

## RESEARCH ARTICLE

# The relationship between the biovars and the antimicrobial resistance of *Ureaplasma urealyticum* in female patients with urogenital infections

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**Background:** *Ureaplasma urealyticum* is considered as one of the main pathogens found in women with urogenital infection. This study aimed to investigate the relationship between the biovars, serovars, and their antimicrobial resistance against antibiotics in female patients with urogenital infection.

**Methods:** Two hundred and forty-six cervical secretion samples (125 female outpatients as the patient group, 121 healthy female subjects as the control group) were first collected and analyzed for *U. urealyticum* using the Mycoplasma Identification and Antimicrobial Susceptibility Testing; then polymerase chain reaction (PCR) was carried out to identify the biovars and serovars of *U. urealyticum*-positive samples.

**Results:** The prevalence of *U. urealyticum* in the patient group (57.60%) was higher than that in the control group (24.79%,  $P < .01$ ). The main biovar was biovar 1, and the main serovars were 1 (S1), 3 (S3), 6 (S6) in biovar 1. Mixed infection was observed in biovar 2. According to the results of Antimicrobial Susceptibility Testing in the patient group, biovar 1 shows more resistance to minocycline, doxycycline, and azithromycin than biovar 2 ( $P < .05$ ). Serovars S1, S3 and S6 have the highest resistant rate to ofloxacin (84.38%), roxithromycin (84.62%), and azithromycin (90.90%), respectively.

**Conclusions:** A high prevalence of *U. urealyticum* was observed in female patients with urogenital infections. And the biovar 1 and the serovars 1, 3, 6 were the main types of pathogens.

**KEYWORDS**biovar, drug resistance, serovar, *Ureaplasma urealyticum*

## 1 | INTRODUCTION

*Ureaplasma urealyticum* is a kind of microorganism with the size between the size of virus and bacteria, which can exist independently or parasitically. Recently, the *U. urealyticum* infection incidence has risen up year by year and becomes an important pathogen in the female genitourinary tract infections, such as vaginosis, urethritis, cervicitis, pelvic inflammatory, and pyelonephritis. Moreover, the infection may further cause infertility, prematurity, and spontaneous abortion. The common treatment for the *U. urealyticum* is drug therapy, which however has become much more difficult as the drug

resistance nature is getting much and more serious.<sup>1</sup> It is known that *U. urealyticum* consists of two types of biovars<sup>1</sup> and 14 types of serovars. The biovar 1 named tiny *Ureaplasma urealyticum* (*U. parvum*, Up), biovar 2 named *Ureaplasma urealyticum* (Uu). The serovars 1, 3, 6, 14 belong to Up type of biovar 1 and serovars 2, 4, 5, 7 - 13 belong to Uu type of biovar 2. Several studies<sup>2,3</sup> have indicated that different biovars maybe responsible for the different responses to antimicrobial agents.

However, the correlation between serovars and antimicrobial agents were nearly reported. In this study, we will detect the *Ureaplasma urealyticum* biovars and serovars in female patients with

urogenital infections, and determine the relationship between different types of pathogens and their antimicrobial susceptibility.

## 2 | MATERIALS AND METHODS

### 2.1 | Samples

This study has been approved by the Ethical Committee of our hospital, and before the study, all the study subjects have given informed consent for participation. About 246 female outpatients (age 20-55) including 125 female outpatients as the patient group and 121 healthy subjects as the control group from the gynecology and obstetrics clinics in our hospital were involved in the study. All cervical secretion specimens were examined within 48 hours after collection from July 2013 to July 2014.

### 2.2 | Reagents

The commercial Mycoplasma Identification and Antimicrobial Susceptibility Testing kit (Zhuhai Lizhu reagent Co., Zhuhai, China) was used to test all the samples for the presence of *U. urealyticum* according to the manufacture's protocol. The principle was simple as follows: the growing *U. urealyticum* in the culture could metabolize urea, which changes the color of the culture medium from yellow to red. The positive results were as follows: a color change of more than  $10^4$  units was an evidence of infection. Susceptibility tests included nine antibiotics: tetracyclines (doxycycline, minocycline); macrolides (azithromycin, roxithromycin, clarithromycin, and josamycin); fluoroquinolones (ofloxacin, levofloxacin, sparfloxacin, and azithromycin). The possible results were shown as "susceptible," "intermediate," and "resistant." Bacterial growth was evaluated after a 24-48 hours of incubation of culture at 37°C.

