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ORIGINAL ARTICLE

Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in female outpatients, 2009—2013

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Purpose: The aim of this study was to estimate the prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* among female outpatients treated for genital infection at a Chinese hospital from January 1, 2009 to December 31, 2013.
Methods: Samples from 6051 female outpatients were analyzed using *Mycoplasma* Identification and Antimicrobial Susceptibility Testing (ID/AST).
Results: The overall prevalence of *U. urealyticum* was higher than the prevalence of single *M. hominis* infection (31.2% vs 0.7%) and coinfections (31.2% vs. 1.9%). The percentage of *U. urealyticum* and/or *M. hominis* detected in the 30—39 year age group was greater than in the other age groups. More than 94.6% of the *U. urealyticum* isolates, 100% of the *M. hominis* isolates, and 84.3% of the isolates from coinfections were susceptible to doxycycline, minocycline, and tetracycline. More than 69.2% of the *U. urealyticum* isolates were susceptible to azithromycin, erythromycin, clarithromycin, and roxithromycin, but $>$ 95.6% of the *M. hominis* isolates and 89.6% of the isolates from coinfections were resistant to these antibiotics. Acetylspiramycin, sparfloxacin, levofloxacin, ciprofloxacin, and ofloxacin were inactive against more than one-half of the isolates. More than 75.6% of the *M. hominis* isolates were susceptible to spectinomycin, but $>$ 87.1% of the *U. urealyticum* and 93.3% of the coinfection isolates were resistant to this antibiotic. Isolates from three coinfections were completely resistant to the 14 antibiotics.
Conclusion: The determination of antimicrobial susceptibility of these mycoplasma species is often crucial for optimal antimicrobial therapy of infected outpatients.

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Introduction

Ample evidence from clinical studies using culture, serology, and polymerase chain reaction (PCR) assays in humans, and from experimental infection of laboratory animals indicates that *Ureaplasma urealyticum* and *Mycoplasma hominis* are etiologic agents of a variety of urogenital diseases in women. These diseases include urinary calculus formation, pyelonephritis, bacterial vaginosis, pelvic inflammatory disease, infertility, chorioamnionitis, spontaneous abortion, prematurity, intrauterine growth retardation, postpartum fever, and extragenital disease.[1] Antibiotic resistance among urogenital mycoplasmas develops via gene mutation or the acquisition of new genetic material. The prevalence of these pathogens and the results of surveillance of their antibiotic resistance profiles change in relation to the patient's country of origin. Testing should be performed for optimal antimicrobial therapy of infected patients, and to monitor the spread of resistant organisms.[2–4] Some related research studies have been performed in China. However, the resistance characteristics were different and may be related to local antibiotic use regulations. It is important to perform a detailed analysis on the characteristics of the area in which the resistant strains occur. The aim of this study was to estimate the prevalence of *U. urealyticum* and *M. hominis* infections and the antimicrobial susceptibilities of isolates of these bacteria. Analyses of patient age groups and some multidrug-resistant strains were also included.

Materials and methods

Between January 1, 2009 and December 31, 2013, a total of 6051 specimens were examined by the Department of Clinical Laboratory at the Xiyuan Hospital, China Academy of Chinese Medical Sciences (Beijing, China). Consistent with the manufacturers' guidelines and standard laboratory protocols, all specimens were immediately transported to the laboratory without additional transport medium. They were then refrigerated and examined within 48 hours after collection. The microbiological principle used by *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 14 antibiotics: erythromycin, acetylspiramycin, josamycin, tetracycline, doxycycline, minocycline, roxithromycin, levofloxacin, ofloxacin, azithromycin, clarithromycin, sparfloxacin, ciprofloxacin, and spectinomycin. The three possible results were "susceptible", "intermediate", and "resistant". Bacterial growth was evaluated after a 2-day incubation period (at 37°C). The results were interpreted as follows: a negative result was clear and a color change of more than 10^4 units was evidence of infection. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to categorize the results for bacterial susceptibility or resistance to antibiotics.[2,5] The breakpoints for the 14 antibiotics (mg/L) were: erythromycin, $S \leq 1$, $R \geq 4$; acetylspiramycin, $S \leq 1$, $R \geq 2$; josamycin, $S \leq 2$, $R \geq 8$; tetracycline, $S \leq 4$, $R \geq 8$; doxycycline, $S \leq 4$, $R \geq 8$; minocycline, $S \leq 4$, $R \geq 8$; roxithromycin, $S \leq 1$, $R \geq 4$; levofloxacin, $S \leq 1$, $R \geq 4$; ofloxacin, $S \leq 1$, $R \geq 4$; azithromycin, $S \leq 0.12$, $R \geq 4$; clarithromycin, $S \leq 1$, $R \geq 4$; sparfloxacin, $S \leq 1$, $R \geq 4$; ciprofloxacin, $S \leq 1$, $R \geq 2$; and spectinomycin, $S \leq 4$, $R \geq 8$. For all analyses, $p < 0.05$ indicated statistical significance. Ethics Committee approval and informed patient consent were not required for this study.

