ORIGINAL RESEARCH PAPER

Anatomy

ANTIMICROBIAL SUSCEPTIBILITY OF MYCOPLASMA AND UREAPLASMA STRAINS FROM GENITAL TRACT

KEY WORDS: *Ureaplasma urealyticum; Mycoplasma hominis;* sensitivity pattern; urethritis.

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ABSTRACT

The aim of this study was to determine the sensitivity pattern of *Mycoplasma hominis* and *Ureaplasma urealyticum* strains from the genital tract among male and female asymptomatic patients. The study series included 99 samples, isolated between 2008-2010 in outpatient clinic, from which 39 positive, 34 *U. urealyticum* and 5 *M. hominis,* respectively. Samples examined were urethral endocervical swab. Detection, quantification and antimicrobial susceptibility testing were performed by Mycoplasma IST-2 test (BioMerieux, Marcy L'Etoile) against nine antimicrobial agents supplied in the kit. *U. urealyticum* was resistant to Ofloxacin (73.5%) and Ciprofloxacin (85.2%) and *M. hominis* to: Erythromycin, Clarithromycin, Azithromycin, Ofloxacin, Ciprofloxacin (100%). Significantly more *M. hominis* were resistant to Tetracycline (60%) than Ureaplasma urealyticum (11.7%). Doxycycline is the drug of first choice in the treatment of U. urealyticum infections, and may be used for co infection with M. hominis; this fact correlates with data from literature, in which this agent is used in the empirical therapy.

Introduction

The genital *Mycoplasmas* occur with important frequency in healthy, sexually active adults. *Ureaplasma urealyticum* can be cultured from the cervix of 40-80% of healthy asymptomatic women and *Mycoplasma hominis* from 21-53% [1].

M. hominis and *Ureaplasmas* colonize the genital tract of humans. They are associated with genital or urinary tract infections (urethritis, cervicitis, cystitis, and bacterial vaginosis) and appear to have an etiological role in postpartum infections in mothers and newborns[2]. Both *Mycoplasma* and *Ureaplasma* genus belong to the family *Mycoplasmataceae*, class *Mollicutes* ("soft skin")[3].

Mollicutes are contained by a trilaminated cell membrane and do not possess a cell wall, making them unique among prokaryotes and differentiating them from bacterial L forms for which the lack of cell wall is a temporary reflection of environmental conditions.

Due to the lack of cell wall, Mollicutes:

- are insensitive to the activity of beta lactam antibiotics
- have pleomorphic forms
- are non-responsive to Gram stain technique[4].

The extremely small genome and limited biosynthetic capabilities explain the parasitic or saprophytic existence of these organisms, sensitivity to environmental conditions and their fastidious growth requirements which can complicate cultural detection[3].

Material and methods

Detection, quantification and sensitivity testing was done by Mycoplasma IST-2 System (Marcy L' Etoile, France). Sample prelevation was done between 2008-2010 in outpatient clinic-Cervical and urethral swabs prelevated from patients by the gynecologist or dermatologist were immediately placed in R1 medium – R1 contains broth with special nutrients that inhibit Gram positive and Gram negative flora and is used for resuspension of R2 vial- and then added to R2 medium, supplied with the kit. The Mycoplasma strip inoculated with rehydrated R2 growth medium and the remaining broth were incubated for 48 hours at 37o C. After 48 hours of incubation, the system provides information on counting, identification and sensitivity testing of Mycoplasma hominis and Ureaplasma urealyticum as presented in tables 1 and 2. The tested antibiotics were: Doxycycline, Josamycin, Ofloxacin, Erythromycin, Tetracycline, Ciprofloxacin, Azithromycin, Clarithromycin and Pristinamycin. Interpretation was done according to manufacturer's indications.

Table No 1. Overview of the test - counting and identification

Cupule no.	Test	Substrate
1	0 (control)	Phenol red (0.05g/L)
2	*Ureaplasma urealyticum*	Phenol red (0.05g/L) Lincomycin
3	*Mycoplasma hominis*	Erythromycin
4	*U. urealyticum* > 10^4 *CFU/ml*	Phenol red (0.05g/L) Lincomycin
5	*M. hominis* > 10^4 *CFU/ml*	Erythromycin Inhibition agent

Table No 2. Sensitivity testing read in cupules 6 - 22 against nine antibiotics

Cupule	Antibiotic		Concen trations mg/L
6..7	Doxycycline	4	8
8.9	Josamycin	2	8
10.11	Ofloxacin	1	4
12.13	Erythromycin	1	4
14.15	Tetracycline	4	8
16.17	Ciprofloxacin	1	2
18.19	Azythromycin	0.12	4
20.21	Clarithromycin	1	4
22	Pristinamycin	2	

Our studied series comprised 99 samples, out of which 39 were positive: 34 *U. urealyticum* and 5 *Mycoplasma hominis.*

Results

Sensitivity pattern is presented in table 3. The most active agents were: Doxycyclin and Pristinamycin with U. urealyticum 8.82% and 5.8% resistant respectively and M. hominis 40% resistant. *U. urealyticum* is resistant to Ofloxacin (73.5%) and Ciprofloxacin (85.2%) and M. hominis to: Erythromycin, Azithromycin, Ofloxacin, Ciprofloxacin in 100%.

