Epidemiology of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the semen of male outpatients with reproductive disorders

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Abstract. The aim of the present study was to investigate the association between *Mycoplasma* infection and infertility in male outpatients among a Chinese population. Epidemiological data, including prevalence, age distribution and antibiotic resistance profile of patients with an *Ureaplasma urealyticum* or *Mycoplasma hominis* infection were collected between 2009 and 2012. Among the 7,374 individuals analyzed, 3,225 patients (43.7%) were determined to be positive for infection with *U. urealyticum*, *M. hominis* or for both *Mycoplasmas*. Among the positive cultures, *U. urealyticum* was detected most frequently, while *M. hominis* was rarely found. The age range of 25-34 years was the preferred period for the positive detection. Tetracyclines and josamycin were the most effective agents against both genital *Mycoplasmas,* including in the case of co-infection. Macrolides (erythromycin, roxithromycin, azithromycin, clarithromycin except for josamycin) were effective against the majority of *U. urealyticum* clinical isolates, but were naturally resisted by *M. hominis* in this study. Fluoroquinolones had the lowest activity against *U. urealyticum*, particularly in cases of *M. hominis* co-infection. Furthermore, fluoroquinolones showed a similar pattern of drug resistance against *M. hominis* to that of *U. urealyticum*. Antibiotic resistance did not vary significantly over the test period. Notably, an elevated multi-drug resistance rate was observed in patients co-infected with both *Mycoplasmas*. In light of the epidemiological characteristics of genital *Mycoplasmas* in male infertility patients, the present results may aid Chinese clinicians to implement rational drug usage and avoid the overuse of antibiotics.

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Introduction

Mycoplasmas belong to the class Mollicutes which also contains *Ureaplasmas*, *Acholeplasmas*, *Spiroplasmas* and the newly classified *Haemoplasmas* (1). *Mycoplasmas* are characterized by a small size, lack of cell wall, extremely fastidious *in vitro* environmental requirements and a tendency to form centered colonies on solid media (2).

β-lactam antibiotics and vancomycin are inactive against *Mycoplasmas*, as their target is the cell wall (3). *Mycoplasma* and *Ureaplasma* spp. are currently susceptible to agents that interfere with protein synthesis, including tetracycline, macrolides, aminoglycosides and chloramphenicol, and the fluoroquinolones that inhibit topoisomerases (3,4). However, resistance to these agents is increasing, as a consequence of the spread of the *tetM* gene which confers resistance to tetracycline (5-7). Clindamycin, fluoroquinolones or other macrolides may be used following the failure of therapy with tetracycline or erythromycin (8).

The extent of bacterial resistance varies geographically, depending on the use of different antibiotics and the history of previous antimicrobial exposure among various populations (9). To date, with regard to the rising infertility in China, an elevated incidence of genital *Mycoplasma* infection in female outpatients was identified by Wang *et al* (10); however, there is currently no data regarding the infection prevalence of genital *Mycoplasmas* in the male partners of infertile couples. For these reasons, it is important to implement surveillance studies on the prevalence and antimicrobial susceptibilities of these species in the Chinese male population. The purposes of this study were as follows: i) Analyze the prevalence of urogenital mycoplasma; ii) investigate the susceptibilities of a large number of clinical isolates of *M. hominis* and *U. urealyticum* to various antibiotics; and iii) compare changes in the antibiotic susceptibilities of these microorganisms between 2009 and 2012.

Materials and methods

Patients. Between January 1st, 2009 and December 31st, 2012, a total of 7,374 male outpatients with suspected reproductive disorders were enrolled from the Affiliated Hospital of Nanjing University of Chinese Medicine (Nanjing, China), and underwent an inspection of the reproductive system. The patients

Table I. Distribution of *Ureaplasma urealyticum* and *Mycoplasma hominis* (single- and co-infection) among male patients in different age groups during the study period.

