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Original Article

Prevalence and Treatment of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* in Patients with Non-Gonococcal Urethritis

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SUMMARY: The aim of present study was to evaluate the occurrence of *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* in non-gonococcal urethritis (NGU) and to determine the bacterial resistance to six antibiotics in order to determine the most suitable treatment strategy. A total of 50 patients were enrolled into the study. Urethral samples were taken with a dacron swab placed into urethra 2 - 3 cm in males, and vaginal samples were taken from the endocervical region in women. The patient samples that did not grow *Neisseria gonorrhoeae* were accepted as NGU. Direct immunofluorescence technique was used for the investigation of *C. trachomatis*. Mycoplasma IST was used for the isolation of *M. hominis* and *U. urealyticum*. *U. urealyticum* was isolated from 24 patients. Thirteen of them had only *U. urealyticum*, and the rest had mixed pathogen organisms (7 *U. urealyticum* + *M. hominis*; 3 *U. urealyticum* + *C. trachomatis*, and 1 *U. urealyticum* + *M. hominis* + *C. trachomatis*). *C. trachomatis* was detected in 12 patients. While 8 patients had *C. trachomatis* only, the rest had a mixture of the pathogen organisms listed above. Partner examinations could be performed for only 22 patients' partners. In the evaluation of antibiotic susceptibility, higher resistance was obtained against ofloxacin in *U. urealyticum*, and against erythromycin with *M. hominis*. Our results indicated that doxycycline or ofloxacin should be the first choice when empirical treatment is necessary.

INTRODUCTION

Urethritis is clinically defined as leukorea and urethral inflammation characterized by dysuria and purulent or mucoid discharge. Urethritis is generally classified into two large groups, gonococcal and non-gonococcal urethritis (NGU), as a basis for conventional treatment strategies. The pathogen of gonococcal urethritis is *Neisseria gonorrhoeae*. The vast majority of the etiological microorganisms constituting of NGU is *Chlamydia trachomatis*, followed by *Ureaplasma urealyticum*, *Mycoplasma genitalium*, and *Mycoplasma hominis* (1,2). *Trichomanas vaginalis*, *Candida albicans*, and herpes simplex virus are the other less common pathogens (3,4).

C. trachomatis is usually described as an obligate intracellular pathogen and accounts for 30 - 40% of etiopathogenesis of urethritis. Diagnosis can be made by showing 300 - 400 nm elementary bodies under immunofluorescent microscope (1,3,5). *M. hominis* and *U. urealyticum* are species in the family *Mycoplasmataceae*, which is the smallest bacteria replicating in culture medium and they do not possess peptidoglycan cell walls. These tiny microorganisms can be found commensal in lower genitourinary tracts of sexually active men and women. Moreover, they cause many disorders such as NGU, postpartum fever, infertility, and pelvic inflammatory disease (3,6,7).

Centers for Disease Control and Prevention (CDC) has announced guidelines regarding the epidemiology, changing concepts of diagnostic tools, and treatment strategies of sexually transmitted diseases (8). However, the epidemiological distribution of sexually transmitted diseases based on geographical regions, new diagnostic tests, and new treatment regimens due to bacterial resistance have expanded our standard knowledge (4,7).

We evaluated the occurrence of *C. trachomatis*, *M. hominis*, and *U. urealyticum* in NGU and searched for bacterial resistance against six antibiotics in order to determine the most suitable treatment strategy.

MATERIALS AND METHODS

Between July 2000 and July 2001, *N. gonorrhoeae*, *M. hominis*, and *U. urealyticum* were investigated in patients who had been admitted to the Departments of Urology and Infectious Disease and Clinical Microbiology, with signs of urethral discharge and/or urethral discomfort and lower urinary tracts sysmptoms including dysuria, pollakiuria, and nocturia.

