Indian J Med Res 136, December 2012, pp 991-996

# *Ureaplasma* serovars & their antimicrobial susceptibility in patients of infertility & genital tract infections

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Received April 27, 2011

***Background & objectives*: Ureaplasmas have been implicated in a variety of clinical conditions. However, only certain serovars of ureaplasmas are disease associated. Only a few classes of antimicrobial agents are available for the treatment of mycoplasmal infections in humans. Increase of resistance of genital mycoplasmas to antimicrobials has been reported worldwide. The aim of the present study was to determine the occurrence of *Ureaplasma* serovars in patients with infertility and genital tract infections with polymerase chain reaction (PCR)–based serotyping. The antimicrobial susceptibilities of *Ureaplasma* spp. and *Mycoplasma hominis* were also assessed to determine the most suitable treatment strategy.**

***Methods*: Sexually active adults (n=147) with symptoms of genital tract infections and 115 infertile women were enrolled. Endocervical swabs from women and urethral swabs from men were subjected to culture and multiplex PCR for detection of genital mycoplasmas. Serotyping of *Ureaplasma* was done by PCR and antimicrobial susceptibility to doxycycline, azithromycin, josamycin and ofloxacin was done by microbroth dilution method.**

***Results*: Ureaplasma was detected in 25.8 per cent patients with genital tract infections and 20.8 per cent in infertile women. Serovar 3/14 was the most frequent isolate followed by serovar 1 and serovar 6. The majority of *Ureaplasma* isolates were susceptible to doxycycline (91%) and josamycin (86%) followed by ofloxacin (77%) and azithromycin (71%). All the isolates of *M. hominis* were uniformly susceptible to doxycycline, josamycin and ofloxacin.**

***Interpretation & conclusions*: The predominance of *Ureaplasma* serovar 3/14 suggests their possible pathogenic role in genital tract infections and infertility. For empirical treatment, doxycycline could be the drug of choice for genital mycoplasmas.**

**Key words** Antimicrobial susceptibility - PCR - ureaplasma serovars

Ureaplasmas belong to the normal commensal flora of the genital tract of human beings, with colonisation rates between 60 and 80 per cent worldwide[1]. However, ureaplasmas are also implicated in invasive diseases such as urethritis, postpartum endometritis, chorioamnionitis, spontaneous abortion and premature

birth, as well as low birth weight, pneumonia, bacteremia, meningitis, and chronic lung disease in prematurely born infants[2]. In studies conducted earlier in India *Ureaplasma urealyticum* was isolated from 32[3] and 29 per cent[4] of women. In a previous study from our centre, *U. urealyticum* was recovered from 47 and 45 per cent of men and women with symptoms of genital discharge[5]. In another Indian study[6] *U. urealyticum* was the predominant organism (56%) isolated from women with chronic cervicitis.

Because of the frequency with which ureaplasmas occur in healthy asymptomatic individuals, it has been suggested that only certain subgroups of the species are pathogenic. The majority of human *Ureaplasma* isolates belong to *U. parvum* (biovar 1). *U. urealyticum* (biovar 2) is isolated less often[7]. We have also reported *U. parvum* (biovar 1) as the predominant biovar in patients with genital tract infections[8]. *Ureaplasma* consists of 14 serovars. *U. parvum* includes serotypes 1, 3, 6 and 14, whereas *U. urealyticum* comprises the remaining 10 serotypes[7]. Some *Ureaplasma* serovars have been found to be more frequently associated with clinical diseases; however, the data are limited and conflicting because of the difficulties with traditional genotyping methods[9]. No studies are available from India on the prevalence of *Ureaplasma* serovars in clinical diseases.

*Mycoplasma* infections require the therapeutic use of antimicrobials. Tetracyclines, macrolides and quinolones are the major antibiotics used in the treatment of genital mycoplasmas[10]. However, their therapeutic efficacy may be unpredictable due to increasing resistance[11]. The aim of the present study was to determine the distribution of *Ureaplasma* serovars in patients with infertility and genital tract infections by polymerase chain reaction (PCR) – based serotyping. The antimicrobial susceptibilities of *Ureaplasma* spp. and *M. hominis* isolates were also assessed to determine the most suitable strategy for treating these infections.

