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Mycoplasma Infections and Their Resistance Phenotypes in a Southwestern Area of China

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Abstract

The aim was to determine the prevalence and antibiotics resistance of Uroaplasma urealyticum (*U.urealyticum*) and Mycoplasma hominis (*M. hominis*) isolated in a southwestern area of china. A total of 1093 patients with Chronic Prostatitis/ Chronic Pelvic Pain Syndrome (CP/CPPS) were included and Expressed Prostatic Secretion (EPS) were collected from the subjects. Antibiotic resistance tests were conducted by using the mycoplasma kits. Of the individuals studied, 17.29% (189/1093) and 3.66% (40/1093) samples were respectively identified to be positive for *U.urealyticum* and *M. hominis* in EPS. *U.urealyticum* were less than 10% (0-8.99%) resistance to doxycycline, minocycline, azithromycin and josamycin, while they were higher than 60% (60.85%-73.54%) resistance to ciprofloxacin, ofloxacin and gatifloxacin, and the resistance incidence of erythromycin to *U.urealyticum* was 39.15%). The resistance rate of *M. hominis* was less than 10% (0-2.5%) to doxycycline, minocycline and josamycin, while they were higher than 60% (62.50%-87.50%) to azithromycin, erythromycin, ciprofloxacin and ofloxacin, and that of gatifloxiacin was 32.50%. In conclusion, testing for both *U.urealyticum* and *M. hominis* in EPS of patients with CP/CPPS should been encouraged and doxycycline, minocycline and josamycin were recommended to treat infections of these strains in patients with CP/CPPS in a southwestern area of china. Further investigations should be focus on the new promising antibiotics against *M. genitalium* due to increasing resistance of antimicrobial.

Keywords: *Uroaplasma urealyticum*; *Mycoplasma hominis*; Chronic prostatitis/ chronic pelvic pain syndrome; Resistance

Introduction

Mycoplasma, a slow growing organism, is the smallest prokaryote capable of independent replication, and much about its natural features is still unknown until it was first isolated from urethral exudates of male patients [1], *Uroaplasma urealyticum* (*U.urealyticum*) and *Mycoplasma hominis* (*M. hominis*) are well recognized as two major organisms causes Non-Gonococcal Urethritis (NGU) in male patients, with an increasing body of evidence to suggest it also play an important role in occurrence of prostatitis, orchitis, epididymitis, and infertility [2].

There are three antibiotic families, including tetracyclines, macrolides and fluoroquinolones and related antibiotics, were regarded as potent drugs against *U.urealyticum* or *M. hominis*, but high resistance of tetracycline was found due to presence of the tet (M) gene [3]. The macrolide azithromycin is considered as the first-line treatment for *U.urealyticum* or/and *M. hominis* in most of western countries, but a rapid decline of its efficacy was documented, from 85% before 2009 to 67% since 2009, and the highest incidence of resistance was reported in the Asia-Pacific area [4]. Fluoroquinolones was deemed as the second-line treatment, but, unfortunately, recent decades has seen a reducing cure rate from 96% prior to 2010 to 89% after 2010 [1].

Reportedly, prostatitis symptoms of more than 80% patients with positive *U.urealyticum* or/and *M. hominis* in Expressed Prostatic Secretion (EPS) were markedly improved with azithromycin in ShangHai, an eastern metropolis in china [5]. However, Josamycin, doxycycline, and minocycline were recommended for the clinical treatment of patients infected with *U.urealyticum* or *M. hominis* according to antibiotic susceptibility in XI`an, a western metropolis in china [6]. It suggests that there is different antibiotics susceptibility against *U.urealyticum* or *M. hominis* in various areas, may be due to unique environment and genetic heterogenetic.

The aim of the present study was to determine the prevalence of *U.urealyticum* or *M. hominis* and the resistance levels of tetracyclines, macrolides, fluoroquinolones and related antibiotics against these strains in patients with category III or

Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) in southwestern area of china.