Ordinary PCR assay of the existence of MBA (multiple-banded antigen) gene was conducted to determine the biovars and serovars of *U. urealyticum* from the patient group (YE Xiang-qun et al.)<sup>4</sup>. The amplified products were subjected to 2% gel electrophoresis; a 2000 kb molecular marker was used to identify the product band sizes, which were 320 bp and 470 bp in size for *U. parvum* and *U. urealyticum*, respectively.

Statistical analysis was performed by using SPSS 17.0 software, Beijing, China.  $P < .05$  indicates the statistical significance.

## 3 | RESULT

In total, 102 (41.46%) of 246 subjects were positive for *U. urealyticum*, among them, 72 were from the patient group (57.60%, 72/125) and 30 were from the control group (24.79%, 30/121,  $P < .01$ ).

Among the 102 positive isolates, 75 have biovar 1 and 23 have biovar 2, the remaining four were found in four patients co-infected with both the biovars. The main serovars were 1, 3, 6 in biovar 1, and a mixed infection was observed in biovar 2. The distribution of *U. urealyticum* according to the biovars is given in Table 1.

**TABLE 1** Distribution of *Ureaplasma urealyticum* according to the biovars and serovars

| Biovars           | Infection mode | Isolates | Rates (%) |
|-------------------|----------------|----------|-----------|
| Biovars 1 (n=75)  | S1             | 11       | 14.67     |
|                   | S3             | 26       | 34.67     |
|                   | S1 + S3        | 6        | 8.00      |
|                   | S6             | 32       | 42.67     |
| Biovars 2 (n=23)  | S1' + S3'      | 14       | 60.87     |
|                   | S2' + S3'      | 9        | 39.13     |
| Coinfection (n=4) | S3 + S1'       | 3        | 75.00     |
|                   | S6 + S2'       | 1        | 25.00     |

The distribution of resistant isolates in two biovars is depicted in Table 2, biovar 1 was less resistant to sparfloxacin (\*\* $P < .01$ ) and more resistant to azithromycin, minocycline, and doxycycline than biovar 2 (\* $P < .05$ ).

The distribution of drug-resistant isolates in different serovars from biovar 1 is given in Table 3. The serovars S1, S3 and S6 have the highest resistant rate to azithromycin (90.90%), roxithromycin (84.62%), and ofloxacin (84.38%), respectively.

### 3.1 | Limitations of this study

We did not perform the Antimicrobial Susceptibility Test in the control group. Hence, we have no idea about the differences existing between the patient group and the control group.

## 4 | DISCUSSION

From the detection of *U. urealyticum* in liquid culture isolated from the female patients, we found that the total positive rate of *U. urealyticum* was 41.46%, and the infection rate of patient group (57.60%) was higher than that of the control group (24.79%), the difference was statistically significant ( $\chi^2 = 16.550$ ,  $P < .01$ ). Qing-Yong et al.<sup>5</sup> reported that single infections with *U. urealyticum* were the most prevalent in

**TABLE 2** Distribution of drug-resistant isolates in two biovars (R = drug-resistant isolate, Total = all positive isolate)

| Drugs          | Biovar 1 (%=R/Total) | Biovar 2 (%=R/Total) | $\chi^2$ | P      |
|----------------|----------------------|----------------------|----------|--------|
| Azithromycin   | 60 (80.00)           | 12 (52.17)           | 6.992    | .008** |
| Josamycin      | 27 (36.00)           | 8 (36.25)            | 0.010    | .915   |
| Clarithromycin | 29 (38.67)           | 8 (36.25)            | 0.113    | .737   |
| Roxithromycin  | 45 (60.00)           | 14 (60.86)           | 0.006    | .941   |
| Minocycline    | 12 (16.00)           | 0 (0.00)             | 4.193    | .041*  |
| Doxycycline    | 13 (17.33)           | 0 (0.00)             | 4.596    | .032*  |
| Sparfloxacin   | 45 (60.00)           | 19 (82.61)           | 3.971    | .046*  |
| Ofloxacin      | 45 (60.00)           | 14 (62.50)           | 0.006    | .941   |
| Levofloxacin   | 38 (50.67)           | 12 (52.17)           | 0.016    | .899   |