## Material & Methods

All consecutive sexually active adults attending the STD outpatient clinic with symptoms of urethral or cervical discharge, genital pruritis or dysuria and 115 women attending the infertility clinic at the All India Institute of Medical Sciences (AIIMS), New Delhi, India, during January 2010 to December 2010 were included in this study. The medical records of each patient were examined. Patients who had been treated with antibiotics or antifungal agents within the past four weeks were excluded, as were patients who tested positive for *Neisseria gonorrhoeae*, bacterial vaginosis and *Mycobacterium tuberculosis*.

A total of 147 patients (59 males, 88 females) with genital tract infections and 115 women with infertility were eligible for enrollment and screened for infection with genital mycoplasmas *viz.*; *Ureaplasma* spp and *M. hominis*. All female patients with genital tract infection were also screened for candidial infection by means of 10% potassium hydroxide (KOH) examination of vaginal secretions.

Ethics Committee approval for study protocol and written informed patient consent were taken for this study.

The specimens included two endocervical swabs from women and two urethral swabs from men. Samples were transported in 2 ml of Pleuropneumonia like organisms medium (PPLO) broth (Difco, USA) containing urea for *U. urealyticum* and arginine for *M. hominis*. Serial 10-fold dilutions starting from 1:10 to 1: $10^5$ were prepared. The broths were incubated at 37°C under 5 per cent $CO_2$ and were inspected twice daily for 14 days before discarding as negative. The highest dilution which changed the colour of the indicator present in the broth represented the number of the organisms in the sample in colour changing units per ml (CCU/ml). The reference strains from National Collection of Type Culture *Ureaplasma* (NCTC10177) and *M.hominis* (NCTC10111) were used as positive controls.

In addition to culture, multiplex PCR was performed for detection of genital mycoplasmas with primers specific for urease gene of *Ureaplasma* and 16S rRNA gene of *M. hominis* to detect the presence of DNA of these two organisms using the protocol by Stellrecht *et al*[12]. Briefly, the 50 µl amplification reaction mixture contained 5.00 µl of 10× PCR buffer [1× PCR buffer is 10 mmol/l Tris–HCl (*p*H 8.8 at 25°C), 50 mmol/l KCl, and 0.1% Triton X-100], 3.0 mM $MgCl_2$, 1.25 U of Taq polymerase (GeneiTaq, Bangalore Genei, India), 400 µmol/l (each) deoxynucleoside triphosphate mixture, 25 pmol of each primer, 16 µl of sample DNA and ultrapure sterile water. The PCR conditions used were initial denaturation at 95°C for 10 min, followed by 35, two-step cycles of 95°C for 15 sec and 60°C for 60 sec, followed by 5 min at 72°C (Fig I). All the isolates of *Ureaplasma* were further biotyped in a second PCR targeting the multiple banded antigen (MBA) gene[13]. The PCR conditions used were initial denaturation at

95°C for 5 min, cyclic denaturation at 94°C for 1 min, annealing at 56°C and elongation at 72°C for 1 min for 35 cycles and final extension at 72°C for 5 min in a thermocycler. PCR positive for biovar 1 were further subtyped into serovars as described earlier[7]. Briefly, the 50µl amplification reaction mixture contained 10× PCR buffer [1× PCR buffer is 10 mmol/l Tris-HCl (*p*H 8.8 at 25 °C), 1.5 mM/l $MgCl_2$, 50 mmol/l KCl, and 0.1% Triton X-100], 1 U of Taq polymerase (GeneiTaq, Bangalore Genei, India), 200 µmol each dCTP, dGTP, dATP, dTTP, 10 pmol of each primer, 5 µl of sample DNA and ultrapure sterile water. The PCR conditions used were initial denaturation at 95°C for 5 min, cyclic denaturation at 95°C for 45 sec, annealing at 55°C for 45 sec and elongation at 72°C for 45 sec for 35 cycles and final extension at 72°C for 5 min in a thermocycler. Controls for polymerase inhibition were the DNA for serovar 1, 3/14, and 6 (kindly provided by Dr M.A. De Francisco, Italy). Primers UMS83/UMA269, UMS125/UMA269 and UMS54/UMA269 were used for identification of serovar 1 (398bp), serovar3/14 (442 bp) and serovar 6 (369 bp) respectively (Figs 2-4).

