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Antimicrobial Susceptibilities of *Mycoplasma genitalium* Strains Examined by Broth Dilution and Quantitative PCR▽

Ryoichi Hamasuna,[1,2]* Jørgen Skov Jensen,[3] and Yukio Osada[2]

Department of Urology, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan[1]; Division of Urology, Department of Surgery, Faculty of Medicine, University of Miyazaki, Kiyotake, Miyazaki, Japan[2]; and Mycoplasma Laboratory, Statens Serum Institut, DK-2300 Copenhagen, Denmark[3]

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Only limited information regarding the antimicrobial susceptibilities of *Mycoplasma genitalium* is available because of difficulties in isolating *M. genitalium* strains from clinical specimens. Antimicrobial susceptibilities of 15 clinical isolates, 7 ATCC strains, and an early passage of the M30 strain were examined by the broth dilution method. Azithromycin, clarithromycin, sitafloxacin, and moxifloxacin were the most active drugs against *M. genitalium*, and their MIC_{90}s were 0.002, 0.008, 0.125, and 0.125 mg/liter, respectively.

Mycoplasma genitalium is one of the important pathogens in male nongonococcal urethritis. However, the optimal treatment for the infection has not been clearly established. In vitro antimicrobial susceptibility data are also limited because of the difficulties in isolating *M. genitalium* strains from clinical specimens. In recent clinical trials, azithromycin (AZM) and moxifloxacin (MXF) effectively eradicated *M. genitalium* and improved symptoms (2, 11). We isolated *M. genitalium* strains from clinical specimens by cocultivation with Vero cells (4, 5, 9, 10), and 15 strains were adapted to SP4 mycoplasma medium. In the present study, antimicrobial susceptibility testing was performed by a conventional broth dilution method for these clinical isolates and for ATCC reference strains.

Seven ATCC strains (G37, M30, R32, TW10-5G, TW10-6G, TW48-5G, and UTMB-10G) and an early passage of the M30 strain were obtained from the Mycoplasma Laboratory, Statens Serum Institut, Copenhagen, Denmark. The remaining strains were primary isolates obtained from male urethral or urine sediment specimens from the same laboratory. M2282, M2300, M2321, and M2341 were from Danish men. M6257, M6280, M6285, M6286, and M6328 were from Swedish men. M6090 and M6151 were from two consecutive specimens from a French man. M6282, M6283, M6284, and M6287 were from Japanese men. All strains were regrown in modified SP4 mycoplasma medium in the Laboratory of Microbiology, Frontier Science Research Center, University of Miyazaki, Japan. The modified SP4 mycoplasma medium was made in-house according to Tully's recipe (13) but without any antimicrobials. The pH of the medium was adjusted to 7.5. Antimicrobials for susceptibility testing were sitafloxacin (STX), MXF, levofloxacin (LVX), gatifloxacin (GAT), ciprofloxacin (CIP), norfloxacin (NOR), minocycline (MIN), doxycycline (DOX), tetracycline (TET), clarithromycin (CLR), and AZM. STX and LVX were supplied by Daiichi-Sankyo Co. Ltd., Tokyo, Japan. GAT was supplied by Kyorin Pharmaceutical Co. Ltd., Tokyo, Japan. CLR was supplied by Taisho Pharmaceutical Co., Ltd., Tokyo, Japan. AZM was supplied by Pfizer Global Research and Development, Pfizer Inc., Groton, CT. CIP and MXF were supplied by Bayer Health Care A.G., Leverkusen, Germany. NOR, MIN, DOX, and TET were bought from Sigma-Aldrich Inc., St. Louis, MO.

The antimicrobial susceptibility tests (4) were performed by the modified broth dilution method as described by Hannan (6). In brief, antimicrobials were twofold diluted in SP4 mycoplasma medium. The thawed *M. genitalium* strains were diluted with SP4 mycoplasma medium to contain 10^4 color changing units/0.1 ml. A 0.025-ml volume of the dilution of the antimicrobial was mixed with 0.075 ml of the diluted *M. genitalium* strains in a Nunclon Delta 96-well microtiter plate (96 MicroWell plates; Nunc, Roskilde, Denmark). The microtiter plates were sealed and incubated at 37°C for up to 4 weeks. The MICs were determined when an initial color change in the control wells without antimicrobials was observed.