Results

The total positive rate of infection for the 6051 female outpatients was 33.9% (Table 1). The overall prevalence of *U. urealyticum* infection was greater than the prevalence of *M. hominis* infection (31.2% vs. 0.7%) and was greater than the prevalence of coinfection (31.2% vs. 1.9%).

The results for the distribution of *M. hominis* and *U. urealyticum*, according to age group, are presented in Table 1. During our study period, 49.7% of positive results occurred in the 30—39 year age group, which was significantly higher in comparison to the other age groups ($p < 0.05$).

There was no resistance to any of the three tetracyclines (i.e., tetracycline, doxycycline, and minocycline) for any *M. hominis* isolate, and 91.1% of these isolates were susceptible to the macrolide josamycin. These four antibiotics were also effective against > 87.9% of the *U. urealyticum* isolates and 73% of the bacteria isolated from coinfections. More than 95.6% of the *M. hominis* isolates and 89.6% of the isolates from coinfections were resistant to four of the macrolide antibiotics (i.e., azithromycin, erythromycin, clarithromycin, roxithromycin), but > 69.2% of the *U. urealyticum* isolates were susceptible to these antibiotics.

More than 50% of the bacteria isolated from the study population were resistant to four of the quinolone antibiotics (i.e., sparfloxacin, levofloxacin, ciprofloxacin, and ofloxacin) and one of the macrolide antibiotics (i.e., acetylspiramycin). The bacteria isolated from three of the coinfections (i.e., *U. urealyticum* and *M. hominis*) were completely (100%) resistant to the 14 antibiotics.

Discussion

Mycoplasmas are the smallest free-living microorganisms. They are commonly isolated from the genitourinary tract of symptomatic patients, but also from asymptomatic patients. The aim of this study was to evaluate differences in the prevalence and antibiotic resistance of *U. urealyticum* and *M. hominis*. Single infections with *U. urealyticum* were most prevalent (31.2%), followed by coinfections with *U. urealyticum* and *M. hominis* (1.9%) and single infections with *M. hominis* (0.7%). The infection rate was significantly higher in young women than in older women. This result is consistent with results reported in other studies.[5–7] Except for age and sex distribution of the outpatients, no other demographic or clinical characteristics were examined in this study. Other characteristics will be examined in a follow-up study.

Mycoplasmas are normally susceptible to antibiotics that inhibit protein synthesis, but are resistant to antibiotics that act on bacterial cell wall components because

Table 1 Distribution of *Ureaplasma urealyticum* and *Mycoplasma hominis* (single infection and coinfection) among female outpatients in different age groups during the study period[a]

Infection in different age groups	*Ureaplasma urealyticum*	*Mycoplasma hominis*	Coinfection	Negative	Total
20—29	718 (32.8)	15 (0.7)	47 (2.1)	1408 (64.4)	2188 (100)
30—39	948 (29.6)	24 (0.7)	46 (1.4)	2189 (68.3)	3207 (100)
40—49	193 (34.4)	4 (0.7)	19 (3.4)	345 (61.5)	561 (100)
Others	30 (31.6)	2 (2.1)	3 (3.2)	60 (63.2)	95 (100)
Total	1889 (31.2)	45 (0.7)	115 (1.9)	4002 (66.1)	6051 (100)

[a] "Coinfection" means that the patients were simultaneously infected with *Ureaplasma urealyticum* and *Mycoplasma hominis*.
Data are presented as *n* (%).

