Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in a population of Italian and immigrant outpatients

*Prevalenza e resistenza agli antibiotici di Ureaplasma urealyticum e Mycoplasma hominis in una popolazione di pazienti ambulatoriali italiani e stranieri*

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**INTRODUCTION**

[Ureaplasma urealyticum](https://www.ncbi.nlm.nih.gov/pubmed/27927570) and *Mycoplasma hominis* are microorganisms commonly found in the genitourinary tract of patients experiencing symptoms, but also in asymptomatic subjects [1, 2]. These two bacteria have been associated with increased risk of recurrent miscarriage, infertility, pelvic inflammatory disease, orchitis, epididymitis, prostatitis and non-gonococcal urethritis [3-7]. The epidemiology of mycoplasma infection changes in different geographic areas, with the emergence of resistant isolates [8, 9]. This issue is particularly important in the light of migration flows from other countries, which change the local epidemiological profile of infectious diseases of patients treated by general practitioners and hospital doctors [10]. To date, this ever-changing issue of mycoplasmas in the Italian population has received little treatment in the literature. Because mycoplasmas lack a cell wall, they are inherently resistant to beta-lactams and glycopeptides. They are not susceptible to sulfonamides or trimethoprim because they do not synthesize folic acid. *Mycoplasma hominis* is also naturally resistant to erythromycin. Treatment is therefore restricted to agents such as tetracyclines, macrolides, and fluoroquinolones [11].

Data on antimicrobial susceptibilities of genital mycoplasmas reported by authors from various countries are controversial [12, 13]. The increase in resistance of many of these pathogens to antimicrobial agents has led to steady surveillance for antimicrobial resistance in clinical strains of *U. urealyticum* and *M. hominis* as a pivotal step for determining subsequent effective therapy [14].

The aim of this study was to assess possible differences in prevalence and antimicrobial susceptibilities of these two pathogens, isolated from cervical and urethral swabs collected from native and immigrant outpatients referred to our laboratory complaining of various symptoms.

**PATIENTS AND METHODS**

*Setting and participants*

A total of 433 consecutive Italian and immi-
grant outpatients, 331 females and 102 males, aged 15-80, were examined during an eight-month period (from January 2011 to August 2011). We included in the study all patients referred to our laboratory by family practitioners for detecting the presence of *U. urealyticum* and *M. hominis* reporting symptoms of urethritis or vaginitis (discharge, itching and pain), pelvic pain, urinary frequency, inguinal lymphadenopathy, prostatic tenderness, tenderness or swelling of testes or epididymis, infertility, hematuria, hematospermia, abnormal Pap smear, irregular periods or miscarriage. Exclusion criteria were: detection of mycoplasma mixed infection, history of antibiotic therapy within one month prior to presentation, structural abnormality of the urogenital system, language barrier, homosexuality, psychiatric disorders and physical disability. A questionnaire about age and ethnicity was administered to all patients. The study was performed according to good clinical practice and the Declaration of Helsinki, and consent was obtained from each patient.

**Sampling, culture, and antimicrobial susceptibility testing**

Male patients underwent urethral sampling, females cervical sampling [15]. All the subjects had not urinated for at least 3 hours. Urethral samples in males were collected with a dacron swab placed 2-3 cm in the urethra and turned to obtain as many cells as possible after cleaning external meatus without antiseptics or antibiotics.

In female patients, cervical samples were collected from the endocervical region after inserting a sterile speculum into the vagina. Mucus had been cleaned with a sterile cotton swab without causing any bleeding or using antiseptics. All the samples were taken twice. The first sample was processed for direct microscopic analysis with Gram staining. Only samples in which white blood cells were ≥5 per high power field (1000 X) were considered. The second sample was processed for *U. urealyticum* and/or *M. hominis* detection, and their antimicrobial susceptibilities, by means of the commercially available MYCOFAST® Screening EvolutioN 3 Kit (ELITech MICROBIO, Signes, France).

During growth, *U. urealyticum* and *M. hominis* metabolise urea and arginine respectively, resulting in a colour change of the medium, which contains phenol red indicator, from yellow to red. This colour change is due to liberation of ammonia resulting in an alkaline pH of the medium.