Material and Methods

Clinical samples

A total of 1093 patients with CP/CPPS, whom were outpatients attending two hospitals in a southwestern area of china (the Second Affiliated Hospital of GuiLin Medical University and the Central Hospital of ShaoYang) were analyzed from January 2017 to December 2020.

EPS was obtained from the urogenital tracts of patients with CP/CPPS, in which non-bacteria were cultured, after cleaning of urethral meatus using sanitizer. The inoculation and incubation of samples were conducted according to the manufacture`s guidelines of a mycoplasma IST kit (Upper Bio-Tech, ShangHai, China), and presence and absence of *U.urealyticum* or *M. hominis* and antibiotics susceptibility to doxycycline, minocycline, azithromycin, erythromycin, josamycin, ciprofloxacin, ofloxacin and gatifloxacin was obtained from this kit.

Statistical analysis

The occurrence of strains susceptible and resistance to different antibiotics was compared by chi-squared test or Fisher`s exact test. A p-value<0.05 was considered as statistically significant.

Results

Mycoplasma in EPS was found positive in 229 (20.95%) samples of the 1093 tested specimens. In the positive samples, 189 (82.53%) were positive for *U.urealyticum* and 40 (17.47%) were positive for *M. hominis*. The positive incidence of *U.urealyticum* was significantly higher than that of *M. hominis* in EPS of patients with CP/CPPS (P<0.05).

In the 229 positive sample of mycoplasma in patients with CP/CPPS, *U.urealyticum* were less than 10% (0-8.99%) resistance to doxycycline, minocycline, azithromycin and josamycin, while they were higher than 60% (60.85%-73.54% resistance to ciprofloxacin, ofloxacin and gatifloxacin, and the resistance incidence of erythromycin to *U.urealyticum* was 39.15% (**Table 1**). The resistance rate of *M. hominis* was less than 10% (0-2.5%) to doxycycline, minocycline and josamycin, while they were higher than 60% (62.50%-87.50%) to azithromycin, erythromycin, ciprofloxacin and ofloxacin, and that of gatifloxiacin was 32.50% (**Table 1**). The different in resistance rate of all antibiotics to *U.urealyticum* or *M. hominis* is obvious significantly (P<0.05) (**Table 1**).

Table 1: Antibiotics resistance of *U.urealyticum* or *M. hominis* in 229 positive samples of EPS in patients with CP/CPPS.

Number of strains resistance (%)			
	***U.u* (n=189)**	***M,h* (n=40)**	**p**
doxycycline	1(0.53)	0(0.00)	<0.05
minocycline	3(1.59)	1(2.50)	
azithromycin	17(8.99)	31(77.50)	
erythromycin	74(39.15)	35(87.50)	
josamycin	3(1.59)	1(2.50)	
ciprofloxacin	139(73.54)	27(67.50)	
ofloxacin	115(60.85)	25(62.50)	
gatifloxacin	117(61.90)	13(32.50)	

Discussion

2%-10% of men are suffered from prostatitis, which is classified into four categories (category I-IV prostatitis) according to National Institutes of Health (NIH) classification, and category III or Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) accounts for 90%-95% prostatitis [7]. Patients with CP/CPPS are often suffering from lower urinary tract symptoms, genital pain, abdominal discomfort, ejaculatory pain and erectile dysfunction. Although CP/CPPS patients do not have evidences of bacterial infections in urinary and genital tract according to definition, but many studies had attempted to discover 'hidden' pathogen in urine or EPS, in which *Staphlococcus aureus* and *Burkholderia cenocepacia* were detected but the etiologic significance of these finding is not clarification and more studies should continue.

Some atypical organisms were considered been involved within symptoms of patients with CP/CPPS. Atypical pathogenic microorganisms, including Ureaplasma urealyticum (*U.urealyticum*), Mycoplasma hominis (*M. hominis*), Chlamydia Trachomatis (CT) and nanobacteria, are regarded as important causes of this condition, and *U.urealyticum* was found in 17.0%-22.4% EPS of patients with CP/CPPS without analysis of antibiotics resistance against these strains in previous study [8]. In previous studies, female patients with pain voiding symptoms who have been found to have *U.urealyticum* benefit from erasing of the pathogen, suggesting that *U.urealyticum* may play a role to some extent in pelvic pathology.

