

Antimicrobial Susceptibilities of *Ureaplasma urealyticum* and *Mycoplasma hominis* in Pregnant Women

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Background: *Ureaplasma urealyticum* and *Mycoplasma hominis* are associated with an increased risk of pregnancy complications, such as preterm birth and premature membrane rupture. The purpose of this study was to determine the isolation rates and antimicrobial susceptibilities of genital mycoplasma in a sample of pregnant women from Jinju, Korea.

Methods: Vaginal swabs were obtained from 258 pregnant women between 2004 and 2008 and tested for the presence of *U. urealyticum* and *M. hominis* at Gyeongsang National University Hospital. The identification and antimicrobial susceptibilities of *U. urealyticum* and *M. hominis* were determined with a commercially available kit, the Mycoplasma IST2 Kit (bioMérieux, Marcy-l'Etoile, France), and evaluated according to standards set by the Clinical and Laboratory Standards Institute (CLSI).

Results: *U. urealyticum* only was detected in 105 specimens (38.6%), while *M. hominis* only was detected only in 2 specimens (1.8%). Seven specimens (6.7%) were positive both for *U. urealyticum* and *M.*

hominis. Susceptibilities of *U. urealyticum* to azithromycin, erythromycin, clarithromycin, and doxycycline were 75.2%, 82.9%, 88.6%, and 88.6%, respectively, while almost all of the isolates were susceptible to josamycin (99.0%) and pristinamycin (100%). The susceptibility of *U. urealyticum* to ofloxacin and ciprofloxacin was 56.2% and 15.2%, respectively.

Conclusion: The rate of isolation of genital mycoplasma in pregnant women was 44.2% in Jinju; most of the mycoplasma were *U. urealyticum*. *U. urealyticum* and *M. hominis* were highly resistant to quinolones, but susceptible to josamycin. Therefore, empirical treatment without prior identification and determination of the antimicrobial susceptibility of genital mycoplasma will fail in many cases. (Korean J Clin Microbiol 2009;12:159-162)

Key Words: *Ureaplasma urealyticum*, *Mycoplasma hominis*, Genital mycoplasma, Antimicrobial susceptibilities

INTRODUCTION

Ureaplasma urealyticum and *Mycoplasma hominis* are commensals which can be detected in the lower genitourinary tract of sexually active women, resulting in colonization of genitalia by sexual contact[1,2]. Although most colonized women remain asymptomatic, vaginal colonization with *U. urealyticum* and *M. hominis* are associated with an increased risk of developing certain pathogenic conditions and pregnancy complications, such as bacterial vaginosis, pelvic inflammatory disease, postpartum fever, postpartum septicemia, infertility, premature rupture of the membranes, preterm labor, preterm birth, and systemic neonatal infections[2-5]. Such aggravating conditions require the therapeutic use of antimicrobials. Tetracyclines and quinolones are the drugs

of choice[6-8], but obstetricians empirically use macrolides for treatment of pregnant women in many cases[9]. The antimicrobial susceptibility of genital mycoplasmas has changed over time and is different by geographic area[1,10-15]. It is important to know the antimicrobial susceptibilities of genital mycoplasmas in a specific geographic region for the successful treatment. No such a study regarding the antimicrobial susceptibilities of *U. urealyticum* and *M. hominis* has been reported in Korea so far. The present study was performed to investigate the isolation rates and the antimicrobial susceptibilities of *U. urealyticum* and *M. hominis* in pregnant women who reside in Jinju, a southern area of Korea.

