

Statistical Study of Pathology Associated to Urethral Infection with *Ureaplasma urealyticum* and *Mycoplasma hominis*

Chiujdea Natalia-Simona¹, Tilinca Mariana², Chiujdea A³

¹ County Emergency Clinical Hospital Tîrgu Mureş, Clinic of Gynecology II, Laboratory

² University of Medicine and Pharmacy of Tîrgu Mureş, Department of Cell Biology

³ County Emergency Clinical Hospital Tîrgu Mureş, Clinic of Urology

Introduction: *Ureaplasma urealyticum* and *Mycoplasma hominis* are present as part of the normal flora of the urethra, but in some conditions of multiplication they can cause infections of the prostate or sterility.

Material and method: Two-hundred-fifty patients were tested in the laboratory under the proposed criteria of selection. The Stamey test was done for the differential diagnosis of chronic prostatitis. The harvesting was done with Dacron swab, which was then placed in a transport medium. We used the Mycofast Duo kit for the identification and quantification of Mycoplasmas, as well as for the testing of antibiotic susceptibility.

Results: Of the 43 positive cases, 15 presented only *Ureaplasma* and 28 presented both bacterias. Mycoplasmas were found in 8.57% of urethritis cases and 10.25% of prostatitis cases. 33.33% of patients with a diagnosis of infertility had a positive Mycoplasma test. 23.6% of cases were associated with Gram positive or Gram negative infections and 2 with *Chlamydia trachomatis*.

Discussions: The incidence of *Ureaplasma urealyticum* has been reported to be between 9% and 42% in the literature. It is noted this bacteria has a good sensitivity to the new macrolides, josamycine, pristinamycin and roxithromycin. However, Doxycycline is still on the first place, azithromycin on the 4th place, and fluoroquinolones have the lowest sensitivity of all.

Conclusions: Our results suggest that infertility in men is significantly associated with the presence of the studied Mycoplasmas. The treatment of urogenital inflammatory disease would require the corroboration of all the results from the Stamey test, stained microscopic examination, testing for Gram positive and negative bacteria – *Chlamydia trachomatis* and urogenital Mycoplasmas, and an antibiotic or a combination of targeted antibiotics for each pathological strain detected.

Keywords: *Ureaplasma urealyticum*, *Mycoplasma hominis*, infertility, prostatitis

Introduction

Ureaplasma urealyticum and *Mycoplasma hominis* bacteria are often present as part of the normal flora in the urethra. In some conditions of multiplication they can cause infections, being the second cause of non-gonococcal urethritis, a sexually transmitted disease [1,2].

The incidence of colonization and infection with *Ureaplasma urealyticum* and *Mycoplasma hominis* depends on the frequency of sexual activity, the increasing number of colonies being associated with a deficit of hygiene and the colonization and infection with other organisms. All these conditions may lead to the upward inflammatory reaction of the prostate [3].

At a time when the number of infertile couples is increasing worldwide, and the main or associated causes of these states are intensively explored, various studies conducted in the developed countries have shown that the *Ureaplasma* infection and the presence of genital Mycoplasmas can lead to sterility [4].

The objective of our study is to assess the relationship between the incidence of Mycoplasmas and the presence of chronic prostatitis and infertility.

Material and methods

The study included 250 patients with urogenital pathology which indicated the removal of urethral discharge before

and after prostatic massage, with specification of tests for *Mycoplasma* and *Ureaplasma*. The patients were tested in two private laboratories between September 2007 – December 2010.

The patients were included in the study using the following inclusion criteria:

- ▶ the presence of prostatitis or urethritis or infertility;
- ▶ patients with Stamey test and spermogram;
- ▶ the presence of paraclinic documentation.

The exclusion criteria were:

- ▶ the presence of more than one infection associated with Mycoplasmas;
- ▶ the lack of necessary documentation;
- ▶ the presence of kidney disease.

The Stamey test is the most important test for the differential diagnosis of chronic prostatitis. The first sample is harvested from the first 10 ml of urine. The second sample consists of 10 ml of urine collected from the middle urinary stream and the urethral expressed prostatic secretion (EPS). Finally, a 10 ml urine sample is collected after the urethral secretion.

