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Antibiotic Susceptibility Testing of *Mycoplasma genitalium* by TaqMan 5′ Nuclease Real-Time PCR

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Mycoplasma genitalium is an important pathogen in male nongonococcal urethritis (NGU). Isolation of *M. genitalium* from clinical specimens by axenic culture is very difficult and time-consuming, and very few strains are available for antibiotic susceptibility testing. Primary isolation of *M. genitalium* by coculture with Vero cells improves the isolation rate significantly. However, some strains cannot be adapted to axenic culture. In this study, we determined the antibiotic susceptibility of *M. genitalium* strains grown in Vero cell culture with dilutions of antibiotics. Growth of *M. genitalium* was monitored by a quantitative PCR assay detecting a single-copy region of the *mgpB* adhesin gene. Growth inhibition in the presence of antibiotics was expressed as a percentage of the DNA load of controls grown in the absence of antibiotics. Eighteen strains were examined, including 6 new strains isolated from urethral swab specimens and 4 new strains isolated from urine specimens collected from Japanese men. Eight strains adapted to axenic culture were also tested by the conventional broth dilution method. The two methods had an acceptable correlation. Azithromycin was the most active drug against *M. genitalium*. Among the fluoroquinolones, moxifloxacin had the highest activity, with MICs ranging from 0.03 to 0.5 mg/liter, whereas ciprofloxacin and levofloxacin were considerably less active, with MICs ranging from 0.5 to 16 mg/liter and 0.25 to 4 mg/liter, respectively. MICs for tetracycline ranged from 0.125 to 4 mg/liter. This new method could increase the number of *M. genitalium* strains available for antibiotic susceptibility testing and significantly shorten the time from sampling to MIC results.

Mycoplasma genitalium was first isolated after prolonged incubation from 2 of 13 men with urethritis (36). Despite repeated attempts, new isolates of *M. genitalium* were not obtained from urethral specimens by conventional culture techniques (31, 33), but isolation of *M. genitalium* in cultures of throat and synovial fluid specimens has been reported, although these isolates were mixed with *Mycoplasma pneumoniae* (1, 35). Aside from these reports, a technique for isolation of *M. genitalium* from urethral specimens using Vero cells to initially cultivate *M. genitalium* and subsequent adaptation to conventional broth has been developed (19) and has been used with some success in other laboratories (34). Using this new technique, however, it took more than 50 days to adapt the *M. genitalium* strains to cell-free medium, i.e., axenic culture. Even today, other urogenital isolates are extremely rare and have been difficult to obtain (23).

M. genitalium is gaining increasing attention as one of the important pathogens in men with nongonococcal urethritis (NGU). While *Chlamydia trachomatis* can be found in 30 to 40% of NGU patients, *M. genitalium* has been detected in 15 to 30% (3, 11, 15, 20). Several studies have shown a significant association between the presence of *M. genitalium* DNA and symptoms and/or signs of urethritis (reviewed in reference 17).

Patients with NGU are usually treated empirically with the same antibiotics regardless of the detection of *C. trachomatis*. Depending on local recommendations, tetracyclines, macrolides, or quinolones are used. However, several open studies have shown that *M. genitalium* may persist after treatment with tetracyclines or quinolones (9, 21) and that failure of eradication was associated with recurrence (25). Although resistant strains of *C. trachomatis* have been characterized in vitro (26), very few reports linking such resistance to clinical treatment failure have been reported (32). However, we do not have much information about the antimicrobial susceptibility of *M. genitalium*. Pure cultures of *M. genitalium* strains grown in axenic culture are needed for the conventional method for antibiotic susceptibility testing. Consequently, the number of strains available for antibiotic susceptibility testing has been very limited and the trends of antibiotic susceptibilities among current strains are not known. Using the Vero cell culture method, we isolated *M. genitalium* from male urethral swabs or urine within 3 to 5 weeks after initiation of the cultivation compared to at least 6 months for adaptation to axenic culture. Recently, six strains from urethral swabs and four strains from urine have been propagated in the Vero cell culture and a new method for antibiotic susceptibility testing of *M. genitalium* growing only in Vero cell culture was developed and validated.

