Agar plates (Mycotest Agar, Bioprepure, 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 al⁶ and Beeton et al⁶. The strains of *U. urealyticum* 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 in both concentrations.

Results

Regarding the prevalence, *U. urealyticum* spp was detected in high concentration (>10⁴) in 56 (16.1%) out of 347 cul
guished to

Moderate sensitivity is indicated if the colour change is observed in both 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 (comparing 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 Norway⁶. Moreover, recent studies on non-asymptomatic women all over the world presented various results, such as 20.1% of infertile women in the USA³, 46.52% of women with genital infectious diseases in China², 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

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