also reported 1-3% to tetracyclines, 12.2%-18.1% to macrolides, while 86.1% and 53.7% to ciprofloxacin and ofloxacin respectively¹⁸. Researches carried out in various countries indicate resistance percentages at similar levels. Strains isolated in France were 2.2% resistant to tetracyclines¹⁶. In China, resistance to quinolones has increased since 2000 and overtakes 50%, while resistance to erythromycin has been reduced from 63.9% to 20% since 2000, which is attributed to the widespread use of quinolones¹⁴. According to Duffy et al, in U.S.A., ureaplasma isolates showed increased resistance to all fluoroquinolones¹³. It seems that quinolone abuse leads to resistance¹⁴, mainly through mutations to the enzymes targets or throughout an altered membrane permeability of the microorganism⁶. However, strains isolated in U.K. presented equal and very low (1/61) resistance to each one of the three antimicrobial groups mentioned above⁶.

Concluding, *Ureaplasma urealyticum* in asymptomatic women is quite frequent. Its increasing resistance to quinolones raises concerns about detaining the problem but also motivates future research in various levels and directions.

Conflict of Interest

There are no financial or other relationships that might lead to a conflict of interests about this work.

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