The isolates of *Ureaplasma* and *M. hominis* were subjected to antibiotic susceptibility testing against azithromycin (Hi-Media Laboratories, Mumbai, India), doxycycline (Hi-Media Laboratories, Mumbai, India),

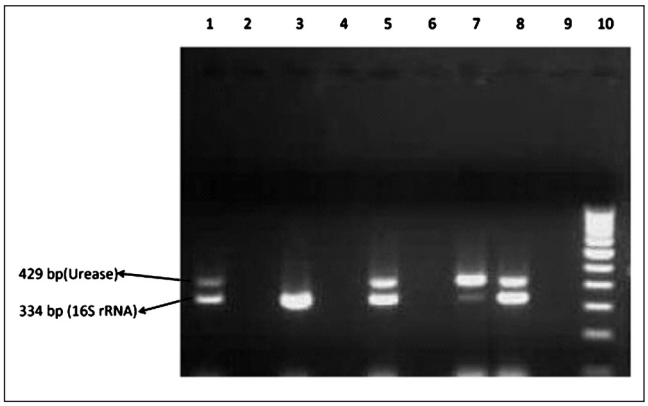


**Fig. 1.** PCR detection of urease and 16S rRNA gene. Lane 1 : *U. urealyticum* positive control (NCTC 10177; urease positive) and *M. hominis* positive control (NCTC 10111; 16S rRNA positive; Lane 2, 4 and 9: Clinical samples (negative); Lane 3 : Clinical samples (*M. hominis* positive); Lane 5, 7 and 8 : Clinical samples (*U. urealyticum* and *M. hominis* positive); Lane 6 : Negative control; Lane 10: 100 bp ladder.

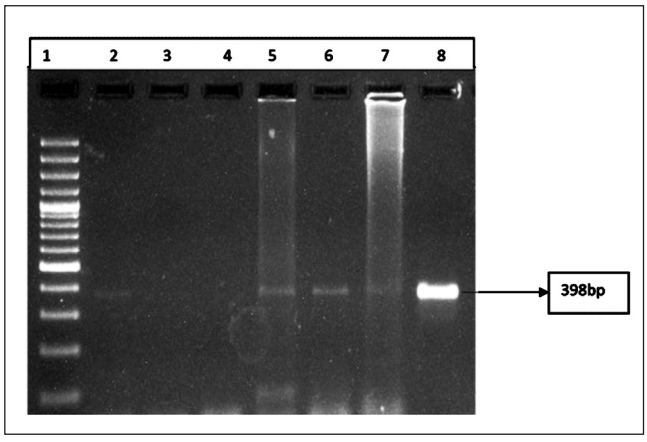


**Fig. 2.** Results of PCR amplification for identification of serovar 1; Lane 1: 100 bp Marker; Lane 3, 4 : Negative clinical samples; Lane 2,5-7: Positive clinical samples; Lane 8: Positive control.

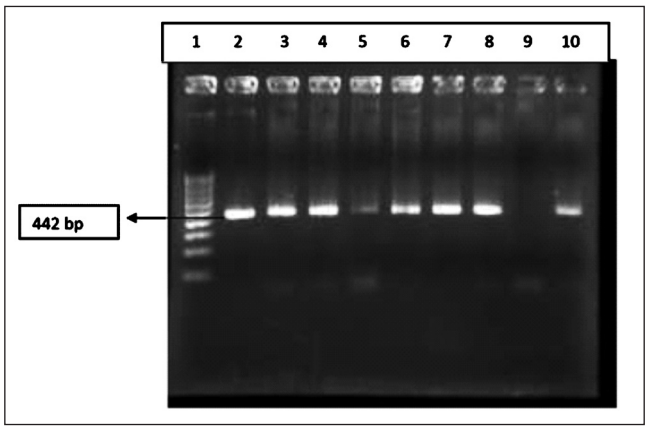


**Fig. 3.** Results of PCR amplification for MBA gene for identification of serovar 3/14; Lane 1: 100 bp marker; Lane 2: Positive control; Lane 3-8, 10: Clinical samples; Lane 9: Negative control.

