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ORIGINAL ARTICLE

Incidence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolated in Brescia, Italy, over 7 years

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Abstract The prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* collected during 2004–2011 were determined. A total of 9956 individuals was analyzed. Identification was performed by use of the mycoplasma IST-2 kit. Antimicrobial susceptibility against doxycycline, josamycin, ofloxacin, eryth romycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin, and pristinamycin was also tested by use of this commercial kit. Our results show a prevalence of 1856 positive patients for genital mycoplasmas (18.6 %). Among positive cultures, 89 and 1.1 % of isolates were *Ureaplasma urealyticum* and *Mycoplasma hominis*, respectively. For 9.8 % of isolates both urogenital mycoplasmas were grown. Doxycycline was the most active tetracycline for mycoplasma infections, and this is still the drug of first choice. Among macrolides, josamycin and clarithromycin are the most active agents against ureaplasmas; josamycin is also active against mycoplasmas and is an alternative to tetracyclines and erythromycin for mixed infections, especially for pregnant women and neonates. Fluoroquinolones had low efficacy against urogenital mycoplasmas. For *Ureaplasma urealyticum*, cross-resistance was found between erythromycin and macrolides (except josamycin) (40–80 %) and between erythromycin and ciprofloxacin (79 %). Antibiotic resistance over the test period did not vary significantly. Because of geographical differences among antibiotic resistance, local in-vitro susceptibility testing is recommended to avoid failure of therapy.

Keywords Susceptibility · Resistance · Antibiotic · *Ureaplasma urealyticum* · *Mycoplasma hominis*

This work is dedicated to the memory of the late Professor Nino Manca.

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Introduction

Mycoplasmas belong to the class Mollicutes which also contain Ureaplasmas, Acholeplasmas, Spiroplasmas, the newly classified Haemoplasmas, and other wall-less bacteria. They are characterized by small size, lack of cell wall, extremely fastidious in-vitro environment requirement, and tendency to form centered colonies on solid medium.

Mycoplasma hominis and *Ureaplasma urealyticum* are part of the normal commensal flora of the genital tract of sexually active healthy women, with the incidence of colonization reaching 80 % in some areas of the world [1]. They are associated with genitourinary tract infections (urethritis, cervicitis, cystitis, bacterial vaginosis) and seem to be involved in the aetiology of postpartum infections of mothers and newborns [2–5]. The incidence of infection is affected by the menstrual cycle, pregnancy, and the use of vaginal contraceptives [6]. The prevalence of these organisms is significantly associated with socioeconomic conditions, for example poverty, and large numbers of sexual partners [4, 7, 8].

Beta-lactam antibiotics and vancomycin are completely inactive because their target is the cell wall. *Mycoplasma* and *Ureaplasma* spp. are currently susceptible to agents that interfere with protein synthesis, for example tetracycline, macrolides, aminoglycosides, and chloramphenicol,

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and to the fluoroquinolones that inhibit topoisomerases [4, 9, 12]. Mycoplasma and ureaplasma infections are usually treated with tetracycline, except for pregnant women, neonates, and children, for whom erythromycin is recommended [11, 13]. However, resistance to these agents is increasing, as a consequence of the presence of the tetM resistance determinant [11, 13] or chromosomal mutation [14–17]. Clindamycin, fluoroquinolones. or other macrolides can be used after failure of therapy with tetracycline or erythromycin [13, 15].

The extent of resistance varies geographically, depending on the use of different antibiotics and the history of previous antimicrobial exposure of different populations [11, 18]. For these reasons, it is important to implement surveillance studies on the antimicrobial susceptibilities of these species.

The purposes of this study were:

1. to analyze the prevalence of urogenital mycoplasma;
2. to investigate susceptibilities of a large number of clinical isolates of *M. hominis* and *Ureaplasma urealyticum* to different antibiotics; and
3. to compare changes in the antibiotic susceptibilities of these microorganisms over several years, from 2004 to 2011.

Materials and methods

Clinical specimens

Endocervical, urethral, and vaginal swabs were obtained from the urogenital tracts of adults aged between 18 and 43 years (median 29 age) who visited Brescia's main hospital (Spedali Civili) for urogenital mycoplasma analysis between January 2004 and June 2011. A total of 9956 individuals were analyzed during this period. All were consecutive outpatients who for gynecologic health care control, for normal routine screening during pregnancy or infertility problems, or for the presence of symptoms of genital infections had attended Brescia's hospital.

Culture for genital mycoplasmas and antimicrobial susceptibility testing

Culture of genital mycoplasmas was performed by use of a Mycoplasma IST 2 kit (Biomerieux, Marcy l'Etoile, France). Because this method does not enable discrimination of *Ureaplasma parvum* (biovar 1) from *Ureaplasma urealyticum* (biovar 2), in this paper *Ureaplasma urealyticum* is used for both biovars. The strips provided information about the presence or absence of *M. hominis* and *U. urealyticum*, an estimate of the density of each organism ($>10^4$ CFU), and antimicrobial susceptibility to doxycycline, josamycin, ofloxacin, erythromycin, and tetracycline.

Transport medium (R1) was inoculated with clinical specimens in accordance with the manufacturer's instructions. They were vortex-mixed rapidly, and 3 ml R1 was used to rehydrate the lyophilized growth medium R2. A Mycoplasma IST strip, consisting of 22 wells, was inoculated with the rehydrated R2 growth medium (55 ml per well, overlaid with two drops of mineral oil).

The remainder of the broth and the inoculated strip were incubated at 37 °C and observed for color changes from yellow to red as a result of alkalization of the medium after incubation for 24 and 48 h. The development or absence of red color on the relevant part of the strip provided an index of resistance or susceptibility to each antimicrobial agent, according to the guidelines of the CLSI. The breakpoints for the antimicrobials tested were: tetracycline $S \leq 4$, $R \geq 8$; doxycycline $S \leq 4$, $R \geq 8$; clarithromycin $S \leq 1$, $R \geq 4$; azithromycin $S \leq 0.12$, $R \geq 4$; erythromycin $S \leq 1$, $R \geq 4$; josamycin $S \leq 2$, $R \geq 8$; ciprofloxacin $S \leq 1$, $R \geq 2$; and ofloxacin $S \leq 1$, $R \geq$.

Statistical analysis

The Chi-squared test was used to compare the occurrence of strains susceptible to or resistant to different antibiotics. $p < 0.05$ was regarded as statistically significant.

Results

Prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis*

Of the 9956 specimens tested, 1856 (18.6 %) were positive for genital mycoplasmas. Of these, 1652 were positive for *U. urealyticum* (89 %), 21 were positive for *M. hominis* (1.1 %), and 183 were positive for both (9.8 %). In 1141 (69 %) of 1652 positive clinical samples, *U. urealyticum* was grown as a single pathogen ($p < 0.001$), in 10 (48 %) of 21 *M. hominis* was grown as a single pathogen, and in 107 (58.8 %) of 183 both urogenital mycoplasmas were grown alone ($p < 0.05$). For the remaining 596 specimens (32.1 %) there was mixed growth including genital mycoplasmas and other pathogens.

In particular, microorganisms mostly detected in association with genital mycoplasmas were *Gardnerella vaginalis*, *Candida* spp., and *Streptococcus agalactiae* group B. Statistically significant association ($p < 0.05$) was observed between *Gardnerella vaginalis* and mycoplasmas (Fig. 1). The distribution of *M. hominis* and *U. urealyticum* according to age group is shown in Table 1. The prevalence of *U. urealyticum* and of both mycoplasmas was

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