In present study, *U.urealyticum* and *M. hominis* were respectively found in 17.29% (189/1093) and 3.66% (40/1093) EPS of patients with CP/CPPS. In the patients infected by mycoplasma, *U.urealyticum* infection (82.53%) is mostly found, followed by *M. hominis* single infection (17.47%). The positive rates and distribution of these infections types were mostly in line with previous studies conducted in Xi`an, China and Africa [6, 9]. In study conducted by Rerambiah, incidence of mixed infection of U.urealyticum and M. hominis is higher than monoinfection of M. hominis but lower than *U.urealyticum* [9]. The samples of Africa study, including sperm, urine, ureteral or vaginal swabs, is different from this study, which may be contributed to discrepancy between two studies.

Macrolides, tetracyclines and fluoroquinolones are selected to use for treatment of mycoplasma infections. C14 macrolides (erythromycin, clarithromycin, azithromycin etc.) is considered resistant to *M. hominis*, whereas moderate resistance to *U.urealyticum* is found [9] Ours results in present study were

consistent with these facts. In the macrolides, josamycin showed the lowest resistance against both *U.urealyticum* (1.59%) and M. hominis (2.50%), and *M. hominis* was less active to both erythromycin and azithromycin than *U.urealyticum*.

In present study, tetracyclines, including doxycycline and minocycline, was proved to be more active against both *U.urealyticum* and M. hominis than macrolides and fluoroquinolones, and doxycycline showed the lowest resistance (0.53% and 0%, respectively) in all antibiotics, which was in agreement with previous study [9]. However, tetracycline reportedly has a decreasing activity against mycoplasma and the emergence of tetracyclines resistance should raise concerns [10].

Ciprofloxacin, ofloxacin and gatifloxacin were found to be less effective against *U.urealyticum* than macrolides, doxycycline and minocycline, but less resistant to *M. hominis* than macrolides in present study. Gatifloxacin showed more activity against *M. hominis* than ciprofloxacin and ofloxacin, whereas significant difference was not found in resistance against *U.urealyticum* in ciprofloxacin, ofloxacin and gatifloxacin, all which have an intermediate (I) to resistant (R) profile. Consistent with other reports, ciprofloxacin a have ineffective against majority of *U.urealyticum*, but contrary to these study, ofloxacin proved to be relatively ineffective against *U.urealyticum* [3,9].

Huerta reviewed the literature published from 2012 and 2018 updates antimicrobial resistance data in *M. genitalium* in Europe, he found that exceeding 50% of resistance rate in macrolides and increasing tendency of resistance in fluoroquinolone [11]. Miyake and associates found that more advanced prostate cancer was demonstrated in patients with positive *M. genitalium* that negative ones and significantly higher incidence of *M. genitalium* in biopsy samples of prostate cancer compared with benign prostatic hyperplasia was detected by Erturhan [12,13]. Given high resistance of antimicrobial to *M. genitalium* in Asia, Africa and Europe and possible role of *M. genitalium* in etiopathogenesis of prostate cancer, it is suggested that the new promising antibiotics are urgently required to overcome resistance against *M. genitalium* in the future.

Conclusion

Testing for both *U.urealyticum* and *M. hominis* in EPS of patients with CP/CPPS should been encouraged due to relatively high incidence of infection with these strains. Doxycycline, minocycline and josamycin were recommended to treat infection of *U.urealyticum* and *M. hominis* attributed to less resistance of these strains than other antibiotics in southwestern area of china. However, it should be kept in mind that selection of antibiotics should be based on results of antimicrobials resistance analysis and antibiotics resistance profiles are different from one area to another due to difference in environment, races and practice of clinicians, and so on.

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Conflict of Interest

None to disclose.

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