Table No 3. Sensitivity testing – results for the studied strains.

Antimicrobial agent	*U. urealyticum*			*M. hominis*		
	S, n (%)	I, n (%)	R, n (%)	S, n (%)	I, n (%)	R, n (%)
Doxycyclin	31 (91.1)	0	3 (8.82)	3 (60)	0	2 (40)
Josamycin	30 (88.2)	0	4 (11.7)	2 (40)	0	3 (60)

Ofloxacin	8 (23.5)	1 (2.94)	25 (73.5)	0	0	5 (100)
Erythromycin	21 (61.7)	1 (2.94)	12 (35.2)	0	0	5 (100)
Tetracycline	29 (85.2)	1 (2.94)	4 (11.7)	0	0	3 (60)
Ciprofloxacin	5 (14.7)	0	29 (85.2)	0	0	5 (100)
Azithromycin	27 (79.4)	1 (2.94)	4 (11.7)	0	0	5 (100)
Clarithromycin	28 (82.3)	1 (2.94)	5 (14.7)	0	0	5 (100)
Pristinamycin	31 (91.1)	1 (2.94)	2 (5.8)	2 (40)	1 (20)	2 (40)

Discussion

In our study there are differences in resistance patterns of the two bacterial species.

The *U. urealyticum* strains showed high resistance to quinolones, 73.5%, 85.2% to Ofloxacin and Ciprofloxacin respectively. For macrolides, resistance rates were: 35.2%, 14.7& and 17.6% for Erythromycin, Clarithromycin and Azithromicin. For the other tested antibiotics, resistance was significantly lower: Doxycycline 8.82%, Josamycin 11.7%, Tetracycline 11.7% and Pristinamycin 5.8%.

For *M. hominis* we encountered a 100% resistance for: Erythromycin, Clarithromycin, Ciprofloxacin and Ofloxacin followed by Josamycin, Tetracycline (60%) and Doxycycline and Pristinamycin (40%).

Our results are comparable with those obtained by Mares et al in 2011 for North East Romania- with high resistance rates in *M. hominis* and *U urealyticum* against Ciprofloxacin (77.27% and 51.72% respectively)[5]. Compared to our results, Singapore *M. hominis* strains were sensitive to fluoroquinolones and Clindamycin but resistant to Azithromycin6. M. hominis from Israel showed high sensitivity to Doxycyline and Tetracycline, but high resistance to macrolides[7].

Concerning *U. urealyticum*, our results conclude that Doxycyclin, Josamycin, Clarithromycin are the most potent agents against this agent, as previously reported[8,9,10]. Results reported from Turkey are comparable with ours – U. urealyticum sensitive to Josamycin and resistant to Ciprofloxacin (40.5%)[11].

The susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* to antibacterial in different regions of the world is quite difficult to compare, because of the different test methods used; less resistance correlates with less usage of a certain antibiotic. In our study, the high resistance ratio found in Ciprofloxacin and Ofloxacin is explained by the extensive use of these antibiotics in Suceava region, for other infections - e.g. urinary tract infections, otitis, pneumonia; these agents can be still bought after self-indication, are cheap and effective in a wide range of situations. Doxycycline and Pristinamycin were active on both species, making these agents the first choice therapy for *Mycoplasma* and *Ureaplasma infection.*

Concerning Tetracycline resistance, this was reported since 1980, mediated by test M gene codifying a ribosome binding protein that protects against this agent. Tetracycline resistance varies with geographical distribution and preexposure to antibiotics[8,12.]

Although several effective antibiotics are available, there is not a unique antibiotic with activity against the high variety of resistance. Therefore, antimicrobial susceptibility tests, correlation between clinicians and laboratories, development of more rapid diagnostic methods, and continuous monitoring of drug resistance are important priorities[13].

Conclusion

This study reveals the trend in antibiotic resistance also for species less studied and reported. Also, the IST - kit used, make possible detection of resistance and the therapeutic choice, correlated with reports from the studied region.

Advantages of using IST 2 system are clear: time for setting and reading the test is 48 hours, compared to culture - 2-6 weeks; gives information on both species identification, counting and sensitivity testing.

The susceptibility patterns seen in our study seem to suggest that it is absolutely necessary to obtain sensitivity reports before initiation of antibiotic therapy.

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