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MATERIALS AND METHODS

Culture of Vero cells. Vero cells were maintained in Eagle's minimal essential medium (MEM) supplemented with 2% Ultroser G serum substitute (Ciphergen, Cergy-Saint-Christophe, France) and 3% sodium bicarbonate, without any antibiotics. Vero cells were propagated in 15-ml or 50-ml plastic culture flasks (NUNC, Roskilde, Denmark) at 37°C.

M. genitalium strains and culture. *M. genitalium* strain G37[T], an early passage of the M30 strain (18), and *M. genitalium* strains isolated in our laboratory,

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designated M2282, M2300, M2321, M2341, M6090, and M6151, were grown in modified Friis's mycoplasma broth medium containing horse serum (19).

M. genitalium strains growing in broth medium were divided in small portions and stored at −80°C for the conventional antibiotic susceptibility test. In addition, these strains were regrown in Vero cell culture in 15-ml flasks before being divided into small portions and stored at −80°C for the new antibiotic susceptibility test.

Ten new *M. genitalium* strains were grown in the Vero cell culture system to high titers. Five were urethral swab specimens from Swedish male attendees of sexually transmitted disease clinics (R5G, R6G, R65G, R66G, and R67G), one (M6312) was from a French male patient with recurrent urethritis from whom strains M6090 and M6151 (18) were previously isolated, and four were from urine specimens from Japanese male patients with NGU (R53G, R69G, R70G, and R74G). When the strains reached titers of >500,000 genome equivalents (geq) as determined by TaqMan 5′ nuclease real-time PCR, they were frozen at −80°C as described above.

Growth curve of *M. genitalium* in cell culture tubes. In order to establish the growth pattern of *M. genitalium* strains in Vero cell culture, the *M. genitalium* DNA loads were determined in the supernatant and in the cell-containing medium. Vero cells were adjusted to 2.5×10^4/ml in Eagle's MEM supplemented with 2% Ultroser G. *M. genitalium* strains were cultured with 2 ml of the Vero cell suspensions in Nunclon Delta flat-bottom, screw-cap culture tubes (Nunc, Roskilde, Denmark) at 37°C. At 1, 2, and 3 weeks of incubation, 100 μl of the supernatant was transferred from a culture tube to 300 μl of 20% Chelex 100 slurry (Bio-Rad Laboratories, Hercules, CA). In addition, Vero cells were scraped from the bottom of the same tubes and 100 μl of medium containing Vero cells was transferred to 300 μl of 20% Chelex 100 slurry. Each week, the supernatant and cell-containing medium was harvested from five tubes and the *M. genitalium* DNA load in each sample was determined by a TaqMan 5′ nuclease real-time PCR as previously described (18). In brief, a 78-bp fragment of the *mgpB* adhesin gene was amplified and detected with a 6-carboxyfluorescein-labeled minor groove binder (MGB) probe.

Antibiotics for susceptibility testing. The antibiotics used for susceptibility testing were doxycycline hydrochloride (DOX), tetracycline hydrochloride (TET), erythromycin (ERY), clarithromycin (CLR), azithromycin (AZM), ciprofloxacin hydrochloride (CIP), levofloxacin (LVX), and moxifloxacin hydrochloride (MXF). DOX and ERY were bought from Sigma-Aldrich Denmark, Vallensbaek Strand, Denmark, and TET was bought from Fluka, Buchs, Switzerland. CLR was provided from Abbott Laboratories, North Chicago, Ill. AZM was supplied by Groton Laboratories, Pfizer Inc., Groton, CT. CIP and MXF were supplied by Bayer Health Care, Lyngby, Denmark, and LVX was supplied by Daiichi Pharmacology Company, Tokyo, Japan.