After obtaining detailed histories, male patients were evaluated by means of rectal examination for prostatic disease, and female patients had gynaecological examination for infections and cystocele. Urine analysis and urine culture were performed in all patients. In addition, prostate size was evaluated by transrectal ultrasonography, and bladder outlet obstruction was investigated by uroflowmetry and a postmicturation ultrasonography in male patients. After these investigations, 50 patients were enrolled into the study. Male patients who had positive urine cultures, prostate disease, or bladder outlet obstruction, and females with cystocele and urinary tract infection were excluded from the study.

Urethral samples were taken with a dacron swab placed into the urethra 2 - 3 cm and turned to obtain as many cells as possible after cleaning the external meatus without using antibiotics. The subjects had not urinated for at least 3 h. In women, vaginal samples were taken from the endocervical region after exocervical mucus had been cleaned with a swab

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without using local antiseptics. Urethral samples were taken three times from all patients. The first sample was prepared for direct microscopic analysis involving staining with methylane blue and Gram, and were plated on modified Thayer-Martin agar and chocolate agar and incubated at medium CO_2 of 5%. Colonies were evaluated after 24 or 48 h of incubation. When the amount of white blood cell (WBC) were greater or equal to 5 WBC per high power field in urine sediment or urethral smear, male patients with urethral cultures Gram stain-negative for gonococci were accepted as NGU (3,8).

The patients that did not grow *N. gonorrhoeae* were accepted as NGU, and *C. trachomatis*, *M. hominis*, and *U. urealyticum* were investigated in these patients. The direct immunofluorescence (DIF) technique was used for the investigation of *C. trachomatis* from the second urethral samples. In this method, a sample was prepared into fluorescence slide glass. After being dried in air, the samples were fixed with cold methanol for 5 min. Twenty-five microliters *C. trachomatis* monoclonal antibody (Chlamydia-Cel IF test, Cellabs, Brookvale, Australia) was dropped on these slides, which were incubated at 37°C for 30 min in a dark and humidified condition. After washing with phosphate buffered saline for 1 min, mounting substance including glycerol was dripped. The preparation was examined using an immunfluorescent microscope.

Mycoplasma IST (BioMerieux, Marcy L'etoile, France) was used for the isolation of *M. hominis* and *U. urealyticum*. The third samples taken from patients were placed in R1 medium, which inhibits the growing of Gram-positive and -negative bacteria and includes nutrients. Then, 3 ml from this mixture were added to R2 medium including maya extract, horse serum, urea, arginin, polyVitex, and antibiotics. This solution was vortexed until lyophilized pellet was definitely melted. A Mycoplasma IST strip, consisting of 16 wells, was then inoculated with the rehydrated R2 growth medium (50 μl per well, overlaid with mineral oil). The remainder of the broth and the inoculated strip were incubated at 37°C and observed for color changes, and the results were interpreted after 24 and 48 h of incubation. The strips provided information regarding the presence or absence of *M. hominis* and *U. urealyticum*, an estimate of the density of each organism ($\geq 10^4$ CFU) and its antimicrobial susceptibilities to doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, and pristinamycin.

RESULTS

Of the 50 patients enrolled in the study, 43 (86%) were men and 7 (14%) were women. Their average ages were 39.2 ± 12.6 and 37.3 ± 10.5, respectively. On swab samples, microorganisms showing Gram and methylene blue staining were not detected. More than 10 WBC on each high power microscope area were found in 23 patients (46%) and less than 10 in 27 patients (54%).

U. urealyticum was isolated from 24 patients. Thirteen of them had only *U. urealyticum*, and the rest had mixed pathogen organisms (7 *U. urealyticum* + *M. hominis*; 3 *U. urealyticum* + *C. trachomatis* and 1 *U. urealyticum* + *M. hominis* + *C. trachomatis*). *C. trachomatis* was detected in 12 patients. While eight patients had *C. trachomatis* only, the rest had a mixture of the pathogen organisms listed above. Finally, pathogen microorganisms were isolated or detected from 32 patients. Partner examinations could be performed for only 22 patients' partners. The distributions of isolated or detected microorganisms from the patients and their partners are shown in Table 1.