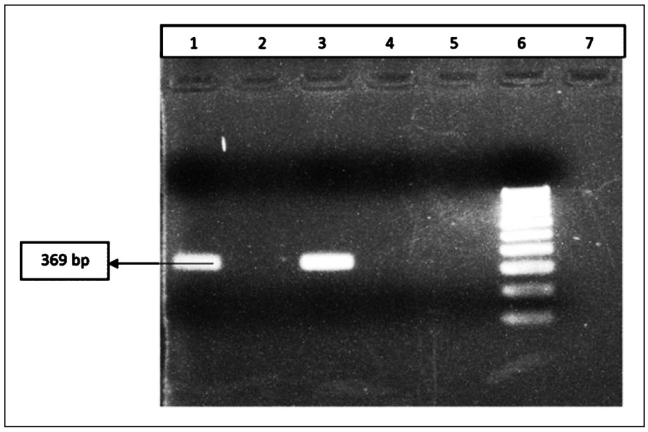


**Fig. 4.** Results of PCR amplification for identification of serovar 6; Lane 1: Positive control; Lane 2,4: Negative clinical samples; Lane 3: Positive clinical samples; Lane 5 : Negative control; Lane 6: 100 bpMarker.

ofloxacin (Sigma Aldrich, USA) and josamycin (Alexis Biochemicals, Switzerland) by microbroth dilution method[14]. Cut-off MICs for susceptibility, intermediate and resistance were based on the values described by Krausse *et al*[15].

*Statistical analysis*: Pearson Chi-square was used for significance analysis.

## Results

Ureaplasma was detected in 38 of 147 [25.8%; 95% confidence interval (CI), 19-34] patients with genital tract infection, of whom 27 of 88 (31%) were females and 11 of 59 (19%) were males and in 24 of 115 (20.8%; 95% CI: 14-29%) infertile women. *M. hominis* was detected in 18 (12.2%; 95% CI, 7.5-18.6%; 13 females, 5 males) and 8 (6.9%; 95% CI: 3.0-13.2%) patients, respectively. Co-infection with *Ureaplasma* and *M. hominis* was detected in five patients (4 females, 1 male) with genital tract infection and one woman with infertility by culture and/or PCR. In addition, microscopic examination showed candidiasis in four (4.5%) of the 88 women with genital tract infection.

Of the 115 infertile women, 53 were cases of primary infertility and 62 of secondary infertility. *Ureaplasma* was detected in 9 of 53 (17%) and 15 of 62 (24.2%) cases of primary and secondary infertility. *M. hominis* was detected in three (5.7%) and five (8.1%) cases of primary and secondary infertility, respectively.

*U. parvum* (biovar 1) was predominant in 38 of 147 (25.8%; 95% CI: 19-34%) patients with genital tract infection and in 24 of 115 (20.8%; 95% CI: 14-29%) infertile women. None of the patients were infected with both biovars. *U. parvum* isolates were further subtyped into different serovars (Table I). Serovar 3/14 (29/62, 46.8%) was the most frequent isolate in both group of patients followed by serovar 1 (18/62, 29.0%) and serovar 6 (15/62, 24.2%).

The majority of *Ureaplasma* isolates were susceptible to doxycycline (91%; 95% CI: 77-98%) and josamycin (86%; 95% CI 69.7-95.2%), 77 per cent were susceptible to ofloxacin (CI: 59.8-89.6%) and 71 per cent to azithromycin (CI: 54-85%) (Table II). Resistance to ofloxacin and azithromycin was of intermediate nature in all the *Ureaplasma* isolates. The serovars of *U. parvum* showed no significant difference in their susceptibility patterns to the four antibiotics tested. All the isolates of *M. hominis* were resistant to azithromycin. No resistance was observed to doxycycline, josamycin and ofloxacin (Table II).

**Table I**. Distribution of *Ureaplasma parvum* serovars (n=62) among patients with genital tract infection and women with infertility

| | *Ureaplasma parvum* No. (%) | | |
|---|---|---|---|
| Study group | Serovar 1 | Serovar 3/14 | Serovar 6 |
| Genital tract infections (n=38) | 10 (26.3) | 17 (44.7) | 11 (29) |
| Infertile women (n=24) | 8 (33.3) | 12 (50.0) | 4 (16.7) |
| Total (n=82) | 18 (29.0) | 29 (46.8) | 15 (24.2) |

$P<0.05$

**Table II.** Susceptibility rates of *Ureaplasma* spp. and *M. hominis* to four different antibiotics

| Antibiotics | *Ureaplasma* spp. (%) n=35 | *M. hominis* (%) n=20 |
|---|---|---|
| Doxycycline | 91 | 100 |
| Azithromycin | 71 | 0 |
| Josamycin | 86 | 100 |
| Ofloxacin | 77 | 100 |

## Discussion

Detection of ureaplasmas is possible by characteristic growth on appropriate culture media but species identification of *U. parvum* and *U. urealyticum* along with serovar identification by molecular method is important, especially for correct interpretation of laboratory results and evaluation of pathogenicity. *U. parvum* was found to be predominant isolate in both our study groups as reported by other also[9].