Age group (years)	*M. hominis*	*U. urealyticum*	*M. hominis* + *U. urealyticum*	Total
18-24	2 (0.0)	445 (14.1)	8 (10.8)	453 (100)
25-29	17 (100.0)	1,047 (33.3)	23 (31.1)	1,073 (100)
30-34	9 (0.0)	1,128 (35.8)	31 (41.9)	1,159 (100)
35-39	1 (0.0)	476 (15.1)	7 (9.5)	483 (100)
≥40	0 (0.0)	52 (1.7)	5 (6.8)	57 (100)
Total	29 (100.0)	3,148 (100)	74 (100)	3,225 (100)

Results are presented as n (%).

ages ranged between 18 and 47 years. The present study was approved by the ethical committee of the Nanjing University of Chinese Medicine.

Specimens, culture and antimicrobial susceptibility testing. All semen samples were obtained by masturbation and inoculated into the Mycoplasma Susceptibility kit (cat. no. 20140317119877; Autobio Diagnostics Co., Ltd., Zhengzhou, China) within 1 h, according to the manufacturer's guidelines. The microbiological principle used by the *Mycoplasma* identification verification and antibiotic susceptibility testing kits was as follows: During growth, *U. urealyticum* and *M. hominis* metabolize urea and arginine, respectively, which changes the color of the culture medium (e.g., from yellow to red). Susceptibility results were obtained at two concentrations for 12 antibiotics: Erythromycin, roxithromycin, josamycin, tetracycline, doxycycline, minocycline, levofloxacin, ofloxacin, azithromycin, clarithromycin, ciprofloxacin and sparfloxacin (Thermo Fisher Scientific Oxoid, Ltd., Basingstoke, UK). The susceptibility of the bacteria to each antibiotic was graded as either 'susceptible', 'intermediate' or 'resistant' (11). Bacterial growth was evaluated following a two-day incubation period at 37˚C. The results were interpreted as follows: Negative result was clear and a color change of $>10^4$ units was considered to indicate infection. Clinical and Laboratory Standards Institute guidelines were used to categorize the results for bacterial susceptibility or resistance to antibiotics (12).

Statistical analysis. SPSS software, version 12.0 (SPSS, Inc., Chicago, IL, USA) was used to conduct the data analysis. The χ^2 test was used to compare the occurrence of strains susceptible or resistant to different antibiotics. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Prevalence of U. urealyticum and M. hominis. Among the 7,374 specimens tested, 3,225 (43.7%) were positive for genital *Mycoplasmas*. Of these, 3,122 specimens were positive for *U. urealyticum* (42.3%), 29 were positive for *M. hominis* (0.4%) and 74 were positive for both (1.0%) (Fig. 1). The distribution of *M. hominis* and *U. urealyticum* according to age group is shown in Table I. The prevalence rates of *U. urealyticum* and of both *Mycoplasmas* were highest in patients aged 25-34 years, whereas *M. hominis* occurred predominantly in patients between the ages of 25 and 29 years.

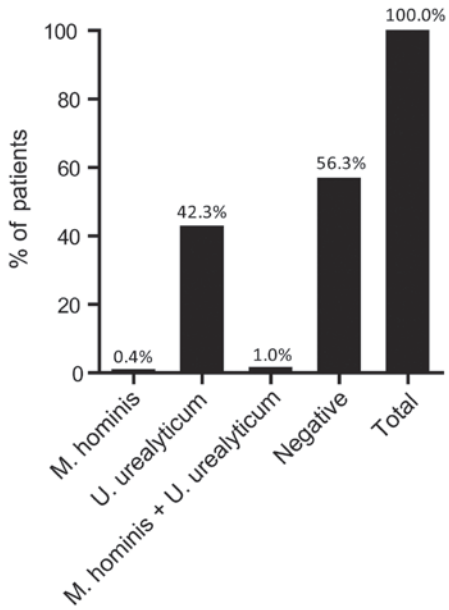

Figure 1. Constituent ratio of *U. urealyticum*, *M. hominis* and co-infection. *U. urealyticum*, *Ureaplasma urealyticum*; *M. hominis*, *Mycoplasma hominis*.

