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Original article

Prevalence and antimicrobial resistance of *Mycoplasmas* and *Chlamydiae* in patients with genital tract infections in Shanghai, China

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ABSTRACT

The infections of *Mycoplasmas* and *Chlamydiae* are still severe in patients with genital tract diseases and antimicrobial resistance for these organisms has been changing in recent years. In this study, we reported the prevalence status of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Chlamydia trachomatis* in 965 patients with genital tract infection in Shanghai from January 2011 to December 2014 and analyzed the antimicrobial resistance of *U. urealyticum* and *M. hominis* to 12 kinds of antimicrobial drugs by using commercial kits and SPSS13.0 software. Here, we found the infection of *U. urealyticum* was the most frequent among these three organisms. The total infection rate for containing any organisms of them was 49.5%, and it has been increasing in recent 4 years. Positive rate in female (53.3%) was higher than male's (34.8%), and the high risk population was 20–39 years old (56.7%). Besides, *U. urealyticum* and *M. hominis* displayed relative lower resistance rates to minocycline, doxycycline, josamycin and gatifloxacin (6.5%, 7.2%, 13.5% and 8.6%, respectively). However, for erythromycin, roxithromycin, thiamphenicol and clindamycin, the resistance rates were relatively high (41.9%, 47.2%, 62.3% and 74.9%, respectively). *U. urealyticum* and *M. hominis* displayed a declined trend of the antimicrobial resistance to 12 kinds of drugs detected in this study. In total, these preliminary data showed the prevalence of *Mycoplasmas* and *Chlamydiae* in patients and the antimicrobial resistance status of *Mycoplasmas*, which has use for reference on both prevention and treatment of diseases caused by them.

1. Introduction

Ureaplasma urealyticum and *Mycoplasma hominis*, belonging to *Mycoplasmataceae* are mainly two types of pathogens leading to Nongonococcal urethritis (NGU) [1]. *Chlamydia trachomatis* is also one of the most common agents of sexually transmitted genital infections in statistics [2,3]. These infectious microorganisms often have been associated with infertility (including miscarriages, dysplasia, stillbirth, premature birth and postabortal fever) [4,5], and genital tract infections (including pyelonephritis, pelvic inflammatory disease, chorioamnionitis, postpartum, and inflammatory urethral opening) [6–9]. Among these three kinds of pathogenic bacteria, *U. urealyticum* was the most frequent species [10,11], and it was firstly isolated from the urethral discharge of men with NGU by Shepard in 1954 [12,13]. In addition, the pathogen of 45% patients with NGU has not been clear so far [14,15].

Many factors, including age, gender, socioeconomic status, menstrual cycle, pregnancy and the use of vaginal contraceptives affect detection rate of *U. urealyticum*, *M. hominis* and *C. trachomatis* [16,17]. In general, the presence of these microbes in female is higher than male's, and the infected population is mainly concentrated in married young couples [18]. According to the previous studies, the overall infection rate of these organisms has been changing, but not dramatically in recent years [19]. *Mycoplasmas* have a natural resistance to the drugs of beta lactam such as penicillin due to their lack of cell wall. Antibiotics such as tetracycline, quinolone and macrolides acting on the ribosome or synthesis of DNA molecules are clinically used to treat the infection of *U. urealyticum* and *M. hominis*. However, their drug resistance is

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becoming more and more severe owing to the abuse of antibiotics, non-standard and incomplete treatment, *etc* [20]. In addition, a number of previous studies have confirmed that *U. urealyticum* can also form biofilms, leading to antimicrobial resistance [21–23]. Thus, studying on the antibacterial drugs of *Mycoplasmas* is very meaningful to treat with genital tract infectious diseases caused by them at present.

Over the past two decades, rapid pace of economic and social changes has been followed by a resurgent epidemic of STDs in China [24–26]. While nowadays the statistics about prevalence of *Mycoplasmas* and *Chlamydiae*, and antimicrobial resistance to *Mycoplasmas* in patients with genital tract infection have not been reported in Shanghai, China. In order to provide guidelines for both prevention and treatment of genital tract infection, we determined the prevalence status of *U. urealyticum*, *M. hominis* and *C. trachomatis*, and analyzed the antimicrobial resistance of *Mycoplasmas* in this study.

2. Patients and methods

2.1. Study population

The study group was consisted of 965 patients suspected with genital tract infectious diseases in Fengcheng Hospital of Shanghai from January 2011 to December 2014. Their average age was 33.3 years old (range: 15–75 years). Infection types of the patients mainly included some or all of the following symptoms including vaginitis, cervicitis, dysuria, urinary frequency and pelvic inflammatory disease, *etc*.