Although doxycycline was administered to 24 (75%) patients and ofloxacin to 8 (25%) as empirical treatments, these antibiotic treatments were replaced with other treatment alternatives according to later antibiotic susceptibility results. However, these changes were necessary in two patients using doxycyline and one patient using ofloxacin. The antibiotic susceptibility in the partners was the same in the patients. At the end of the treatment, all cases were cured microbiogically and clinically in follow up. In evaluation of antibiotic susceptibility, higher resistance was seen against ofloxacin by *U. urealyticum*, and against erythromycin by *M. hominis* (Table 2). No intermediate resistance was observed.

Table 1. Results of Mycoplasma IST kit or direct immonofluorescence (DIF) test from patients and their partners

	Patients n =50	Evaluated partner n = 22	Detected or isolated microorgansim from partners of patients n = 22
U. urealyticum only[2]	13 (26%)	10	*U. urealyticum* only 6 (60%)[1] *U. urealyticum* + *M. hominis* 3 (30%) No detected 1 (10%)
M. hominis only[2]	–	–	
C. trachomatis only[3]	8 (16%)	8	*C. trachomatis* only 5 (62.5%) No detected 3 (37.5%)
Mixed[4]	11 (22%)	4	No detected 4 (100%)
U. urealyticum + *M. hominis*	7	–	
U. urealyticum + *C. trachomatis*	3	3	
M. hominis + *C. trachomatis*	–	–	
U. urealyticum + *M. hominis* + *C. trachomatis*	1	1	
Total	32 (64%)	22	14 (63.6%)

[1] Percentage of partner was calculated based on isolated and detected microorganisms in partners.
[2] Mycoplasma IST kit.
[3] DIF test.
[4] Mixed.

Table 2. Resistance rates of *U. urealyticum* and *M. hominis* to six different antibiotics

Antibiotics	*U. urealyticum* (%) *n* = 24	*M. hominis* (%) *n* = 8
Doxycycline	4.2	0
Tetracycline	4.2	12.5
Ofloxacin	16.7	12.5
Erythromycin	12.5	50
Josamycin	0	0
Pristinamycin	8.3	37.5

DISCUSSION

In spite of the progress in the development of diagnostic methods, the number of NGU cases with unknown etiology has been increasing (1,2,9). NGU has shown up with different epidemiological characteristics due to different cultural features. Therefore, the distribution of agents and the susceptibility of the antibiotics changed in terms of the time and geographical region (7,10,11,12). Mycoplasma IST is a commercial kit that identifies *Mycoplasma* and shows sensitivity to antibiotics. There was not any difference between the results of culture and those of the Mycoplasma IST kit in regard to the isolation of *Mycoplasma* (9). Therefore, we preferred using the Mycoplasma IST kit alone in the present study.

In our study, *C. trachomatis* was observed in 12 (24%) patients, four of which had mixed infections (Table 1). This ratio was similar to the ratios reported in the literature (5.8 - 38%) (1, 9, 13).

U. urealyticum has been reported to be between 9 and 42% in previous studies (14,15). It has been known that *U. urealyticum* can be found as commensal in healthy populations (3). For that reason, *U. urealyticum* can be found not only as a pathogen but also as a colonization. However, colony number was found to be more than 10^4 per ml in all cases. Therefore, WBC number is helpful to clarify the diagnosis of commensal mycoplasmal and ureaplasmal infection (8,13). In our study, WBC number was more than 10 cells per ml in 23 (46%) of our patients.

While the rate of colonization of *M. hominis* in urogenital tracts was reported to be between 4 - 13% in men and 21 - 54% in women, it was found between 2 - 84% in patients with NGU in different studies (14-16). It has been thought that these great differences in ratio are due to different laboratory methods, and to various geographical and cultural features in selected patients.