Harvesting is done with Dacron swabs, with calcium alginate or polyester, plastic and aluminum handle, since

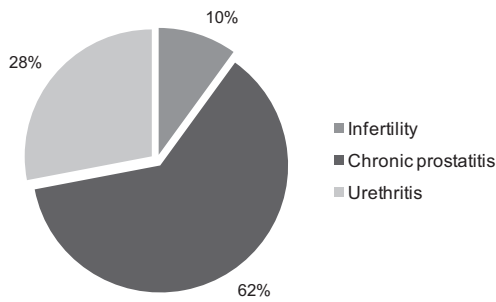


Fig. 1. The distribution of clinical diagnosis

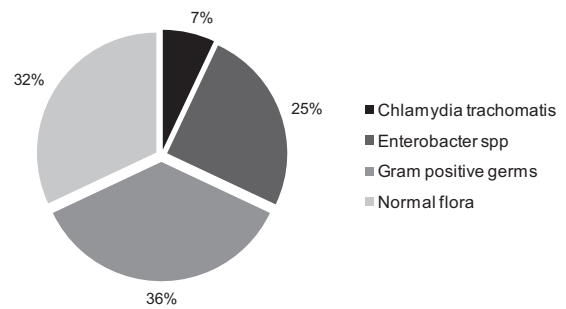


Fig. 2. Bacterial analysis of positive Mycoplasma cases

other materials may have inhibitory effect on Mycoplasmas. The swab has to vigorously rotated in the urethra, in order to obtain cells potentially infected with Mycoplasmas. One swab will be placed in the transport medium, because microorganisms are very sensitive to temperature, humidity and UV rays. The other swab will be used for Chlamydia tests. The rapid method for testing the Chlamydia recognizes the markers from bacteria within 20 minutes. The Chlamydia Rapid Test Device is a qualitative, lateral flow immunoassay for the detection of Chlamydia antigen from male urethral swab. In this test, the antibody specific to the Chlamydia antigen is coated on the test line region of the test. During the testing, the extracted antigen solution reacts with an antibody to Chlamydia that is coated on to particles. The mixture migrates up, to react with the antibody to Chlamydia on the membrane and generates a colored line in the test line region. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. Samples can be stored for 48 hours at 2–8 °C. No local antiseptics or lubricants must be used before. The Mycofast Duo kit combines the organisms in the micro-culture in selective broth with 22 biochemical tests for identification and quantification of Mycoplasmas and antibiotic susceptibility testing. If a broth culture is positive, then the specific substrates (urea for Ureaplasma urealyticum and arginine for Mycoplasma hominis) and the phenol red indicator present in the broth will change colour from yellow-orange to red, as a result of increasing pH. After inoculation, the broth is dispersed into the strip, and the reading is made at 24 h and 48 h. The results simultaneously provide the identification and semi-quantitative

assessment of the antibiotic susceptibility testing. The criterion for positivity for Mycoplasma hominis is $\geq 10^4$ CFU/ml, and for Ureaplasma urealyticum there are three variants: 10^3 , 10^4 and $\geq 10^5$. Reference values: Negative Ureaplasma urealyticum and negative Mycoplasma hominis.

The statistical processing of data was performed using two software: Microsoft Office Excel 2003 for some of the processing (graphics, sorting, etc) and Epi Info 6 for other statistical tests (chi-square, etc). Note that it was considered statistically significant $\chi > 3.84$ and $p < 0.05$.

Results

Out of the 250 patients included in the study, we found 43 cases positive for Ureaplasma urealyticum and/or Mycoplasma hominis.

Three clinical diagnosis were found: chronic prostatitis, infertility and urethritis (Figure 1).

Out of the 43 positive cases, 15 were positive for Ureaplasma and 28 were positive for both Mycoplasmas.

In 19 of the 28 patients positive for both Mycoplasmas, we also found other pathogens in the urethral secretion (Figure 2).

Among the tested antibiotics were the macrolids azithromycin, erythromycine, roxitromycin, pristamycin and josamycin and the quinolones ofloxacin and ciprofloxacin. It is noted that the studied bacteria have a good sensitivity for new macrolides, with a 93.02% sensitivity for josamycin, 86% for roxitromycin and 83.72% for pristamycin. Doxycycline is still on the first place with 95.35% sensitivity. No microbial agent has 100% sensitivity. Given their poor response, sulphamide and erythromycin were tested only on a few cases and were excluded from the final statistics (Figure 3).

To determine whether there are any statistical differences between the cases with urogenital disease associated with one or both studied microorganisms and the cases with the presence of the disease, we used the Pearson test and 2x2 contingency tables to calculate the risk ration (RR), odds ratio (OR), chi test and p (probability). The results are presented in Table I.