Cell-culture-based antibiotic susceptibility test. Vero cells grown in 50-ml flasks were trypsinized and resuspended in Eagle's MEM with 2% Ultroser G and adjusted to 1.7×10^4/ml. A 2.2-ml portion of the Vero cell suspension was dispensed in the wells of Multiwell 24-well tissue culture plates (Becton Dickinson, France). Each antibiotic was diluted with Eagle's MEM with 2% Ultroser G in twofold steps, and 0.2 ml of the dilution was added to each well. Triplicate control wells received 0.2 ml of the same medium without antibiotics. After thawing, *M. genitalium* strains cultured on Vero cells were diluted in cell culture medium and adjusted to contain from 1,000 to 3,000 geq of *M. genitalium* per 0.1 ml and 0.1 ml of the dilution was used as inoculum, resulting in a total volume of 2.5 ml medium in each well. The plates were covered with sterile sealing tape (Nunc, Roskilde, Denmark) to prevent evaporation of the medium and incubated in an atmosphere with 5% CO_2 at 37°C. At 1, 2, and 3 weeks after the inoculation, 100 μl of the supernatant was harvested from each well and added to 300 μl of 20% Chelex 100 slurry, and the *M. genitalium* DNA load was determined by the TaqMan PCR assay. Inhibition rates of the antibiotics were calculated by the formula inhibition rate (%) = [(average of DNA loads in control wells − DNA load in test well)/(average of DNA loads in control wells)] × 100.

The MIC was defined as the lowest concentration of antibiotic causing 99% inhibition, and the minimum bactericidal concentration (MBC) was defined as the lowest concentration causing 99.9% inhibition.

The initial test ranges of drug concentrations were determined by previous antibiotic susceptibility studies of *M. genitalium* (2, 9, 20, 26). If the MIC or MBC was not within the initial test range, the strain was reexamined after expansion of the range.

Conventional antibiotic susceptibility tests. The conventional antibiotic susceptibility test was performed by the broth dilution method, essentially as described by Hannan (14). Briefly, antibiotics were twofold diluted with SP-4 mycoplasma medium and *M. genitalium* strains which could grow in axenic culture were diluted in SP-4 medium to contain 10^4 color-changing units/0.1 ml.
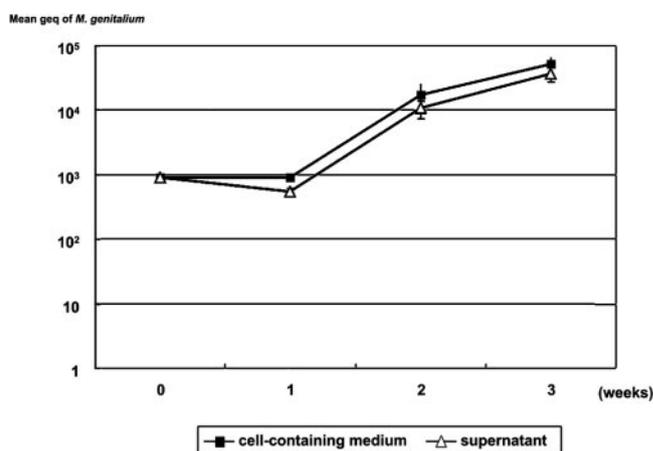

FIG. 1. Growth of *M. genitalium* strain R6G in Vero cell culture. *M. genitalium* strain R6G was grown with Vero cells in air with 5% CO_2. The *M. genitalium* DNA load was determined at 1, 2, and 3 weeks of incubation from the supernatant and from a mixture of supernatant and Vero cells scraped from the bottom of the same tubes.

A 0.1-ml volume of the dilution of the antibiotic was mixed with 0.1 ml of the diluted *M. genitalium* strains in a Nunclone Delta 96-well microtiter plate (96 MicroWell plates; Nunc, Roskilde, Denmark). The microtiter plates were sealed and incubated at 37°C. The plates were inspected at regular intervals for change of color. The initial MIC was read when the change of color in the broth was first observed in the control wells (usually about 10 to 14 days). The final MIC was read at the time when no further color change in the wells containing antibiotics was observed after approximately 4 weeks.