MATERIALS AND METHODS

Vaginal swabs were obtained from 258 pregnant women (range, 20~46 years; mean, 29.6 years) between 2004 and 2008 and were tested for the presence of *U. urealyticum* and *M. hominis* at Gyeongsang National University Hospital. Identification and antimicrobial susceptibilities of *U. urealyticum* and *M. hominis* were

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Table 1. Antimicrobial susceptibilities (%) of *U. urealyticum* and *M. hominis* from vaginal swabs

	<i>U. urealyticum</i> (N=105)			<i>M. hominis</i> (N=2)			<i>U. urealyticum</i> + <i>M. hominis</i> (N=7)		
	S	I	R	S	I	R	S	I	R
Tetracycline	81.0	7.6	11.4	50	0	50	42.9	28.6	28.6
Doxycycline	88.6	4.8	6.7	100	0	0	85.7	0	14.3
Azithromycin	75.2	18.1	6.7	0	0	100	0	0	100
Clarithromycin	88.6	2.9	8.6	0	0	100	0	0	100
Erythromycin	82.9	4.8	12.4	0	0	100	0	0	100
Josamycin	99.0	1.0	0	100	0	0	85.7	14.3	0
Ciprofloxacin	15.2	62.9	21.9	0	50	50	0	57.1	42.9
Ofloxacin	56.2	39.0	4.8	0	100	0	42.9	57.1	0
Pristinamycin	100	0	0	100	0	0	100	0	0

The breakpoints (mg/L) according to CLSI are as follows: tetracycline S \leq 4, R \geq 8; doxycycline S \leq 4, R \geq 8; azithromycin S \leq 0.12, R \geq 4; clarithromycin S \leq 1, R \geq 4; erythromycin S \leq 1, R \geq 4; josamycin S \leq 2, R \geq 8; ciprofloxacin S \leq 1, R \geq 2; ofloxacin S \leq 1, R \geq 4; pristinamycin R \geq 2.

determined with a commercially available Mycoplasma IST2 kit (bioMerieux, Marcy-l'Etoile, France), as indicated by the manufacturer. Briefly, the cotton swab included in the kit was inoculated in R1 transport medium, which inhibits most Gram-negative and -positive bacteria. The inoculated R1 medium was vortexed rapidly and 3 mL was added to the growth R2 medium, which contained 1 mL of lyophilized urea/arginine broth. After reconstitution and shaking, 55 μ L was dispensed into each of the 22 test wells on the strip. Two drops of mineral oil were added to each well. The remainder of the R2 medium and the inoculated strip were then incubated at 37°C and observed for color changes at 24 and 48 h. The antimicrobial susceptibility testing included tetracycline, doxycycline, erythromycin, azithromycin, clarithromycin, josamycin, ofloxacin, ciprofloxacin, and pristinamycin. The development or absence of red color on the relevant part of the strip provided an index of resistance or susceptibility to each antimicrobial agent, respectively, according to the guidelines of the CLSI. The breakpoints for the antimicrobials tested are given in Table 1.

RESULTS

U. urealyticum and *M. hominis* were detected in 114 of 258 cultures (44.2%). *U. urealyticum* was detected in 105 cultures (38.6%) and *M. hominis* was detected in 2 cultures (1.8%). Seven cultures (6.7%) were positive both for *U. urealyticum* and *M. hominis*. The susceptibilities of *U. urealyticum* to azithromycin, erythromycin, and clarithromycin were 75.2%, 82.9%, and 88.6%, respectively, while nearly all of the strains were susceptible to josamycin (99.0%) and pristinamycin (100%). The susceptibilities of ofloxacin and ciprofloxacin were shown to be 56.2% and 15.2% against *U. urealyticum*. Two strains of *M. hominis* were susceptible to doxycycline, josamycin, and pristinamycin, while resistant to azithromycin, erythromycin, and clarithromycin. The antimicrobial susceptibility pattern of the mixed strains was similar to that of *M. hominis* (Table 1).