Clinical samples were placed in R1 transport medium which inhibits the growing of Gram-positive and Gram-negative bacteria. The inoculated R1 medium was vortexed and 3 mL added to the growth R2 medium, containing lyophilized urea/arginine broth. After reconstitution, 100 µL was inoculated into each of the 20 wells of MYCOFAST Evolution 3 tray and overlaid with paraffin oil. The remainder of the R2 medium and the inoculated tray were then incubated at 37°C and observed for colour changes at 24 and 48 hrs. The strips provided:

1) information about presence or absence of *U. urealyticum* or *M. hominis*, based upon susceptibility to lincomycin, trimethoprim/sulfamethoxazole and erythromycin;

2) an estimate of the bacterial load, based upon enzyme kinetics (colour-changing units [CCU]/mL): for *U. urealyticum* 10³, 10⁴, ≥10⁵ CCU/ml, for *M. hominis* ≥10⁴ CCU/ml. Pathological thresholds quoted for *U. urealyticum* and *M. hominis* are ≥10⁴ CCU/ml for urethral/cervical specimens;

3) information on susceptibility to seven antibiotics (doxycycline, pristinamycin, roxithromycin, azithromycin, josamycin, ciprofloxacin and ofloxacin) at two concentrations, with three possible interpretations: susceptible, intermediate, resistant [16].

**Statistical analysis**

Statistical analysis was performed by SPSS 13.0 version. Patient age was compared between different groups by the Mann-Whitney U-Test for independent samples. Categorical variables were analyzed by the Pearson Chi-square test ($\chi^2$), or, where appropriate, with Yates-corrected Chi-square. When a cell value of <5 was encountered, a 2-tailed P value was obtained by means of the Fisher’s exact test. An alpha level of 0.05 was established as a criterion for statistical significance.

**RESULTS**

The study population comprised 433 patients, and the mean age in the whole population was 33.3±9.6 years; 312/433 (72.1%) patients were native, and 121/433 (27.9%) were immigrants.
Of these, 51.2% were from Eastern Europe, 27.3% from Africa, 20.6% from South America, and 0.8% from Asia. No differences were found in the mean values of age between positives and negatives (respectively: 32.8±10.8 vs 34.2±11.7; p=0.328), females and males (respectively: 32.7±8.8 vs 37.2±12.8; p=0.219) nor between natives and immigrants (respectively: 31.8±6.5 vs 34±10.9; p=0.193).

Total positive samples were 158/433 (36.5%). For *U. urealyticum* 152/433 (35.1%), for *M. hominis* 6/433 (1.4%). Prevalence of positive samples according to sex and origin by country are summarized in Table 1. Of the 433 patients, 331 (76.4%) were females. There were significantly more positive samples in females than in males both for *U. urealyticum*: 41.9% vs 12.7%; χ²=29.80; p=0.0001, and for total positive samples: 43.5% vs 13.7%; χ²=29.83; p=0.0001. Comparing immigrants with natives, no significant differences were found regarding positive samples for *U. urealyticum* or *M. hominis* (respectively: p=0.175, p=0.183). Comparing immigrants with natives according to country, there were more positive samples for *U. urealyticum* and total positive samples in African patients: *U. urealyticum*: 51.5% vs 33.3%; Yates-corrected chi-square=3.98; p=0.046; total positive isolates: 54.5% vs 34.3%; Yates-corrected chi-square=4.45; p=0.035. No other significant differences were found.

In Table 2 the antibiotic resistance profiles are displayed according to the microorganism: 66.4% (101/152) of *U. urealyticum* isolates were resistant to ciprofloxacin, whereas 27.6% (42/152) were resistant to ofloxacin. No resis-

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**Table 1 - Prevalence of isolates according to gender and origin by country.**

<table>
<thead>
<tr>
<th></th>
<th><em>U. urealyticum</em> (n=152)</th>
<th><em>M. hominis</em> (n=6)</th>
<th>Total (n=158)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (n=102)</td>
<td>13 (12.7)</td>
<td>1 (0.9)</td>
<td>14 (13.7)</td>
</tr>
<tr>
<td>Females (n=331)</td>
<td>139 (41.9)</td>
<td>5 (1.5)</td>
<td>144 (43.5)</td>
</tr>
<tr>
<td>Italy (n=312)</td>
<td>104 (33.3)</td>
<td>3 (0.9)</td>
<td>107 (34.3)</td>
</tr>
<tr>
<td>Immigrants (n=121)</td>
<td>48 (39.6)</td>
<td>3 (2.5)</td>
<td>51 (42.1)</td>
</tr>
<tr>
<td>Eastern Europe (n=62)</td>
<td>24 (38.7)</td>
<td>2 (3.2)</td>
<td>26 (42)</td>
</tr>
<tr>
<td>Africa (n=33)</td>
<td>17 (51.5)</td>
<td>1 (3)</td>
<td>18 (54.5)</td>
</tr>
<tr>
<td>South America (n=25)</td>
<td>7 (28)</td>
<td>0 (0)</td>
<td>7 (28)</td>
</tr>
</tbody>
</table>

Data are count data. Values in parentheses represent percentages of individuals in each row category.