Statistical methods. Differences between MIC determinations by the cell culture and the conventional method were compared using the Wilcoxon's signed-rank test.

RESULTS

Growth curves of *M. genitalium* strains in cell culture tubes. The growth curve of *M. genitalium* strain R6G in a Vero cell culture grown in flat-bottom culture tubes is shown in Fig. 1. R6G did not grow after 1 week of incubation, but the DNA load increased after 2 weeks. There was no difference in the *M. genitalium* DNA load between the supernatant and in the cell-containing medium of the Vero cell culture. Therefore, we considered monitoring of the DNA load in the supernatant indicative of the total growth in the Vero cell culture.

Growth of *M. genitalium* strains in tissue culture plates without antibiotics. The change of *M. genitalium* DNA loads was monitored by harvesting the supernatant from the control wells of a 24-well tissue culture plate. The DNA loads of R6G slightly decreased at 1 week after the inoculation and started to increase after 2 weeks of incubation. R5G, R53G, R65G, R69G, R70G, R74G, M6312, M30, M2321, M2300, M6090, and M6151 had the same growth pattern as R6G. G37[T], M2341, and M2282 had more rapid growth patterns. At 1 week after incubation, DNA loads of these strains slightly increased and continued to increase at 3 weeks. R66G and R67G had a slow growth pattern. The DNA loads of these strains did not increase at 2 weeks after inoculation. At 3 weeks, DNA loads increased dramatically, but at 4 weeks, all Vero cells were detached from the bottom of the wells.

Inhibition of growth of *M. genitalium* on Vero cell culture with antibiotics. The inhibition rate of LVX is shown in Fig. 2