In the literature, while the pathogenic microorganisms have not been detected in 20 - 45% of patients with urethritis, mixed pathogens have been found approximately 7.4 - 23% of them. In these cases, *U. urealyticum* + *M. hominis* or *C. trachomatis* + *U. urealyticum* were generally found (14,15). In our study, although *M. hominis* was not isolated alone in any patient, it was detected together with *U. urealyticum* in eight (16%) patients. *C. trachomatis* and *U. urealyticum* were observed together in four patients (8%). Three microorganisms coexisted only in one patient (2%). Thus, 11 patients had mixed cultures and our result is similar to those of previously reported studies (14,15).

Although many different treatment alternatives are available for the treatment of NGU, doxycycline is the most commonly used antibiotic (8,12). It was found to be effective in the treatment of *U. urealyticum* in approximately 89 - 90% of cases (7,10,11,14). However, resistance to tetracycline due to the tetM determinant has been observed in both *U. urealyticum* and *M. hominis* throughout the world (3,12). Ullmann et al. (7) reported that the susceptibility of doxycycline was decreased for both *U. urealyticum* and *M. hominis*. Macrolides and, especially, quinolons have been new treatment alternatives with very high efficacy (7,10,11). In the present study, we have chosen two antibiotics, which are primary and secondary antibiotics for the treatment of NGU according to the recommendations of the CDC (8).

While the resistance of *U. urealyticum* against tetracycline was very low (4.2%) in our patients, contrary to current information regarding this drug, *U. urealyticum*'s resistance to ofloxacine was 16.7% (7). We thought that this condition depended on the development of resistance due to common widespread usage of quinolones in urinary tact infections. The resistance to erythromycin against *U. urealyticum* was found to be 12.5%. Erythromycin resistance was very high against *M. hominis* (50%). Although we did not observe any resistance against a new macrolide josamycin, which has not been in the market in Turkey, there was a high resistance rate against pristinamycin, which is another macrolide that has not been used in our country. There was no resistance against doxycycline (Table 2).

Since sexual transmission may not always be present, routine partner examination should not be suggested for urethritis except in cases of *Chlamydia* infection. However, Keane et al. (1) found that partner positivity was 26%, and Lin et al. (5) reported that 65% of *C. trachomatis* detected in the partners of patients was the same serovar. This high difference was attributed to cultural factors, and fewer sexual partners decrease the incidence of contamination. In our study, the partner positivity rate was 41.6% (5/12) for *C. trachomatis* and 64.3% (9/14) for *U. urealyticum* and *M. hominis*. Since our series displayed a high partner-positive ratio, the evaluation of partners can be justified and is suggested.

In conclusion, all patients with urinary symptoms should be evaluated for these three pathogens as the cause of NGU. *U. urealyticum* is the most common pathogen of NGU because of its ease of transmission with sexual intercourse and easy diagnosis using antigenic methods and cultures (4,12,16). Although the investigation of *C. trachomatis* using the DIF method is sensitive and fast, cultures have still been needed to increase diagnostic accuracy. Partner examination must be performed for effective treatment and prevention of the distribution of NGU.

Our results indicated that it should be kept in mind that empiric treatment has been ineffective in many cases, although in the past it was used commonly and deemed to be effective. Furthermore, antibiotics such as erythromycin and tetracycline, believed to be standard treatments of NGU, have not been effective in empirical treatment. However, when empiric treatment is considered, quinolones and doxycycline seem to be the most suitable treatment alternatives. Though new-generation drugs, such as josamycin which is 100% effective against NGU, have been developed, their effectiveness in some geographical areas may have shifted due to the drugs' long term usage because of rapid resistance development (12). Opinion is increasing that *U. urealyticum* and *M. hominis* have resistance to pristinamycin, which is another new generation antibiotic that has not been marketed in Turkey because of cross-border interaction with resistant agents.

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