Among the different serovars of *U. parvum*, serovar 3/14 was the most frequent serovar detected in both the study groups. Though the difference in detection rates of the different serovars of *U. parvum* was not statistically significant, predominance of serovar 3/14 was consistent with previous reports[7] and suggests a possible pathogenic role of *U. parvum* serovar 3/14.

It is possible that the combination of variable serovar specific genes of *Ureaplasma* with generally known virulence factors determines the development of pathological processes on the mucosal surface of the human genital tract. Further studies are needed to confirm the serovar distribution in different clinical settings and their possible pathogenic role.

Although many different treatment alternatives are available for the treatment of genital mycoplasmas,

doxycycline is the most frequently used antibiotic[16]. However, resistance to tetracycline due to *tet*M determinant has been observed in both *Ureaplasma* and *M. hominis* throughout the world[16]. Macrolides and especially quinolones have been new treatment alternatives with very high efficacy[17]. In the present study, doxycycline, azithromycin and ofloxacin were tested as these are the major antibiotics used in the treatment of genital tract infections caused by mycoplasmas[10]. A further purpose of choosing these antimicrobial agents was because these are conventionally being used for the routine treatment of sexually transmitted infections[15]. In addition, a new macrolide, josamycin was also tested.

Doxycycline was the most active agent against both *Ureaplasma* and *M. hominis*. This finding is consistent with those of other studies conducted in China and Turkey[18,19]. Though doxycycline resistance has been reported in both *Ureaplasma* and *M. hominis*[18], it was found to be the most active agent against both these pathogens.

*Ureaplasma* has been considered susceptible to macrolides[11]. However, in our study *Ureaplasma* was moderately susceptible to azithromycin. Similar to our findings, azithromycin resistant strains of *Ureaplasma* are now being reported with increasing frequency[1]. Resistance to all the isolates of *M. hominis* to azithromycin was not surprising as *M. hominis* is known to be intrinsically resistant to macrolide[1]. No resistance was seen against the new macrolide, josamycin for *M. hominis*. Our results are in agreement with those reported earlier[11,19]. However, a high rate of resistance to josamycin was observed for *Ureaplasma* strains. Though resistance to josamycin has been reported for *Ureaplasma* and *M. hominis*[18], the development of resistance of *Ureaplasma* isolates in our study was not clear, since the antibiotic is unavailable in our pharmacy and not prescribed in our hospital.

The quinolones are considered useful in the treatment of mycoplasma infection as these are potentially effective against pathogenic species and also including strains resistant to other drugs such as doxycycline[20]. Although all *M. hominis* isolates were uniformly susceptible to ofloxacin, it proved to be ineffective against 23 per cent of *Ureaplasma* isolates. In addition, the doxycycline resistant strains were also resistant to ofloxacin. Similar rates of resistance to ofloxacin has been observed in clinical isolates of *Ureaplasma* in most of the studies[19].

Treatment of mycoplasma infection is imperative to prevent the occurrence of complications. Empirical therapy is important in the treatment of mycoplasmas, since culture and antimicrobial susceptibilities of mycoplasmas are not routinely done in Indian laboratories. Our results indicate that doxycycline should be the first choice drug when empirical treatment is required. Results from previous reports regarding the antimicrobial susceptibilities of genital mycoplasmas, originating from various countries, are controversial[17,19]. Therapeutic failures can be avoided by implementation of empiric treatment regimens based on the determination of antimicrobial susceptibility of genital mycoplasmas in a given geographical area. Thus, it is important to offer the sensitivity test screening periodically and to use drugs on the basis of regularly available sensitivity results.

## Acknowledgment

The authors thank the Indian Council of Medical Research, New Delhi for financial support.