Mean geq of *M. genitalium*

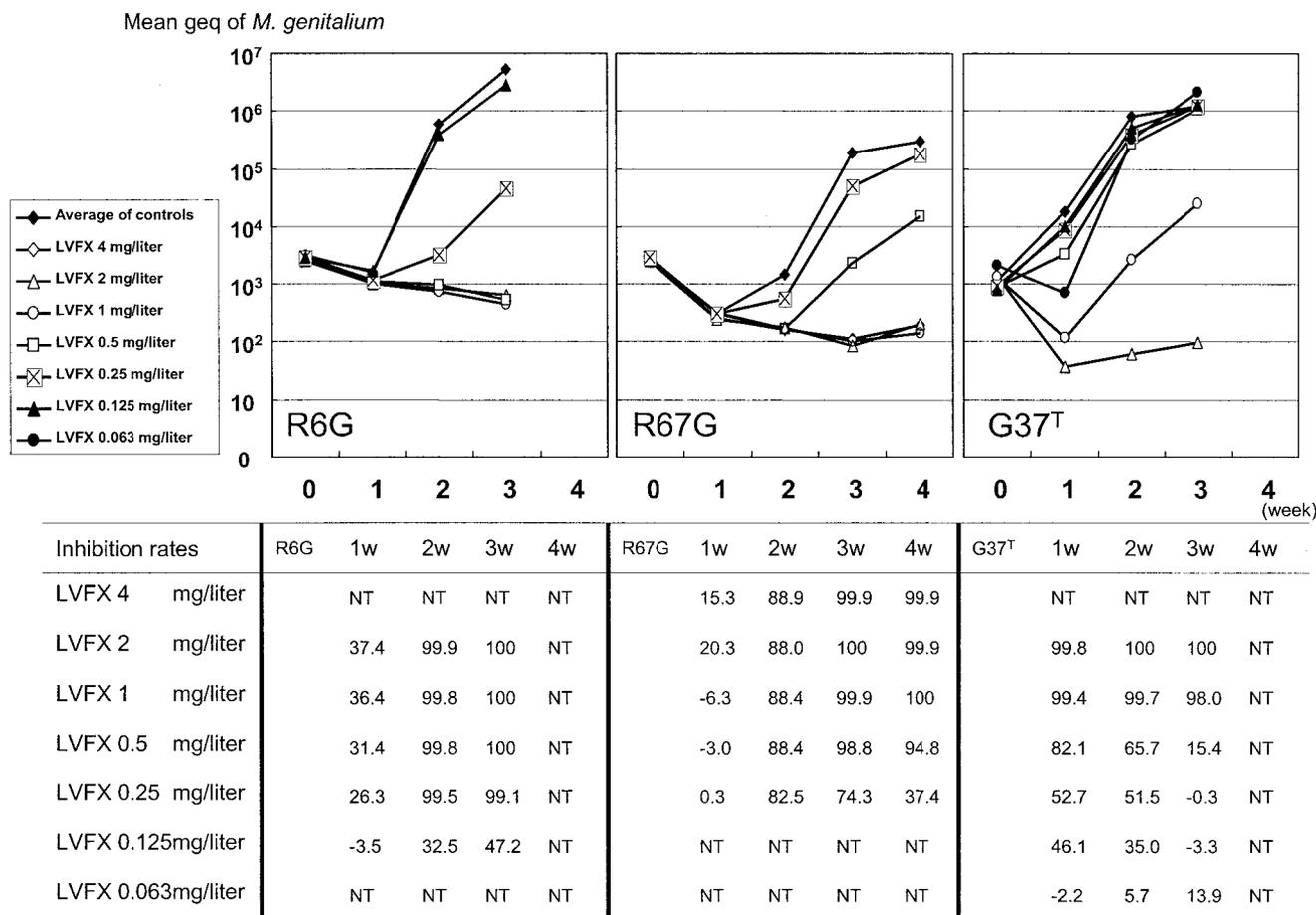| Inhibition rates	R6G	1w	2w	3w	4w	R67G	1w	2w	3w	4w	G37ᵀ	1w	2w	3w	4w
LVFX 4 mg/liter		NT	NT	NT	NT		15.3	88.9	99.9	99.9		NT	NT	NT	NT
LVFX 2 mg/liter		37.4	99.9	100	NT		20.3	88.0	100	99.9		99.8	100	100	NT
LVFX 1 mg/liter		36.4	99.8	100	NT		-6.3	88.4	99.9	100		99.4	99.7	98.0	NT
LVFX 0.5 mg/liter		31.4	99.8	100	NT		-3.0	88.4	98.8	94.8		82.1	65.7	15.4	NT
LVFX 0.25 mg/liter		26.3	99.5	99.1	NT		0.3	82.5	74.3	37.4		52.7	51.5	-0.3	NT
LVFX 0.125 mg/liter		-3.5	32.5	47.2	NT		NT	NT	NT	NT		46.1	35.0	-3.3	NT
LVFX 0.063 mg/liter		NT	NT	NT	NT		NT	NT	NT	NT		-2.2	5.7	13.9	NT

FIG. 2. Inhibition of growth of *M. genitalium* by levofloxacin. *M. genitalium* strains grown in Vero cell suspensions with various concentrations of levofloxacin. Three strains with different growth rates in Vero cell culture are shown. At 1, 2, 3, and 4 weeks of incubation, the *M. genitalium* DNA load in the supernatant was determined. The inhibition rate was calculated by the formula inhibition rate (%) = [(average of DNA loads in control wells − DNA loads in test well)/(average of DNA loads in control wells)] × 100. NT, not tested.

for three strains which had different growth patterns in the Vero cell culture. For R6G, MIC and MBC were determined to be 0.25 mg/liter and 2 mg/liter, respectively, at 2 weeks after the inoculation. At 3 weeks, MIC and MBC were 0.25 mg/liter and 0.5 mg/liter, respectively. G37ᵀ had a rapid growth pattern. However, MBC could not be determined at 1 week after inoculation of G37ᵀ. MIC and MBC were 1 mg/liter and 2 mg/liter at 2 weeks, respectively. MIC and MBC were 2 mg/liter at 3 weeks. R67G grew slowly, and MIC and MBC could not be determined at 1 and 2 weeks. The MIC and MBC were 1 mg/liter at both 3 and 4 weeks after the incubation. Based on these experiences with strains with different growth patterns, it was decided to determine the MIC and MBC at 3 weeks of incubation. MICs for all strains determined at this time point are shown in Table 1.