Antimicrobial susceptibility patterns over the test period. The antimicrobial susceptibilities of *M. hominis* and *U. urealyticum* are shown in Fig. 2. Tetracyclines (tetracycline, minocycline, doxycycline) were the most active agents against both genital *Mycoplasmas* even in the case of co-infection. Macrolide (erythromycin, roxithromycin, azithromycin, clarithromycin and josamycin) remained effective against the majority of *U. urealyticum* clinical isolates. However, macrolides, with the exception of josamycin, were naturally resisted by *M. hominis*. Likewise, in the persons infected with both genital *Mycoplasmas*, the antibacterial activity of macrolides except for josamycin were not significant. Josamycin was the only macrolide observed to be effective against *M. hominis* and co-infection. Fluoroquinolones (ciprofloxacin) had the lowest activity against *U. urealyticum*, particularly in patients with

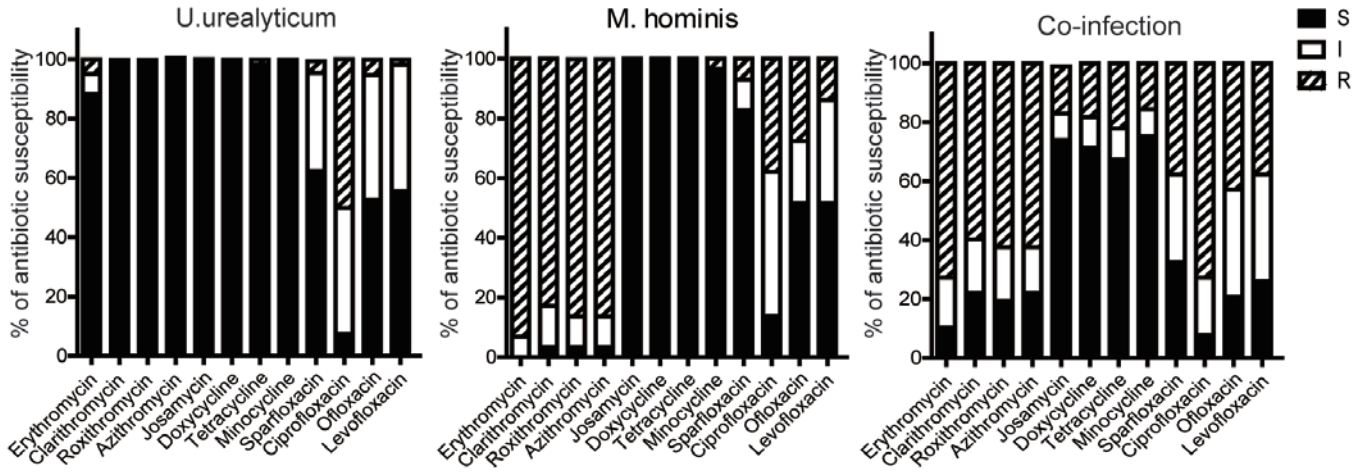

Figure 2. Antimicrobial susceptibility of *U. urealyticum*, *M. hominis* and co-infection, respectively. *U. urealyticum*, *Ureaplasma urealyticum*; *M. hominis*, *Mycoplasma hominis*; S, susceptible; I, intermediate; R, resistant.

