

ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *MYCOPLASMA HOMINIS* AND *UREAPLASMA UREALYTICUM* ISOLATED DURING A POPULATION-BASED STUDY CONCERNING WOMEN INFERTILITY IN NORTHEAST ROMANIA

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ABSTRACT

The study was carried out on 1068 infertile women under initial evaluation. For *Mycoplasma hominis*, the highest resistance rates were registered for ciprofloxacin (72.22%), followed by macrolides and ofloxacin. For *Ureaplasma urealyticum*, the ciprofloxacin resistance was also high (51.72%), while the resistance rates to other tested antibiotics were significantly lower.

Key words: Infertility, fluoroquinolones resistance, *Mycoplasma hominis*, *Ureaplasma urealyticum*

Infertility primarily refers to the biological inability of a person to procreate and, nowadays it is estimated that this condition affects approximately 72.4 million women worldwide (4). The childlessness has usually a lot of negative psychosocial consequences that may vary from fear, guilt, self-blame, marital stress, helplessness and depression to loss of social status, divorce or even violence-induced suicide (14).

The evaluation of an infertile individual comprise a broad panel of investigations – a detailed history, a complex physical examination and exhaustive laboratory analyses, in order to identify the main cause of the patient’s impossibility to contribute to a child conception (11). Genital infections are common cause of infertility, often undiagnosed because of their non-specificity of clinical manifestations. Among the micro organisms involved in women’s infertility, the literature

cites the following bacterial species: *Chlamydia trachomatis* (CT), *Ureaplasma urealyticum/Ureaplasma parvum* (UU), *Mycoplasma hominis* (MH) and *Neisseria gonorrhoeae* (NG) (11). Both UU and MH are sexually transmitted bacterial pathogens undoubtedly implied in impairment of reproductive status, although numerous and often contradictory papers concerning their real pathogenic potential have been published last years. Spreading to other body areas is also possible, MH and UU being recovered from synovial fluid in high amounts – up to 10⁷ CFU/ml (13).

Unlike conventional bacteria, MH does not have a rigid cell wall. Hence, they are not susceptible to penicillins and other antibiotics that act on this structure. They are, however, susceptible to a variety of other broad-spectrum antibiotics, most of which only inhibit their multiplication and do not kill

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them. The tetracyclines have always been in the forefront of antibiotic usage, particularly for genital tract infections, but the newer macrolides, the ketolides and the newer quinolones have equal or sometimes greater activity. Mycoplasmas may be difficult to eradicate from human or animal hosts by antibiotic treatment because of resistance to the antibiotic, or because it lacks cidal activity, or because there is invasion of eukaryotic cells by some mycoplasmas. The quinolones also have the advantage of exhibiting some cidal activity (16). Fluoroquinolones are also attractive choices for treating genitourinary tract *Ureaplasma* infections. Data on antimicrobial resistance in ureaplasmas are very limited, because *Ureaplasma spp.* Cultures are rarely obtained for clinical purposes and *in vitro* susceptibilities are almost never performed. Different studies are showing that the level of resistance to doxycycline, josamycin, tetracycline, azithromycin, clarythromycin and pristinamycin is generally low, but the rate of resistance to fluoroquinolones (ofloxacin, ciprofloxacin) is showing an increasing rate in different studies. For example, Xie and Zhang reported >50% resistance in a large number of strains isolated during 1999 and 2004 (19). Clinical isolates of fluoroquinolone-resistant *Ureaplasma spp.* have been also described thus far from France (1, 2) and USA (6). Duffy *et al.* reported the first case of naturally occurring fluoroquinolone resistance in *Ureaplasma spp.* from the United States, probably developed as a result of mutations in the *gyrA* and *parC* genes of the DNA gyrase/topoisomerase IV complex that occurred in the presence of antimicrobial selective pressure (6).

The purpose of this paper was to determine the antibiotic susceptibility profile of MH and UU isolated during a population-based study concerning women infertility in northeast Romania and to identify the most prevalent resistance markers in the respective strains.