Comparison between results obtained by the cell culture method and by the conventional method for MIC determination. For strains which could be adapted to axenic culture, the MICs determined by the cell culture method and by the conventional method were compared (Table 2). The MICs determined by the cell culture method were similar to the initial MICs by the conventional method for DOX (median, 0.375 versus 0.25 mg/liter; $P = 0.6$) and TET (median, 0.25 versus 0.25 mg/liter; $P = 0.19$) but significantly higher for ERY (median, 0.125 versus 0.004 mg/liter; $P = 0.008$) and LVX (median, 1.5 versus 0.5 mg/liter; $P = 0.03$).

DISCUSSION

The antibiotic susceptibility pattern of *M. genitalium* has been examined in only a few studies (2, 8, 13, 28), and a limited number of strains have been included. Bebear and Renaudin (2, 28) applied an agar dilution method, whereas Hannan (13) and Duffy (8) used broth dilution. Only in the study by Hannan (13) were the more recently isolated Danish strains M2288, M2300, M2321, and M2341 included. This is in contrast to the other studies that tested the G37 strain and other strains that have been shown to be genetically very closely related to it, such as the respiratory tract isolates (18, 22, 24).

Compared with previous studies, our results differ somewhat in their conclusions. Studies examining only the G37 strain and the respiratory tract isolates (2, 8, 28) find *M. genitalium* susceptible to the tetracyclines, whereas we and Hannan (13) find a number of strains that would be considered resistant or at

TABLE 1. MICs of *M. genitalium* strains as determined by TaqMan 5′ nuclease real-time PCR

Strain or MIC parameter	MIC (mg/liter)							
	Doxycycline	Tetracycline	Erythromycin	Clarithromycin	Azithromycin	Ciprofloxacin	Levofloxacin	Moxifloxacin
G37	0.5	0.5	0.125	0.031	0.008	8	2	0.125
M30 (early)	0.5	0.25	0.125	0.031	0.008	8	2	0.125
M2282	0.25	0.25	0.063	0.016	0.004	4	1	0.125
M2300	0.25	0.25	0.125	0.031	0.008	16	4	0.125
M2321	0.5	0.5	0.063	0.016	0.004	8	4	0.25
M2341	1	4	0.25	0.031	0.008	2	1	0.25
M6090	0.125	0.125	0.063	0.016	0.004	2	1	0.063
M6151	0.25	0.25	0.125	0.031	0.008	4	1	0.125
M6312	0.25	0.25	0.063	0.031	0.004	8	2	0.125
R5G	0.125	0.25	0.063	0.016	0.004	1	0.5	0.063
R6G	0.25	0.5	0.063	0.031	0.008	0.5	0.25	0.125
R65G	0.5	2	0.125	0.016	0.004	1	0.5	0.125
R66G	0.25	0.5	0.125	0.031	0.004	2	1	0.125
R67G	0.25	0.25	0.031	0.016	0.002	2	1	0.031
R53G	0.125	0.5	0.031	0.008	0.002	4	2	0.5
R69G	0.25	0.25	0.125	0.063	0.004	4	1	0.125
R70G	0.25	0.5	0.125	0.063	0.008	1	1	0.125
R74G	0.5	2	0.063	0.031	0.004	2	1	0.125
MIC$_{50}$	0.25	0.25	0.063	0.031	0.004	2	1	0.125
MIC$_{90}$	0.5	2	0.125	0.063	0.008	8	4	0.25