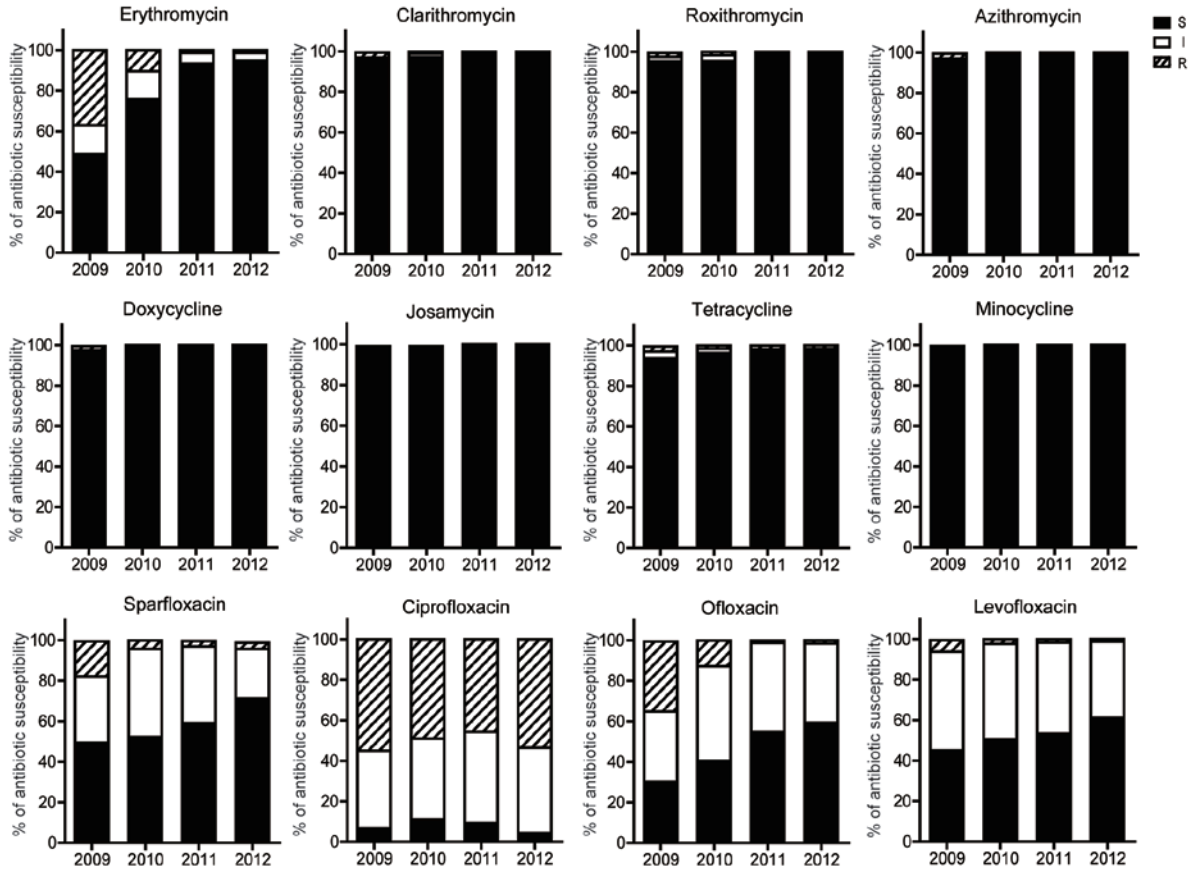

Figure 3. Antimicrobial susceptibility of *Ureaplasma urealyticum* to different classes of antibiotic over the study period. S, susceptible; I, intermediate; R, resistant.

M. hominis co-infection. The susceptibility profiles against both *Mycoplasmas* over the test period did not change significantly, despite the efficacy of ciprofloxacin to *U. urealyticum* becoming increasingly diminished (Figs. 3 and 4).

Multi-drug resistant (MDR) bacteria have been identified in numerous cases and MDR *Mycoplasmas* are defined as those strains resistant to at least one agent in ≥3 antimicrobial categories (13). In the present study, the incidence of MDR single *U. urealyticum* infection was significantly reduced, as compared with that of co-infection (1.09 vs. 33.78%; $P<0.05$), indicating the presence of cross-resistance in the co-infection patients (Fig. 5).

Discussion

Worsening environmental contamination and the increasing incidence of sexually transmitted disease, which may lead to infertility, have meant that an increasing number of couples