2.2. Specimen collection, culture and detection for U. urealyticum, M. hominis and C. trachomatis and analysis of antimicrobial resistance for Mycoplasmas

The specimens for testing *U. urealyticum*, *M. hominis* and *C. trachomatis* were collected according to the principle of aseptic manipulation strictly. For male patients, the samples mainly contained urine, urethral secretions. All urine specimens for culture and future analysis were obtained from the mid-stream urine samples, and urethral secretions were taken with a sterile swab placed into the anterior urethra 1–2 cm and turned to gain as many cells as possible after cleaning the external meatus. For female patients, the cervical samples were totally inflammatory secretions taken from the cervical canal 1–2 cm after exocervical mucus had been wiped out by swabs.

The culture, detection and antimicrobial resistance of *U. urealyticum* and *M. hominis* were carried out with a commercially available *Mycoplasmas* kit produced by Zhengzhou Biological Engineering Co. Briefly, 100 μl basic solution was added into the micropore of drug sensitive test board as a blank control, and the clinical samples were dissolved by extruding and spining the sterile swabs with samples in media for several times. Usually, if the liquid sample was urine, it was often centrifuged (3000 g, 10 min, room temperature) before taking 0.2 ml precipitation into the media. For other liquid samples, adding 0.2 ml of them into the media directly. Mixing the inoculated media fully and adding 100 μl mixture into micropores accurately by pipette. A part of micropores were used to detect infections of *U. urealyticum* and *M. hominis* and the rest of them for analysis of antimicrobial susceptibility. Next, The micropores for antimicrobial resistance were added with 12 kinds of drugs including minocycline (MIN), doxycycline (DOX), erythromycin (ERY), azithromycin (AZI), clarithromycin (CLA), roxithromycin (ROX), josamycin (JOS), thiamphenicol (THI), clindamycin (CLI), sparfloxacin (SPA), levofloxacin (LEV) and gatifloxacin (GAT). Then, adding 1 or 2 drops of oil on all micropores including the blank control. The incubated drug sensitive test plate with lid were put in the incubator at 35–37 °C for 24 h and 48 h respectively and any color changes noted. In general, the color from yellow to pink was regarded as evidence of *U. urealyticum* and *M. hominis* infection and the color of yellow indicated negative results. Besides, when the color change was not obvious, the culture should be appropriately prolonged for 12–24 h before interpreting the results. For interpretation of antimicrobial resistance, the antibacterial agent was drug resistant when the high and low concentration of it in wells were both positive. The antibacterial agent was moderate sensitive when the high concentration of drug was negative and low concentration was positive. The antibacterial agent was drug sensitive when the high and low concentration of drug in wells were both negative.

The culture and detection for *C. trachomatis* were carried out with the British Liming unisex *Chlamydiae* rapid test kit according to the manufacturer's guidelines. The procedure mainly included antigen extraction and detection of somatic antigen. Adding five drops of antigen extraction in the specimen window of test block and reading the test results after incubating for 15 min. Appearance of one line indicated the infection of *Chlamydiae*.

2.3. Statistical analysis

The infection status of *Mycoplasmas* and *Chlamydiae* in different gender and age groups as well as the resistance of *Mycoplasmas* to 12 kinds of antibacterial drugs were analyzed by SPSS13.0 software and GraphPad Prism software version 6.0. Chi-square test was used for statistical analysis. $P < 0.05$ indicates statistical difference; $P < 0.01$ indicates significantly statistical difference.

3. Results

3.1. Prevalence of Mycoplasmas and Chlamydiae from 2011 to 2014

The overall infection rates of these organisms were 41.0%, 58.4%, 47.3% and 49.6% from 2011 to 2014 (Table 1). The most prevalent organism detected in this study was *U. urealyticum* (Table 2). Among the 965 specimens, 478 cases were positive for containing any organism of *U. urealyticum*, *M. hominis* and *C. trachomatis* (the total infection rate was 49.5%). Mono-infection of *U. urealyticum*, *M. hominis* and *C. trachomatis* were 294, 19 and 3 cases, respectively (the infection rates were about 30.5%, 2.0% and 0.3%, respectively). Co-infection of *U. urealyticum* and *M. hominis*, *U. urealyticum* and *C. trachomatis*, were 116 cases (12.0%) and 23 cases (2.4%), respectively. Co-infection rate of all three microbes was also 2.4% (Table 2).

3.2. Distribution of the infectious population

Of the 767 samples in female examined, the total positive rate for containing anyone of these three organisms was 53.3%, which was higher than male's whose infection rate was only 34.8%. For female, the positive rates in all infection types including *U. urealyticum*, *M. hominis*, *C. trachomatis*, *U. urealyticum* and *M. hominis*, *U. urealyticum* and *C. trachomatis*, *U. urealyticum* and

Table 1
Trend of the positive rates of *Mycoplasmas* and *Chlamydiae* in endocervical or urethal specimens from patients each year.

Year	2011	2012	2013	2014	χ^2	*P*
Positive cases	75	128	89	186		
Sample size	183	219	188	375	12.7	<0.01
Positive rates (%)	41.0	58.4	47.3	49.6		