least with decreased susceptibility to the tetracyclines. The attainable concentration of DOX after standard dosage has been determined in the human prostate and was found to be <2 mg/liter (27). Consequently, strains with MICs of >2 mg/liter would be considered having decreased susceptibility. Obviously, this threshold has not been evaluated on a patient basis in *M. genitalium*-infected individuals. However, since the cure rate after DOX therapy is only about 35%, it seems reasonable to expect the breakpoint to be significantly lower than 2 mg/liter. Among the macrolides, AZM is the most potent drug, having a two- to threefold-lower MIC than CLR and a four- to eightfold-lower MIC than ERY. In the present study, we did not find macrolide-resistant strains even after the expanded panel of strains was examined. Reports on sporadic clinical treatment failure after AZM therapy, however, have begun to accrue, and the cell culture method for MIC determination

may prove useful in documenting the correlation between clinical treatment failure and in vitro susceptibility. In accordance with earlier studies, we found the MICs for the quinolones to be relatively high. This was particularly the case for the older fluoroquinolones such as CIP and to a lesser extent LVX. The MICs for LVX were two- to threefold lower than those of CIP; this has also been reported for *Mycoplasma pneumoniae* and *Ureaplasma* spp. (37). However the MIC of M2300 and M2321 for LVX was 4 mg/liter, and these two strains would be considered fluoroquinolone resistant. However, the MICs for MXF of these two strains were 0.125 mg/liter and 0.25 mg/liter, respectively, and thus this newer fluoroquinolone with an extended gram-positive spectrum would be expected to be clinically effective. The potent antimicrobial activity of MXF has also been reported for *M. pneumoniae*, (12), and recently, clinical reports showing that MXF was effective in recurrent or

TABLE 2. Comparison between MICs of *M. genitalium* strains as determined by the cell culture method and conventional broth dilution test

Strain	MIC (mg/liter)											
	Doxycycline			Tetracycline			Erythromycin			Levofloxacin		
	Cell culture[a]	Conventional[b]		Cell culture[a]	Conventional[b]		Cell culture[a]	Conventional[b]		Cell culture[a]	Conventional[b]	
		Initial	Final		Initial	Final		Initial	Final		Initial	Final
G37	0.5	0.125	4	0.5	0.5	2	0.125	0.004	0.031	2	2	4
M30	0.5	0.25	4	0.25	0.125	1	0.125	0.004	0.004	2	0.5	2
M2282	0.25	0.031	0.25	0.25	0.031	0.031	0.063	0.002	0.002	1	0.5	0.5
M2300	0.25	0.25	1	0.25	0.031	0.25	0.125	0.004	0.008	4	0.5	1
M2321	0.5	0.25	4	0.5	0.5	2	0.063	0.002	0.02	4	1	2
M2341	1	2	4	4	2	4	0.25	0.063	0.063	1	0.5	2
M6090	0.125	0.25	2	0.125	0.25	1	0.063	0.008	0.02	1	0.5	1
M6151	0.25	0.25	2	0.25	0.25	2	0.125	0.004	0.02	1	1	1

[a] TaqMan 5′ nuclease real-time PCR. The MIC value was defined as the lowest concentration of antibiotics causing 99% inhibition at 3 weeks after the incubation of *M. genitalium* with Vero cells.
[b] The MIC value was defined as the lowest concentration of antibiotics showing no change of color at the initial and final readings. The initial MIC was read when a change of color in the control wells was first observed, and the final MIC was read when no further color change could be observed.

persistent NGU due to *M. genitalium* occurring after treatment with tetracyclines or macrolides have been presented (C. S. Bradshaw, S. M. Tabrizi, T. R. Read, C. A. Hopkins, M. B. Moss, S. M. Garland, and C. K. Fairley, Program Abstr. Int. Soc. Sex. Transm. Dis. Res., abstr. MO-401, 2005). This is in good agreement with our clinical observations, which have shown a good microbiological and clinical cure rate after MXF treatment in persistent or recurring urethritis.