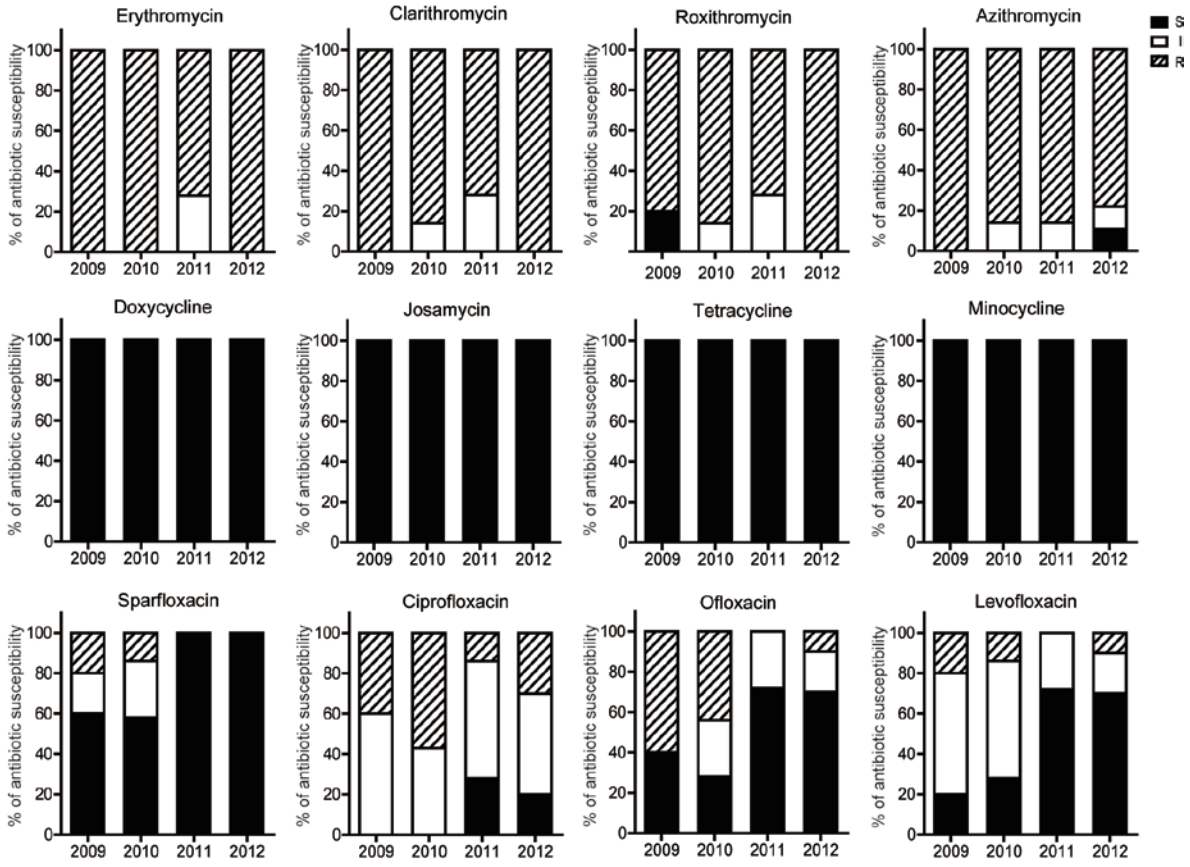

Figure 4. Antimicrobial susceptibility of *Mycoplasma hominis* to different classes of antibiotic over the study period. S, susceptible; I, intermediate; R, resistant.

will not have their first baby without the aid of assisted reproduction (14). Infertility is emerging as a serious public health issue in China (15). As well as physical and chemical factors, sexually-transmitted infections must also be taken into consideration (16). *Mycoplasmas* are among the smallest free-living microorganisms. They are commonly isolated from the genitourinary tract of symptomatic patients, but may be found as commensal bacteria from asymptomatic patients. It has been reported that infection with genital *Mycoplasmas* may lead to pelvic inflammatory disease, puerperal infections, septic abortions, low birth weight, nongonococcal urethritis, prostatitis as well as spontaneous abortion and infertility (17-19). To date, the effect of these microorganisms on male infertility remains unclear, with the exception of limited studies in a few countries (20,21). The present study described the prevalence and antimicrobial susceptibility of *U. urealyticum* and *M. hominis* isolated from semen samples from Chinese patients, and may aid understanding and optimal clinical treatment choice for these pathogens.