Patients

The study has consisted in a screening of 1068 infertile

women presented for initial evaluation in our outpatient clinic from May 2008 to September 2009. The median age of the patients enrolled in the study was 31 years (range 26-42). Approval for the study was granted by the Research Ethics Committee from the "Gr. T. Popa" University of Medicine and Pharmacy, Iasi (Romania). The study was conducted accordingly to the Declaration of Helsinki 2000.

Sample collection

Endocervical samples have been collected in duplicate from all patients using Chlamydia Swab/Brush Collection Kit (Bio-Rad Laboratories, France).

Sample processing

Soon after sampling, the swab has been processed using Mycoplasma IST2 kits (bioMérieux, France) in order to identify MH and UU, and to evaluate the susceptibility of the strains to 9 antibiotics, *i.e.* doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarythromycin, and pristinamycin.

Bacteria detection

The brush has been further submitted to DNA extraction using the DNA-Sorb-A kit (Sacace Biotechnologies, Italy). All extracted DNA samples were processed in an Applied Biosystems 7300 Real Time PCR system (Applied Biosystems, USA) using the *Mycoplasma hominis* Real-TM and *Ureaplasma urealyticum/Ureaplasma parvum* Real-TM kits (Sacace Biotechnologies, Italy). The parameters of amplification were as follows: 95°C for 15 min, followed by 10 cycles of 95°C for 20 s, 65°C for 20 s and 72°C for 20 s, with a last stage of 35 cycles of 95°C for 25 s, 60°C for 30 s and 72°C for 15 s.

After incubation of Mycoplasma IST2 strips, the positive samples have been registered and compared with the results obtained after RT-PCR assay in order to choose only the positive samples for MH and/or UU in the two tests. After the samples processing, we have selected 80 positive samples for

MH and 372 for UU, respectively. For these samples, the susceptibility profiles to the mentioned above antibiotics were analyzed using the manufacturer recommendations.

There were considerable differences in levels of resistance to the antibacterial agents for the two bacterial species. However, the MH strains showed generally higher resistance rates than UU ones. For MH, the highest resistance rates were registered for ciprofloxacin (77.27%), followed by macrolides (azithromycin 38.88%, clarythromycin and erythromycin - 33.33% each) and ofloxacin (27.77%). Lower resistance rates ($p=0.028$) were registered for tetracycline, josamycin, pristinamycin, and doxycycline, *i.e.* 16.66%, 11.11%, 11.11%, and 11.11% respectively (Fig. 1). For UU isolates, the ciprofloxacin resistance was also very high (51.72%), while the resistance rates to the other tested antibiotics were significantly lower ($p=0.022$), *i.e.* ofloxacin (16.09%), erythromycin (16.09%), clarythromycin (9.19%), azithromycin (8.05%), tetracycline (5.75%), pristinamycin (3.45%), josamycin (2.30%) and doxycycline (2.30%) (Fig. 1).

During this study we have reviewed the resistance rates of MH and UU isolated from genital swabs taken from women with infertility problems over the period from May 2008 to September 2009. These bacteria are members of the class *Mollicutes*, commonly referred to as mycoplasmas. The *Mollicutes* are the smallest known free-living microorganisms. MH is involved in the etiology of salpingitis and pelvic inflammatory disease, but its occurrence in sexually active population is lower than UU, with an average of 10% (3, 11, 17). Thus, UU is the most common bacteria of the human urogenital tract (with a detection rate of 67% in sexually active women and 50% in men, respectively) that can cause lower pregnancy rates after *in vitro* fertilization, higher abortion rate of spontaneous pregnancies, increasing of the risk of premature contractions and preterm delivery, puerperal endometritis, orchitis, epididymitis, spermocystitis, prostatitis, urethritis, increased apoptosis in human spermatogens, impairment of semen parameters, less stable chromatin and DNA denaturation

in spermatozoa (5, 7, 8, 10, 12, 15, 18).