The MIC_{50}s, MIC_{90}s, and ranges for the 23 strains as determined by the broth dilution method are shown in Table 1. Macrolide MICs for M6257 were high (MICs of AZM and CLR by the broth dilution method were 250 and 128 mg/liter, respectively). The strain was isolated from a patient failing AZM treatment (9). We have previously presented the antimicrobial susceptibility data for 27 *M. genitalium* strains, determined by measuring growth inhibition of *M. genitalium* in Vero cell culture by quantitative TaqMan PCR (4, 9). This method was also used to report six macrolide-resistant *M. genitalium* strains (3, 9). The MICs for 18 *M. genitalium* strains excluding 5 ATCC strains, as determined by this method, are also shown in Table 1. Some data are lacking because some of the strains adapted to SP4 mycoplasma medium did not grow well in the Vero cell culture. However, it is important to compare data obtained by the two methods in order to be able to compare with other published MICs (1). MICs of macrolides determined by quantitative TaqMan PCR were slightly higher than those determined by the broth dilution method. However, the MICs for macrolide-resistant *M. genitalium* strains of macrolides are extremely high, and the differences between the two methods have no clinical relevance. MICs of fluoroquinolones and TETs determined by the two methods were similar.

* Corresponding author. Mailing address: Department of Urology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807-8555, Japan. Phone: 81 93 691 7446. Fax: 81 93 603 8724. E-mail: hamaryo@med.uoeh-u.ac.jp.

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TABLE 1. MICs of *M. genitalium* as determined by conventional broth dilution and real-time quantitative PCR methods

Antimicrobial	Broth dilution				Quantitative TaqMan PCR			
	n^a	MIC (mg/liter)			n	MIC (mg/liter)		
		Range	50%	90%		Range	50%	90%
STX	23	0.008–0.125	0.063	0.125	12	0.016–0.25	0.063	0.125
MXF	23	0.016–0.25	0.063	0.125	18	0.031–0.5	0.063	0.125
GAT	23	0.031–0.5	0.25	0.25	12	0.063–0.5	0.5	0.5
LVX	23	0.125–2	1	2	18	0.5–4	1	4
CIP	23	0.063–8	4	8	18	1–16	2	8
NOR	23	1–64	32	64	12	4–≥8	≥8	≥8
MIN	23	0.031–0.25	0.125	0.25		NT[b]	NT	NT
DOX	23	0.063–1	0.125	0.25	17	0.125–1	0.25	0.5
TET	23	0.063–2	0.125	0.5	17	0.125–4	0.5	2
AZM	23	0.0002–250	0.001	0.002	17	0.002–≥8	0.004	0.008
CLR	23	0.0005–128	0.004	0.008	17	0.016–≥16	0.032	0.063

[a] *n*, numbers of tested strains.
[b] NT, not tested.

Among the tested antimicrobials, AZM and CLR were the most potent except against macrolide-resistant strain M6257. Among fluoroquinolones, STX and MFX were the most active drugs. AZM should probably be considered the first line of treatment for *M. genitalium* infections. However, several uncontrolled trials suggest that <85% of *M. genitalium*-positive patients are cured by a single dose of 1 g AZM, and recent findings have shown that a high proportion of those failing this treatment may develop macrolide resistance (3, 9). Consequently, AZM should be given as a 5-day treatment with 500 mg on day 1 followed by 250 mg on days 2 to 5 (2, 8). If patients fail this treatment, MFX is at present the only drug presenting a satisfactory cure rate (11). These recommendations reflect the antimicrobial susceptibility data (4). CLR and STX also appear potent in vitro, but clinical trials with these antimicrobials have not been done. STX was developed by Daiichi-Sankyo Co. Ltd., Japan, and has high in vitro activity against many bacteria including mycoplasmas (12) but is at the moment not registered in Europe and the United States. GAT has activity intermediate between those of MFX and LVX. It was withdrawn from the market September 2008, but in a recent study (R. Hamasuna et al., unpublished data) it provided a good clinical and microbiological response in cases of male nongonococcal urethritis.

In the present study, MICs of the ATCC strains are very close (Table 2); this is not surprising as these strains are genetically extremely homogeneous (7). The range of MICs varies between one- and twofold among all tested antimicrobials. For some strains, our data differ slightly from those presented by Bébéar et al., which were based on the agar dilution method (1). One explanation may be that the MICs determined by the broth dilution method depend on the inoculum (6). In the present study, the inocula of the tested strains were examined by quantitative TaqMan PCR and were 2×10^4 to 8×10^4 genome equivalents per milliliter.