**Table 2 - Susceptibility of *U. urealyticum* and *M. hominis* to eight different antibiotics**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>U. urealyticum</em> (n=152)</th>
<th><em>M. hominis</em> (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Azythromycin</td>
<td>152 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8 (5.3)</td>
<td>43 (28.3)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>152 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>152 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Josamycin</td>
<td>152 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>21 (13.8)</td>
<td>89 (58.6)</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>152 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>152 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

S = susceptible; I = intermediate; R = resistant. Data are count data. Values in parentheses represent percentages of isolates of each column category.
tance was found to azithromycin, doxycycline, erythromycine, josamycin, pristinamycin or roxithromycin. Among samples positive for M. hominis, 66.7% (4/6) were resistant to both azithromycin and roxithromycin. No resistance was found to ciprofloxacin, ofloxacin, doxycycline, josamycin or pristinamycin.

**DISCUSSION**

The aim of this study was to evaluate possible differences in the prevalence and antibiotic resistance of genital mycoplasmas, in a population of native and immigrant outpatients. Migration flows to other countries are known to change the prevalence of pathologies, mostly infectious diseases, and both prevalence rates and antimicrobial susceptibilities of mycoplasmas differ according to gender and country [8, 9, 12, 13, 17]. Moreover, multidrug resistant strains of these pathogens have recently been found to be responsible for fatal infection in adults [18, 19]. The data reported in this study showed a higher prevalence of total isolates and U. urealyticum isolates in African immigrants compared to native Italians. This result matches with those reported in a recent paper [20] in which more than half of the patients referred to an Italian Department of Migration Medicine came from Africa, and the second cause of hospitalization was infectious and parasitic diseases, mainly Mycoplasma and Chlamydia urethritis [20]. Similarly, a significant association of U. urealyticum and black race has been reported [21]. The general prevalence of infection found in this report agree in some respects with results of other studies.

Potts et al. in a population of 48 women with chronic urologic symptoms, found a prevalence for U. urealyticum of 45.8% [22]. Guven et al. in 533 women with various gynecologic complaints found a prevalence of U. urealyticum and M. hominis respectively of 11.8% and 0.9% [23]. In 176 men with urethritis, Shigehara et al. found a prevalence of both U. urealyticum and M. hominis of 12% [24].

In this study, for detection of pathogen, we used a method based on culture, commonly used in laboratory routine diagnostics. Since our laboratory provides reports mostly for general practitioners, but also for hospital physicians and health care facilities, our data give a picture of the health status in the general population. Methods based on Polymerase Chain Reaction (PCR) have been recently used in several studies [25]. The molecular approach for detection of bacteria undoubtedly provides in some measure a higher sensitivity/specificity, but gives no information about antibiotic resistance of the isolate, and due to different PCR methods, has not yet been validated for clinical purposes. The prevalence of the antibiotic resistance profile of the isolates in this study differ from those reported in similar studies in other countries. In Greece Kechagia et al. found a great prevalence of intermediate and resistant isolates of U. urealyticum for azithromycin, erythromycin, josamycin, and of M. hominis for ciprofloxacin and josamycin, while we did not obtain similar results [26]. Similarly, Krausse et al. in Germany, reported a considerable number of U. urealyticum isolates resistant to azithromycin (7.3%), roxithromycin (5.6%) and erythromycin (20.7%), whereas we found none [14]. On the other hand, for U. urealyticum, we found a greater prevalence of resistant isolates for ciprofloxacin (66.4% vs 16.2%) and for ofloxacin (27.6% vs 1.7%).

These differences in prevalence are most probably due to the different antibiotic therapies prescribed in the various countries. The data in our study confirm that prevalence of infection sustained by genital mycoplasmas and antibiotic resistance profiles change in relation to the patient’s country of origin. Therefore, detection of local prevalence of these pathogens and surveillance of their antibiotic resistance profile are pivotal for the early cure of patients and prevention of the occurrence of resistant strains.

Conflict of interest: none declared.

Keywords: Ureaplasma, Mycoplasma, infection, immigrants.
**REFERENCES**


[9] Salari M.H., Karimi A. Prevalence of *Ureaplasma urealyticum* and *Mycoplasma genitalium* in men with...