Although mycoplasmas evolved from Gram-positive ancestors, the mycoplasmas lack a cell wall and are usually treated with quinolones, tetracyclines, or erythromycin. However, the number of resistant MH and UU strains is increasing every year following the widespread use of these agents.

The susceptibility rates showed that there was a considerable difference in levels of resistance to various antibacterial agents, and that the rate of change was related to the degree of antibacterial use. Doxycycline is still highly effective against MH and UU. Erythromycin, which is one of the most widely used antibiotics elsewhere, demonstrated high resistance rates in MH. Ciprofloxacin, which was commonly used in treating patients with diarrhoea and other infections has demonstrated a high resistance rate in both tested microorganisms. The high resistance rates to ciprofloxacin observed in our strains could be indeed correlated with the antibiotic treatment history in the analyzed patients. Because the history of the patients reported a treatment with fluoroquinolones in the last 12 months (ciprofloxacin in 68 cases, ofloxacin in 56 cases, both in 28 cases and norfloxacin in 4 cases), it is likely that MH and UU isolates could become resistant to these antibiotics used for the treatment of other bacterial infections. For Romania, the explanation of this high resistance percentage to fluoroquinolones occurred in MH and UU strains isolated from human patients is the frequent prescription of these drugs by the general practitioners for the treatment of urinary and respiratory tract infections, pneumonia or otitis, due to their reduced price and lack of side reactions. The mechanism of resistance is probably the occurrence post exposure to fluoroquinolones, of a target alteration located in the DNA gyrase and topoisomerase IV subunits (1, 9).

Concluding, fluoroquinolones resistance was very high among MH and UU isolates, while macrolides resistance was low in UU and high in MH. Doxycycline was active against both organisms, exhibiting a low percentage of resistance.

These results highlighted the emergence of antibiotic resistance in genital mycoplasmas from northeast Romania. Thus, the

treatment of such infections should be guided by antibiotic susceptibility testing and local antibiotic resistance pattern.

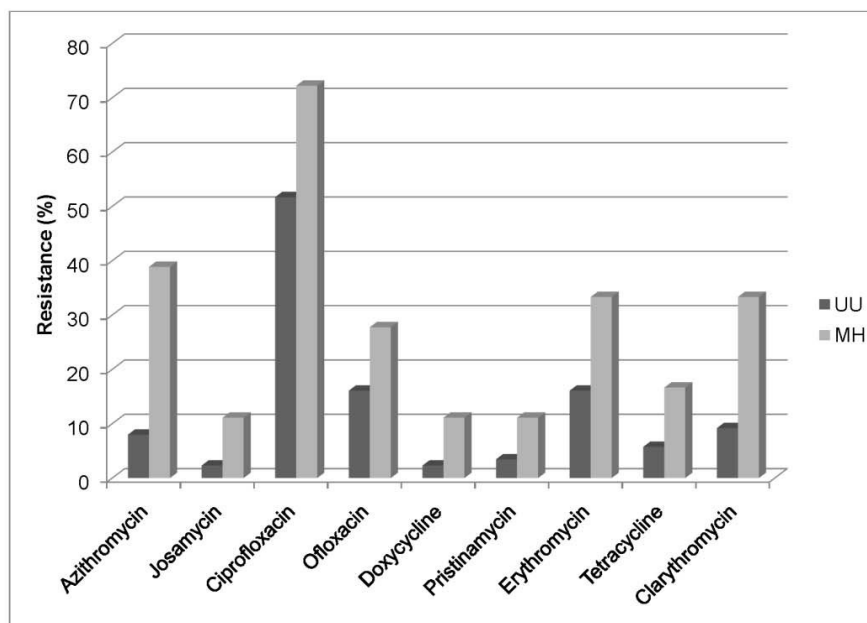



Figure 1. Resistance of *Mycoplasma hominis* and *Ureaplasma urealyticum* strains to the antibacterial agents

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