## References

1. Bayraktar RM, Ozerol HI, Gucluer N, Celik O. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women. *Int J Infect Dis* 2010; *14* : 90-5.
2. Waites KB, Katz B, Schelonka RL. Mycoplasmas and Ureaplasmas as neonatal pathogens. *Clin Microbiol Rev* 2005; *18*: 757-89.
3. Gupta A, Gupta A, Gupta S, Mittal A, Chandra P, Gill AK. Correlation of mycoplasma with unexplained infertility. *Arch Gynecol Obstet* 2009; *280* : 981-5.
4. Jais M, Joshi SG. Correlation of *Ureaplasma urealyticum* with infertility. *Indian J Sex Transm Dis* 1998; *19* : 45-7.
5. Dhawan B, Gupta V, Khanna N, Singh M, Chaudhry R. Evaluation of the Diagnostic Efficacy of PCR for *Ureaplasma urealyticum* infection in Indian adults with symptoms of genital discharge. *Jpn J Infect Dis* 2006; *59* : 57-8.
6. Bhandari H, Malhotra S, Sharma M, Kumar B. Microflora of women with chronic cervicitis. *J Indian Med Assoc* 2000; *98* : 384-6.
7. Francesco De MA, Negrini R, Pinsi G, Peroni L. Detection of *Ureaplasma* biovar and polymerase chain reaction based-subtyping of *Ureaplasma parvum* in women with or without symptoms of genital infections. *Eur J Microbiol Infect Dis* 2009; *28* : 641-6.
8. Gupta V, Dhawan B, Khanna N, Agarwal N, Bhattacharya SN, Sreenivas V, *et al*. Detection and biovar discrimination of *Ureaplasma urealyticum* in Indian patients with genital tract infections. *Diag Microbiol Infect Dis* 2008; *60* : 95-7.
9. Abele-Horn M, Wollf C, Dressel P, Pfaff F, Zimmermann A. Association of *Ureaplasma urealyticum* biovars with clinical outcome for neonates, obstetrics patients and gynaecological

patients with pelvic inflammatory disease. *J Clin Microbiol* 1997; *35*: 1199-1202.

10. Kenny GE, Cartwright FD. Susceptibilities of *Mycoplasma hominis*, *M. pneumonia* and *Ureaplasma urealyticum* to GAR-936, dalfopristin, dirithromycin, evernimicin, gatifloxacin, linezolid, moxifloxacin, quinupristin-dalfopristin and telithromycin compared to their susceptibilities to reference macrolides, tetracyclines and quinolones. *Antimicrob Agents Chemother* 2001; *45* : 2604-8.
11. Stellrecht KA, Woron MA, Mishrik GN, Venezia AR. Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. *J Clin Microbiol* 2004; *42* : 1528-33.
12. Knox CL, Timms P. Comparison of PCR, nested PCR and random amplified polymorphic DNA PCR for detection and typing of *Ureaplasma urealyticum* in specimens from pregnant women. *J Clin Microbiol* 1998; *36* : 3032-9.
13. Waites KB, Duffy LB, Shwartz S, Talkington DF. In: Isenberg H, editor. *Clinical microbiology procedures handbook*, 2nd ed. Washington, D.C. ASM Press; 2004. p. 3.15.1-17.
14. Krausse R, Schubert S. *In vitro* activities of tetracyclines, macrolides, fluoroquinolones and clindamycin against *Mycoplasma hominis* and *Ureaplasma* spp. isolated in Germany over 20 years. *Clin Microbiol Infect* 2010; *16* : 1649-55.
15. Centres for Disease Control and Prevention: Disease characterized by urethritis and cervicitis. Sexually transmitted diseases treatment guidelines. *Morb Mortal Wkly Rep* 2002; *51* : 30-42.
16. Robertz MC, Kenny GE. Dissemination of the tetM tetracycline resistance determinant to *Ureaplasma urealyticum*. *Antimicrob Agents Chemother* 1986; *29* : 350-2.
17. Ullmann U, Schubert S, Krausse R. Comparative *in vitro* activity of levofloxacin, other fluoroquinolones, doxycycline and erythromycin against *Ureaplasma urealyticum* and *Mycoplasma hominis*. *J Antimicrob Chemother* 1999; *43* (Suppl C): 33-6.
18. Lin HP, Lu HX. Analysis of detection and antimicrobial resistance of pathogens in prostatic secretion from 1186 infertile men with chronic prostatitis. *Zhonghua Nan Ke Xue* 2007; *13* : 628-31.
19. Kilic D, Basar MM, Kaygusuz S, Yilmaz E, Basar H, Baiislam E. Prevalence and treatment of *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* in patients with non-gonococcal urethritis. *Jpn J Infect Dis* 2004; *57* : 17-20.
20. Duffy LB, Crabb D, Searcey K, Kempf MC. Comparative potency of gemifloxacin, new quinolones, tetracycline and clindamycin against *Mycoplasma* spp. *J Antimicrob Chemother* 2000; *45* (Suppl S1): 29-33.

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