DISCUSSION

Genital mycoplasmas have been detected more frequently in the group with preterm deliveries compared to the women who deliver at term. The presence of mycoplasma in the lower genital tract early in pregnancy is known as a risk factor for preterm delivery. Among the pregnant women with premature labor, the duration of pregnancy was prolonged after the onset of premature labor in the *M. hominis*-positive group than that in *M. hominis*-negative group[16]. Early pregnancy screening for genital mycoplasmas and following treatment may reduce preterm deliveries[16,17]. In the present study, the isolation rate of *U. urealyticum* and *M. hominis* in pregnant women was 44.2% and our findings are similar to that of other reports, with a higher rate of *U. ureaplasma* colonization (38.6%) and a lower rate of *M. hominis* (1.8%) [18-20]. However, it is difficult to regard all isolates of *U. urealyticum* and *M. hominis* as pathogens or risk factors of preterm delivery because we did not compare the preterm labor group with the term group. We did not review the clinical outcomes after the tests.

There are a limited number of drugs available against genital mycoplasmas in pregnant women. Agents like β -lactams are completely inactive against *U. urealyticum* and *M. hominis* due to the lack of a cell wall. The antimicrobial susceptibilities to macrolides, which are empirical treatment regimen for genital mycoplasma infection, were different between the two species[21-24]. With respect to antimicrobial susceptibilities of genital mycoplasmas, the *U. urealyticum* strains were susceptible to tetracycline and doxycycline (81.0% and 88.6%, respectively). *M. hominis* strains (N=2) were susceptible to tetracycline and doxycycline (50% and 100%, respectively). Among the macrolides, the highest susceptibility against *U. urealyticum* was observed by josamycin (99.0%), followed by clarithromycin (88.6%), and erythromycin (82.9%), while the susceptibility to azithromycin, which is most commonly used antimicrobial for pregnant women in our region, was not as high (75.2%) as the other macrolides. *M. hominis*

strains are known to be naturally resistant to C14 macrolides (erythromycin, clarithromycin, and roxithromycin)[8,21-24], which is fully in agreement with our results. *M. hominis* was resistant to three types of the macrolides tested, except josamycin. All strains of *U. urealyticum* and *M. hominis* were completely susceptible to pristinamycin. Ofloxacin and ciprofloxacin proved to be ineffective against the majority of strains of *U. urealyticum* and *M. hominis* and a significant number of *U. urealyticum* strains were intermediately susceptible (39% for ofloxacin and 62.8% for ciprofloxacin). The pattern of antimicrobial susceptibilities against mixed isolates (*U. urealyticum* and *M. hominis*) was similar to that of *M. hominis*. *U. urealyticum* strains isolated from the women with cervicitis in Athens, Greece were highly susceptible to tetracycline, doxycycline, and pristinamycin, while erythromycin, azithromycin, clarithromycin, ciprofloxacin, and ofloxacin were inactive against most of the strains. *M. hominis* strains were completely susceptible to tetracycline, doxycycline, and pristinamycin[18]. The susceptibilities of macrolides against *U. urealyticum* strains in Greece were much lower (14.4~79.2%) than our results (75.2~99%). Susceptibilities of erythromycin and ofloxacin against *U. urealyticum* strains were only 10.3% and 11.4%, respectively in Bolu, Turkey[19]. However, doxycycline or ofloxacin was used as a first choice in the empirical treatment of *U. urealyticum* and *M. hominis* infections in Japan[1]; we observed that erythromycin was still active, but quinolone derivatives (ofloxacin and ciprofloxacin) were inactive against *U. urealyticum* and *M. hominis* in our region. These discrepancies might be due to the different antimicrobial-use policies, which lead to the emergence of resistance to one or another antimicrobial agents. The empirical treatment can be ineffective for these reasons. Thus, it is difficult to establish common guidelines for the empirical treatment of genital mycoplasma infections. In the present study, we determined the antimicrobial susceptibilities for genital mycoplasma in Jinju, which represents a specific geographic region, rather than whole country. A nationwide survey may enable us to establish new guidelines for the treatment of genital mycoplasma infections in Korea.