mycoplasmas do not possess a cell wall.[8] The results of this study indicated that there was a difference in sensitivity to 14 antibiotics among the isolates from single infections and from the coinfections. Three tetracycline antibiotics (i.e., tetracycline, doxycycline, and minocycline) and one macrolide antibiotic (i.e., josamycin) were active against most of the strains. However, four of the quinolone antibiotics (i.e., sparfloxacin, levofloxacin, ciprofloxacin, and ofloxacin) and one macrolide antibiotic (i.e., acetylspiramycin) were inactive against more than one-half of the strains isolated in this study. Four macrolide antibiotics (i.e., azithromycin, erythromycin, clarithromycin, and roxithromycin) were effective against most *U. urealyticum* isolates, but were ineffective against most bacteria isolated from the single *M. hominis* and coinfections. *M. hominis* is intrinsically resistant to erythromycin, which was a characteristic we observed in our results. Our results also indicated that, except for spectinomycin, the patterns of antimicrobial susceptibilities against coinfection were similar to the patterns displayed by *M. hominis*. Our results indicate that tetracycline, doxycycline, minocycline, and josamycin are the first choice drugs when empirical therapy is required (Table 2). The prevalence of the *U. urealyticum* and *M. hominis* antibiotic resistance profiles in our study differ from profiles reported by authors of similar studies.[6,7,9] The discrepancies in antimicrobial susceptibilities of isolates from various countries are most likely due to differences in antimicrobial use policies.[2] The results of our study indicated that three tetracycline antibiotics (i.e., tetracycline, doxycycline, and minocycline) and one macrolide antibiotic (i.e., josamycin) represent the options for initial empirical treatment. The use of erythromycin must be carefully considered.

Mycoplasmas are innately resistant to penicillins, rifampicins, and some other antibiotics. Some may develop resistance via gene mutation, acquisition of a resistance gene, or while being protected by biofilms. Multidrug-resistant mycoplasma strains have recently been identified.[8,10,11] In our hospital setting, some clinical strains have developed resistance to the 14 drugs (including the 3 resistant mixed isolates in our study), which has increased the difficulty of successfully treating mycoplasma infections. Further study of the efficacy of the use of Chinese medicinal herbs for these infections may result in the discovery of new treatment options.[12,13]

The detection methods for *Mycoplasma* include culture, antigen detection, molecular techniques, and antibody detection. Methods for the simultaneous detection of *U. urealyticum*, *M. hominis*, and other pathogens are extremely useful in a clinical setting arena. Examples of these methods include fluorescence polarization assay, PCR, and multiplex PCR.[14—18] However, although these assays may be useful for detecting coinfections in a hospital setting, they are not useful for detailed information about antibiotic resistance. The commercial mycoplasma kit used in this study was more cost-effective and simpler to use than these methods. However, a limitation of the commercial kit is that it only detects *U. urealyticum* and *M. hominis*; therefore, the development of similar methods for the simultaneous detection of multiple genitourinary infections would be extremely beneficial. Novel and improved technologies may be available in the near future. The emergence of extensively drug-resistant strains of *Mycoplasma* means that antibiotic susceptibility testing is even more important in scientific research and in the clinical setting.[19] *Mycoplasma* identification verification and antibiotic susceptibility testing in the hospital was performed in 2009. So it was important for us to

Table 2 Antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* (single and coinfection) to 14 antibiotics among female outpatients during the study period[a]

Antibiotic	*U. urealyticum*	*M. hominis*	Coinfection
Spectinomycin	12.9	75.6	6.7
Tetracycline	95.2	100	84.3
Minocycline	94.6	100	85.2
Doxycycline	98	100	88.7
Ciprofloxacin	5.8	17. 8	5.2
Ofloxacin	22.1	15. 6	10.4
Sparfloxacin	43.1	37. 8	27
Levofloxacin	39.9	20	20
Azithromycin	69.2	2.2	4.3
Erythromycin	78.1	0	0
Josamycin	87.9	91.1	73
Roxithromycin	96.5	4.4	7.8
Acetylspiramycin	31.7	35.6	9.6
Clarithromycin	98.4	4.4	10.4

[a] "Coinfection" means that the patients were simultaneously infected with *Ureaplasma urealyticum* and *Mycoplasma hominis*.
Data are presented as %.

retrospectively review the computerized database of the Clinical Microbiology Laboratory for the study period. The retrospective analytical approach can effectively provide clinicians with real data about the rational use of antibiotics. We firmly believe that *Mycoplasma* culture verification and antibiotic susceptibility testing will be useful to avoid treatment failure and abuse of antimicrobial agents.

Conflicts of interest

All authors declare that they have no conflicts of interest relevant to this paper.

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