**TABLE 3** Distribution of drug-resistant isolates in mono-serovar from biovar 1

| Drugs          | S1         | S3         | S6         | P     |
|----------------|------------|------------|------------|-------|
| Azithromycin   | 10 (90.90) | 22 (84.62) | 19 (59.38) | .001* |
| Josamycin      | 2 (18.18)  | 10 (38.46) | 12 (37.5)  | .008* |
| Clarithromycin | 8 (72.73)  | 10 (38.46) | 7 (21.90)  | .001* |
| Roxithromycin  | 9 (81.82)  | 22 (84.62) | 19 (59.38) | .000* |
| Minocycline    | 2 (18.18)  | 0 (0.00)   | 5 (15.63)  | .000* |
| Doxycycline    | 2 (18.18)  | 0 (0.00)   | 9 (28.13)  | .000* |
| Sparfloxacin   | 6 (54.55)  | 17 (65.38) | 22 (68.75) | .062  |
| Ofloxacin      | 5 (45.45)  | 16 (61.54) | 27 (84.38) | .001* |
| Levofloxacin   | 6 (54.55)  | 15 (57.69) | 16 (50.00) | .254  |

\*Represents there is significant difference among the serovars S1, S3 and S6 (\*P < .01).

the female outpatients (31.2%). Huang et al.<sup>6</sup> reported that the positive rate of *U. urealyticum* in disease group is significantly higher than that in the healthy group. Gupta et al.<sup>2</sup> found that *Ureaplasma* was more frequently isolated in symptomatic than asymptomatic subjects (48% vs 22%) in Indian patients. However, Redelinghuys<sup>7</sup> reported that 76% (73/96) of the specimens from women contain *Ureaplasma* spp. Thus, the infection rate is regional. The reason why women are easily infected by *U. urealyticum* may be associated with the physiological characteristics that the female reproductive tract is short and is more susceptible to be infected. At the early stage of infection, symptoms are not obvious, so the examination and treatment can be easily ignored. Finally, persistent and cross-infection will come up.

In this study, we aimed to evaluate the relationship between biovars and the antimicrobial resistance of *U. urealyticum* in female patients with urogenital infections. We found that among 102 positive isolates from the patient group, more biovar 1 was found than biovar 2, and four patients were co-infected with both the biovars. Chang-tai et al.<sup>8</sup> reported that biovar 1 was found in 73 (57.94%), which is in accordance with our results. According to the results of Antimicrobial Susceptibility Testing, we found that biovars 1 and 2 were more resistant to azithromycin (80.00%) and sparfloxacin (82.61%), respectively, than other drugs, hence these drugs should not be empirically used. On the other side, we found that both biovars 1 and 2 were more sensitive to minocycline and doxycycline. However, biovar 1 shows much more resistance than biovar 2. The prevalence of antibiotic resistance profiles of *U. urealyticum* in our study differ from those reported by authors of similar studies.<sup>9-11</sup> It is most likely because of the different policies in antimicrobial use in different areas. From the serotyping detection, we found that there were different drug resistances with different serovars. The main serovars were 1, 3, 6 in biovar 1. Mixed infection was seen in biovar 2. The serovars S1/S3/S6 have the highest resistant rate to azithromycin (90.90%), roxithromycin and azithromycin (84.62%), and ofloxacin (84.38%), respectively. There are few reports on the drug resistance of different serovars. So, our study could be helpful in understanding the clinical treatment of the *U. urealyticum* infection.

## 5 | CONCLUSION

In summary, *U. urealyticum* biological type and drug resistance were studied in this paper, which provided the basis for the clinical rational use of drugs in a certain extent. It is also suggested that the detection of biovars and serovars of *U. urealyticum* is necessary in clinical practice. We need to avoid resistant strains when using antimicrobial drugs to refer to the rational use of antimicrobial drugs, as far as possible to avoid resistant strains.

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