In conclusion, the isolation rate of genital mycoplasma in pregnant women was 44.2% in Jinju. Both isolates were resistant to quinolones, but susceptible to josamycin and doxycycline. Characteristically, susceptibility of azithromycin, the empirical treatment regimen for pregnant women in our geographic region, was not as high as we expected. All of the mixed isolates were resistant to azithromycin. Therefore, empirical treatment without the isolation and identification of genital mycoplasma would fail in many cases. *In vitro* determination of the antimicrobial susceptibility of the genital mycoplasma in each clinical case is required to avoid therapeutic failures.

REFERENCES

- Kilic D, Basar MM, Kaygusuz S, Yilmaz E, Basar H, Batislam E. Prevalence and treatment of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* in patients with nongonococcal urethritis. *Jpn J Infect Dis* 2004;57:17-20.
- Krohn MA, Hillier SL, Nugent RP, Cotch MF, Carey JC, Gibbs RS, et al. The genital flora of women with intraamniotic infection. Vaginal infection and prematurity study group. *J Infect Dis* 1995;171:1475-80.
- Koch A, Bilina A, Teodorowicz L, Stary A. *Mycoplasma hominis* and *Ureaplasma urealyticum* in patients with sexually transmitted diseases. *Wien Klin Wochenschr* 1997;109:584-9.
- McDonald HM, O'Loughlin JA, Jolley PT, Vigneswaran R, McDonald PJ. Changes in vaginal flora during pregnancy and association with preterm birth. *J Infect Dis* 1994;170:724-8.
- Waites KB, Rudd PT, Crouse DT, Canupp KC, Nelson KG, Ramsey C, et al. Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* infections of central nervous system in preterm infants. *Lancet* 1988;1:17-21.
- Arai S, Gohara Y, Kuwano K, Kawashima T. Antimycoplasmal activities of new quinolones, tetracyclines, and macrolides against *Mycoplasma pneumoniae*. *Antimicrob Agents Chemother* 1992;36:1322-4.
- Hannan PC. Comparative susceptibilities of various AIDS-associated and human urogenital tract mycoplasmas and strains of *Mycoplasma pneumoniae* to 10 classes of antimicrobial agent in vitro. *J Med Microbiol* 1998;47:1115-22.
- Kenny GE and Cartwright FD. Susceptibilities of *Mycoplasma hominis* and *Ureaplasma urealyticum* to two new quinolones, sparfloxacin and WIN 57273. *Antimicrob Agents Chemother* 1991;35:1515-6.
- Ye Y, Tu S, Li H. Clinic intervention study on urogenital mycoplasma infection of pregnant women. *Zhonghua Liu Xing Bing Xue Za Zhi* 2001;22:293-5.
- Ullmann U, Schubert S, Krause 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.
- Lister PJ, Balechandran T, Ridgway GL, Robinson AJ. Comparison of azithromycin and doxycycline in the treatment of non-gonococcal urethritis in men. *J Antimicrob Chemother* 1993;31(Suppl E):185-92.
- Romanowski B, Talbot H, Stadnyk M, Kowalchuk P, Bowie WR. Minocycline compared with doxycycline in the treatment of nongonococcal urethritis and mucopurulent cervicitis. *Ann Intern Med* 1993;119:16-22.
- Stamm WE, Hicks CB, Martin DH, Leone P, Hook EW 3rd, Cooper RH, et al. Azithromycin for empirical treatment of the nongonococcal urethritis syndrome in men. A randomized double-blind study. *JAMA* 1995;274:545-9.
- Roberts MC and Kenny GE. Dissemination of the tetM tetracycline resistance determinant to *Ureaplasma urealyticum*. *Antimicrob Agents Chemother* 1986;29:350-2.
- Leng Z, Riley DE, Berger RE, Krieger JN, Roberts MC. Distribution and mobility of the tetracycline resistance determinant tetQ. *J Antimicrob Chemother* 1997;40:551-9.
- Wasiela M, Krzemiński Z, Hanke W, Kalinka J. Association between genital mycoplasmas and risk of preterm delivery. *Med Wieku Rozwoj* 2003;7(3 Suppl 1):211-6.
- Park JK, Shin JK, Choi W, Lee SA, Lee JH, Paik WY. Mycoplasma infection of cervicovaginal fluid in women with preterm labor. *Korean J Perinatol* 2005;16:128-36.
- Kechagia N, Bersimis S, Chatzipanagioutou S. Incidence and antimicrobial susceptibilities of genital mycoplasmas in outpatient women with clinical vaginitis in Athens, Greece. *J Antimicrob Chemother* 2008;62:122-5.