The new method for antibiotic susceptibility testing of *M. genitalium* presented here has two major benefits. First of all, this method can expand the number of *M. genitalium* strains available for antibiotic susceptibility testing, since *M. genitalium* strains which cannot be adapted to axenic culture can be tested. In our experience, about 60% of *M. genitalium* strains could grow in Vero cell culture in about 1 month if the DNA load of *M. genitalium* in the specimen was more than 1,000 copies per 0.1 ml of SP-4 medium with urethral swab or urine. The second benefit of this method is the objective validation of the MIC using numeric values instead of a color change in broth or colony formation after a certain period of time. When performing antibiotic susceptibility testing by the broth dilution method, the controversial point is when to read the color change in the broth. Hannan reported both the initial and the final MICs (13, 14), but there is no universal agreement about the clinical relevance of the two different values. For antibiotics with mycoplasmacidal effect such as the fluoroquinolones, the two values tend to be more similar than for mycoplasmastatic drugs such as tetracyclines and macrolides. It could be argued that the final MIC would be clinically most relevant, since a temporary suppression of growth would lead to recurrent symptoms after some time. On the other hand, patients with a normal immune system might be able to efficiently combat the infection when the mycoplasmas are in the suppressed state.

MICs for ERY were significantly higher when determined by the cell culture method as compared to the conventional broth dilution test. This could reflect the poorer intracellular concentration of this drug when compared to the newer macrolides, which may be clinically important since *M. genitalium* may replicate intracellularly (6). This phenomenon has previously been observed for the intracellular pathogen *Legionella pneumophila* (10). We cannot exclude, however, that the decreasing pH in the cell culture medium caused by the growth of the Vero cells may have decreased the activity of ERY.

Unfortunately, no randomized controlled trials comparing different antibiotics have been published, but clinical experience indicates that the cure rate after treatment with tetracyclines or fluoroquinolones is lower than that after macrolides. Falk et al. reported an open comparison of the clinical efficacies of tetracyclines and AZM (9) and found that *M. genitalium* could not be eradicated by tetracyclines in 71% of male patients with urethritis and 63% of female patients with cervicitis, whereas AZM appeared to eradicate *M. genitalium* from all patients investigated. It should be noted, however, that many of the patients treated with AZM had initially received treatment with tetracyclines, although in a subset of the patients, AZM was given as first-line treatment to comparable patients documenting its higher efficacy. Maeda et al. (25) reported that of 12 *M. genitalium*-positive men treated with LVX who were followed up, 8 were positive after treatment: 1 had persistent

NGU, and of the remaining 7, 2 were lost for follow-up but 5 returned with recurrent NGU. Deguchi et al. studied the *M. genitalium gyrA* and *parC* genes from six of these men with persistent positive *M. genitalium* PCR (7) but found the expected mutations in only one of the patients in the pretreatment specimen and in specimens from the same patient and a second patient collected at follow-up. However, since culture was not attempted, no data on the antibiotic susceptibility patterns could be evaluated.

Studies aiming at determining the clinical MIC breakpoints of antibiotics have previously been impossible to conduct for *M. genitalium*; however, by using the techniques presented here, they should be achievable, although very demanding.

Antibiotic susceptibility testing by the use of real-time PCR has been described recently for intracellular bacteria, such as three *Rickettsia* species (*Rickettsia conorii*, *Rickettsia typhi*, and *Rickettsia felis*) (30), *Coxiella burnetii* (5), and *Tropheryma whipplei* (4). The MICs determined using real-time PCR were almost similar to those obtained by conventional methods. Rolain et al. (29) showed that it was possible to speed up the susceptibility tests also for commonly isolated bacteria like *Staphylococcus aureus*, *Haemophilus influenzae*, *Escherichia coli*, and others by real-time PCR. The results were in accordance with those obtained following the CLSI (formerly NCCLS) guidelines. However, the major drawback of this method is the cost. Consequently, at present, we consider this method relevant only for bacteria which are impossible or slow to grow in conventional culture medium, such as *M. genitalium* and other intracellular bacteria.

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