Fisher test statistics include $p = 0.22$ and $\chi^2 = 4.83$, $OR = 2.73$, respectively $RR = 2.40$. These results suggest that infertility is associated in a statistically significant manner with the presence of the studied microorganisms.

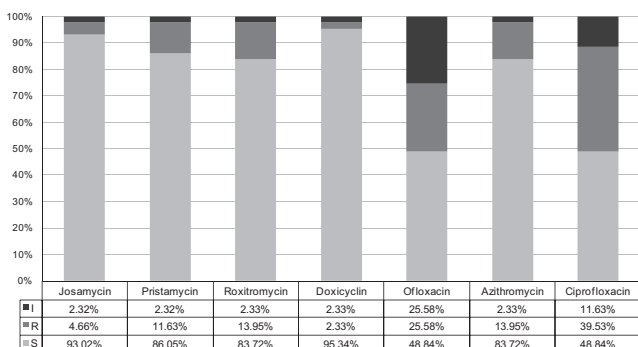


Fig. 3. Antibiogram for Mycoplasmas

Table I. Correlation with the presence of infertility cases

	Infertility	No Infertility	Total
Myc/Ur positive	8	35	43
Myc/Ur negative	16	191	207
Total	24	226	250

Table II. Correlation of prostatitis with the presence of microorganisms by Pearson test

	Chronic prostatitis	No chronic prostatitis	Total
Myc/Ur positive	16	27	43
Myc/Ur negative	140	67	207
Total	156	94	250

Table III. Association with the presence of ureaplasma urealyticum urethritis and / without Mycoplasma hominis

	Urethritis	No urethritis	Total
Myc/Ur positive	6	37	43
Myc/Ur negative	64	143	207
Total	70	180	250

With a χ^2 of 13.99 and $p = 0.0001$, the association of prostatitis with the presence of microorganisms is statistically significant. Although the OR is less than 0.28 and RR 0.55, i.e. the probability of prostatitis is not higher in patients positive for Mycoplasmas, however, between the disease and the presence of microorganisms is a statistically significant correlation.

With a risk of exposure of 13.95% and non-exposure of 30.92%, OR = 0.36, there is no indication that Mycoplasmas represent a risk factor for urethritis, but statistically, the values of χ^2 5.06 and $p < 0.01$ are significant.

Discussions

Ureaplasma urealyticum was present in 15% of the studied cases, in accordance with data from the literature reporting values between 9–42% [5]. Data from the literature also suggest that while no pathogenic microorganisms are found in 25% to 45% of patients with urethritis, mixed pathogens are detected in 7% to 23% of them [6]. In the present work, Mycoplasmas were found in 8.57% of urethritis cases and 10.25% of prostatitis cases.

A study by Naessens and colleagues [7] at a fertility clinic evaluated semen and sperm characteristics in relation to these microorganisms. *Ureaplasma urealyticum* was significantly correlated with disturbances of semen samples ($p < 0.005$). The parameters most affected were the sperm count ($p < 0.005$) and motility ($p < 0.05$).

In our study 33.33% of patients with a diagnosis of infertility were positive for Mycoplasmas. Various studies

conducted in developed countries have shown that infection with genital *Ureaplasma*, *Mycoplasma* and *Chlamydia trachomatis* can lead to sterility [4,8].

Fifteen per cent of the tested group were positive when tested for *Ureaplasma urealyticum* and/or *Mycoplasma hominis*. Among positive cases, 100% were *Ureaplasma* positive and 65.11% were positive for both microorganisms. The secretion was sterile in 59.2% of cases, but in 23.6% of cases a Gram positive or Gram negative infection was present. With regard to *Chlamydia trachomatis*, there were two cases associated with Mycoplasmas.

Conclusions

1. Statistical calculations suggest that infertility in men is significantly associated with the presence of Mycoplasmas. Even if Mycoplasmas do not represent a risk factor for urethritis and prostatitis, there are statistically significant differences among patients with positive Mycoplasma samples.
2. The results of treatment with macrolides, tetracyclines and fluoroquinolones and the presence of resistance to antimicrobial therapy demonstrate the importance of etiologic diagnosis in prostatitis, urethritis and infertility, including Mycoplasmas, before initiating antibiotic treatment.
3. The proper treatment of urogenital inflammatory conditions requires the corroboration of all the results from the Stamey test, stained microscopic examination, testing for Gram positive and negative bacteria – *Chlamydia trachomatis* and urogenital Mycoplasmas, and an antibiotic or a combination of targeted antibiotics for each pathological strain detected.

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