TABLE 2. Differentiation of MIC range of *M. genitalium* between ATCC strains and non-ATCC strains by the broth dilution method

Antimicrobial	MIC range (mg/liter) for:	
	7 ATCC strains	16 non-ATCC strains
STX	0.063–0.125	0.008–0.125
MXF	0.063–0.25	0.016–0.25
GAT	0.125–0.5	0.031–0.5
LVX	1–2	0.125–2
CIP	4–8	0.063–4
NOR	32–64	1–32
MIN	0.031–0.125	0.063–0.25
DOX	0.063–0.25	0.063–1
TET	0.063–0.25	0.063–2
AZM	0.001–0.002	0.0002–250
CLR	0.002–0.008	0.0005–128

REFERENCES

1. **Bébéar, C. M., B. de Barbeyrac, S. Pereyre, H. Renaudin, M. Clerc, and C. Bébéar.** 2008. Activity of moxifloxacin against the urogenital mycoplasmas *Ureaplasma* spp., *Mycoplasma hominis* and *Mycoplasma genitalium* and *Chlamydia trachomatis*. Clin. Microbiol. Infect. **14:**801–805.
2. **Björnelius, E., C. Anagrius, G. Bojs, H. Carlberg, G. Johannisson, E. Johansson, H. Moi, J. S. Jensen, and P. Lidbrink.** 2008. Antibiotic treatment of symptomatic *Mycoplasma genitalium* infection in Scandinavia: a controlled clinical trial. Sex. Transm. Infect. **84:**72–76.
3. **Bradshaw, C. S., J. S. Jensen, S. N. Tabrizi, T. R. Read, S. M. Garland, C. A. Hopkins, L. M. Moss, and C. K. Fairley.** 2006. Azithromycin failure in *Mycoplasma genitalium* urethritis. Emerg. Infect. Dis. **12:**1149–1152.
4. **Hamasuna, R., Y. Osada, and J. S. Jensen.** 2005. Antibiotic susceptibility testing of *Mycoplasma genitalium* by TaqMan 5′ nuclease real-time PCR. Antimicrob. Agents Chemother. **49:**4993–4998.
5. **Hamasuna, R., Y. Osada, and J. S. Jensen.** 2007. Isolation of *Mycoplasma genitalium* from first-void urine specimens by coculture with Vero cells. J. Clin. Microbiol. **45:**847–850.
6. **Hannan, P. C.** 2000. Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. International Research Programme on Comparative Mycoplasmology. Vet. Res. **31:**373–395.
7. **Hjorth, S. V., E. Björnelius, P. Lidbrink, L. Falk, B. Dohn, L. Berthelsen, L. Ma, D. H. Martin, and J. S. Jensen.** 2006. Sequence-based typing of *Mycoplasma genitalium* reveals sexual transmission. J. Clin. Microbiol. **44:**2078–2083.
8. **Jensen, J. S.** 2006. *Mycoplasma genitalium* infections. Dan. Med. Bull. **53:**1–27.
9. **Jensen, J. S., C. S. Bradshaw, S. N. Tabrizi, C. K. Fairley, and R. Hamasuna.** 2008. Azithromycin treatment failure in *Mycoplasma genitalium*-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin. Infect. Dis. **47:**1546–1553.
10. **Jensen, J. S., H. T. Hansen, and K. Lind.** 1996. Isolation of *Mycoplasma genitalium* strains from the male urethra. J. Clin. Microbiol. **34:**286–291.
11. **Jernberg, E., A. Moghaddam, and H. Moi.** 2008. Azithromycin and moxifloxacin for microbiological cure of *Mycoplasma genitalium* infection: an open study. Int. J. STD AIDS **19:**676–679.
12. **Morozumi, M., K. Hasegawa, R. Kobayashi, N. Inoue, S. Iwata, H. Kuroki, N. Kawamura, E. Nakayama, T. Tajima, K. Shimizu, and K. Ubukata.** 2005. Emergence of macrolide-resistant *Mycoplasma pneumoniae* with a 23S rRNA gene mutation. Antimicrob. Agents Chemother. **49:**2302–2306.
13. **Tully, J. G., R. F. Whitcomb, H. F. Clark, and D. L. Williamson.** 1977. Pathogenic mycoplasmas: cultivation and vertebrate pathogenicity of a new spiroplasma. Science **195:**892–894.