19. Karabay O, Topcuoglu A, Kocoglu E, Gurel S, Gurel H, Ince NK. Prevalence and antibiotic susceptibility of genital *Mycoplasma hominis* and *Ureaplasma urealyticum* in a university hospital in Turkey. Clin Exp Obstet Gynecol 2006;33:36-8.
20. Bae HG, Heo WB, Lee NY, Lee WK, Koo TB. Detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* in pregnant women using MYCOFAST(R) evolution 2 and PCR. Korean J Clin Microbiol 2003;6:74-80.
21. Kenny GE and Cartwright FD. Susceptibility of *Mycoplasma pneumoniae* to several new quinolones, tetracycline, and erythromycin. Antimicrob Agents Chemother 1991;35:587-9.
22. Kenny GE and Cartwright FD. Effect of pH, inoculum size, and incubation time on the susceptibility of *Ureaplasma urealyticum* to erythromycin in vitro. Clin Infect Dis 1993;17(Suppl 1):S215-8.
23. Renaudin H and Bébéar C. Comparative in vitro activity of azithromycin, clarithromycin, erythromycin and lomefloxacin against *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. Eur J Clin Microbiol Infect Dis 1990;9:838-41.
24. Rylander M and Hallander HO. In vitro comparison of the activity of doxycycline, tetracycline, erythromycin and a new macrolide, CP 62993, against *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. Scand J Infect Dis 1988;53(Suppl): 12-7.

=국문초록=

임산부에서 비뇨생식기 *Mycoplasma*의 빈도 및 항균제 감수성

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배경: 비뇨생식기 *Mycoplasma*는 조기분만 및 조기양막파열과 같은 산과적 합병증을 일으킬 수 있다. 저자들은 진주지역 임산부에서 *U. urealyticum*과 *M. hominis*의 분리율과 항균제 감수성양상을 알아보자 하였다.

방법: 2004년부터 2008년까지 경상대학교병원에 내원한 258명의 임산부를 대상으로 질에서 면봉 채취를 시행하였다. *U. urealyticum*과 *M. hominis* 동정과 항균제감수성검사는 상품화된 *Mycoplasma* IST2 (bioMerieux, Macrcy-l'Etoile, France)를 사용하였으며 CLSI 기준하에 판정하였다.

결과: *U. urealyticum*은 105검체(38.6%), *M. hominis*는 2검체(1.8%)에서 분리되었다. 두 가지가 혼합 분리된 것은 7검체(6.7%)였다. *U. urealyticum*의 azithromycin, erythromycin, clarithromycin 및 doxycycline에 대한 항균제감수성률은 각각 75.2%, 82.9%, 88.6% 및 88.6%였다. Josamycin (99.0%)과 pristinamycin (100%)에 대해서는 거의 모든 균주가 감수성을 보였다.

결론: 진주지역 임산부에서의 생식기 마이코플라즈마 분리율은 44.2%였고, 대부분은 *U. urealyticum*이었다. *U. urealyticum*과 *M. hominis* 모두 퀴놀론제에 높은 내성률을 보였고 josamycin에는 감수성을 보였다. 생식기 마이코플라즈마의 동정 및 감수성검사 없이 경험적인 항균제를 투여할 경우 치료에 실패할 수 있다. [대한임상미생물학회지 2009; 12:159-162]

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