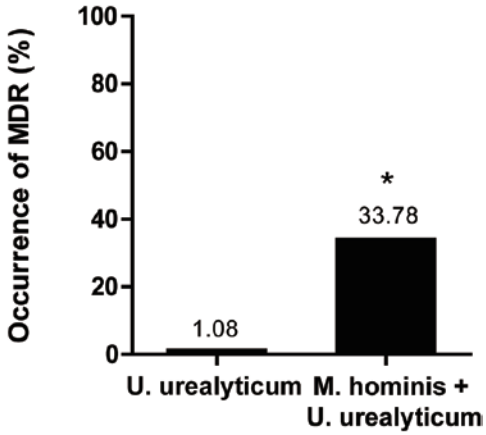

Figure 5. Occurrence of MDR between single *U. urealyticum* and co-infection specimens. *P<0.05 vs. *U. urealyticum* only. MDR, multi-drug resistance; *U. urealyticum, Ureaplasma urealyticum; M. hominis, Mycoplasma hominis*.

This study evaluated differences in the prevalence and antibiotic resistance of *U. urealyticum* and *M. hominis*. The overall prevalence of infection with either type of bacteria was 43.7%. *U. urealyticum* was frequently detected as a single pathogen (42.3%), and was thus significantly associated with symptomatic patients, including loss of sperm, which indicated its potential pathogenicity. By contrast, a single infection of *M. hominis* (0.4%) was rarely found, indicative of disproportionate incidence of these two *Mycoplasmas*. The present findings are consistent with those of other studies performed in Poland (22) and Korea (23); however, they are distinctly different to those of previous studies conducted in Jordan (24) and Italy (25). This discrepancy may be a result of variations in socioeconomic conditions and living standards. In the present study, simultaneous colonization with *M. hominis* and *U. urealyticum* was not common (1.0%).

In this study, the most frequent occurrence rate of genital *Mycoplasma* infection was detected in patients aged between

25 and 34 years old; the age range at which new couples typically conceive their baby. With the exception of the age distribution of the outpatients, no other demographic or clinical characteristics were examined in this study. Other characteristics should be examined in future follow-up studies.

Mycoplasmas are normally susceptible to antibiotics that inhibit protein synthesis, but are resistant to antibiotics that act on bacterial cell wall components because *Mycoplasmas* do not possess a cell wall (26). The results of this study indicated that there was a difference in sensitivity to the 12 antibiotics between the isolates from single infections and co-infections. Three tetracycline antibiotics (tetracycline, doxycycline and minocycline) and one macrolide antibiotic (josamycin) were active against the majority of the strains. However, four of the quinolone antibiotics (sparfloxacin, levofloxacin, ciprofloxacin and ofloxacin) were inactive against more than one-third of the strains isolated in this study, particularly against the co-infection isolates. Four macrolide antibiotics (azithromycin, erythromycin, clarithromycin and roxithromycin) were effective against the majority of *U. urealyticum* isolates, but were inactive against the majority of bacteria isolates of the single *M. hominis* and co-infections. Consistent with that of female *M. hominis* isolates (10,11), semen isolated *M. hominis* in this study displayed similar resistance spectrum to erythromycin, azythromycin, roxythromycin and clarithromycin. However, a small number of single *M. hominis*-positive isolates were calculated in the study; thus, further studies including a greater sample size of single *M. hominis* should be conducted. Although, simultaneous infection with *U. urealyticum* and *M. hominis,* was not often, but leaded an elevated MDR compared with that of single *M. hominis* infection, indicating a crucial role of cross resistance of these microbes with distinct drug resistant spectrum. The present results support the use of tetracycline, doxycycline, minocycline and josamycin as first choice drugs when empirical therapy is required, whereas the use of erythromycin and quinolones must be carefully considered. The prevalence of the *U. urealyticum* and *M. hominis* antibiotic resistance profiles in our study were similar to that of female originated *Mycoplasmas* reported by Wang *et al* (10) in China, indicating a possible transmission of these *Mycoplasmas* between men and women. Additionally, no significant difference in antibacterial activity was observed over the study period, with the exception of the reduced activity of ciprofloxacin, which may be attributed to the excessive usage of the drug. Thus, differences in antimicrobial use policies of various areas may also influence the antimicrobial susceptibility characteristics.

In conclusion, the present study retrospectively analyzed the prevalence and antibiotic susceptibility of *U. urealyticum* and *M. hominis* in semen samples from the Chinese population, in order to provide clinicians with an evidential basis for the rational use of antibiotics, which may be useful for avoiding treatment failure and the abuse of antimicrobial agents.

Acknowledgements

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