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In Vitro Susceptibilities of *Mycoplasma genitalium* to Antibiotics

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The susceptibilities of seven clinical isolates of *Mycoplasma genitalium* and three strains of *Mycoplasma pneumoniae* to a variety of antibiotics were examined by an agar dilution method. Macrolides, pristinamycin, and tetracyclines were very active against both species. Sparfloxacin was the most active quinolone tested. None of the 21 antibiotics tested had differential activity toward the two organisms.

Mycoplasma genitalium is a fastidious mollicute that was first isolated in 1980 from urethral specimens of two homosexual men with nongonococcal urethritis (19). Although experimental urogenital challenge of both male and female primates induced lower genital tract infections (16, 20), attempts to cultivate additional strains from healthy or infected human urogenital tracts continue to be unsuccessful (12, 15, 18). Application of serologic techniques to assess infection is complicated by significant sharing of cross-reacting antigens between this organism and *Mycoplasma pneumoniae* (1, 5, 11, 17). Development of molecular probes for *M. genitalium*, including DNA hybridization (7, 8, 14) and the polymerase chain reaction (9, 12), provided some evidence for the presence of the organism in the male urethra. However, evidence to support involvement of the organism in persistent or recurrent nongonococcal urethritis or in diseases of the female urogenital tract is still lacking. More recently, *M. genitalium* was isolated in culture from human throat specimens in association with *M. pneumoniae* infections (1), but again, it is unclear whether *M. genitalium* plays any role in respiratory disease.

Since *M. genitalium* and *M. pneumoniae* share many properties, including the presence of an organized tip attachment structure, similar biochemical characteristics, adherence capabilities, and the aforementioned serologic cross-reactivities, differentiation in the clinical laboratory is complicated.

M. genitalium reference strain G37 was previously reported to be susceptible to tetracycline, erythromycin, and rosaramicin (11, 16, 17); but the antibiotic susceptibilities of other isolates of the organism, particularly the more numerous strains from the human oral cavity, have not been reported. In attempts to detect any differences in antibiotic susceptibilities between *M. genitalium* and *M. pneumoniae* strains that could be used to prepare a selective culture medium, we examined the in vitro activities of several groups of antimicrobial agents against seven *M. genitalium* and three *M. pneumoniae* isolates (13).

M. genitalium strains included two urethral isolates, G37 (ATCC 33530) and M30 (19); four throat isolates, R32G, TW48-5G, TW10-5G, and TW10-6G (1); and one synovial fluid isolate, UTMB. *M. pneumoniae* strains included one reference strain, FH, and two respiratory isolates. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as controls.

The 21 antimicrobial agents used were obtained from their respective manufacturers. MICs were determined by an agar dilution method, as described previously (2, 3). Although the complex SP-4 culture medium (19) was required for initial isolation of *M. genitalium* strains from patients, for antimicrobial susceptibility testing the strains used here were adapted to a modified Edward (4) medium (pH 7.6) containing 1.7% heart infusion broth (Difco Laboratories, Detroit, Mich.), 2.5% fresh yeast extract, 20% horse serum (GIBCO BRL, Cergy Pontoise, France), and 1% purified agar (Oxoid, Basingstoke, Hampshire, United Kingdom). Twofold serial dilutions (starting at 128 μg/ml and extending to 0.01 μg/ml) of each antibiotic were prepared and added in appropriate amounts to the agar medium. The mycoplasma inocula, which were prepared in SP-4 medium, contained 10^4 to 10^5 color-changing units per spot and were plated onto antimicrobial agent-containing agar plates with a multipoint replicator. A color-changing unit is defined as the minimum number of organisms capable of producing a color change in the inoculum. Plates without antibiotics were included as controls. After incubation of the plates in 5% CO_2 at 37°C, the MIC of each antibiotic that was recorded was the lowest concentration of drug that inhibited mycoplasma colony growth on agar while, at the same time, visible growth was observed on control plates devoid of antibiotic. MICs for *M. genitalium* were generally recorded after a 7-day incubation period; for *M. pneumoniae*, MICs were recorded after 5 days of incubation.

The strains were tested one additional time on a different day to ensure the reproducibility of the results.

The effects of mycoplasma culture conditions on the antimicrobial activities of the antibiotics were studied by comparing the MICs of different antibiotics for the reference *S. aureus* and *E. coli* strains grown either on Mueller-Hinton agar (pH 7.4; Difco) or on modified Edward agar medium. Table 1 shows that culture conditions for mycoplasma testing did not significantly affect the antimicrobial activities of the antibiotics tested.

The MICs of various antimicrobial agents for *M. genitalium* and *M. pneumoniae* are given in Table 2. The results observed with the seven *M. genitalium* and the three *M. pneumoniae* strains were very consistent, indicating that all of the tetracyclines, the macrolides, and the one streptogramin (pristinamycin) were highly inhibitory to all strains. Clindamycin was more active than lincomycin. Among the quinolones tested, sparfloxacin was the most active compound, as was reported previously for the activity of sparfloxacin against other *Mycoplasma* species (10, 21). Both *Mycoplasma* species were resistant to rifampin, as has been noted for a number of other different mollicutes (6), and each

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TABLE 1. MICs of various antibiotics for *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 in different media

Antimicrobial agent	MIC (μg/ml)							
	S. aureus				*E. coli*			
	Mueller-Hinton agar (pH 7.4)		Modified Edward agar (pH 7.6)		Mueller-Hinton agar (pH 7.4)		Modified Edward agar (pH 7.6)	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Erythromycin	0.1	0.2	≤0.01	≤0.01	ND[a]	ND	ND	ND
Spiramycin	2	4	≤0.01	1	ND	ND	ND	ND
Josamycin	0.5	0.5	≤0.01	0.05	ND	ND	ND	ND
Miocamycin	1	1	1	1	ND	ND	ND	ND
Roxithromycin	0.2	0.5	≤0.01	0.02	ND	ND	ND	ND
Azithromycin	0.2	1	≤0.01	0.05	ND	ND	ND	ND
Clarithromycin	0.02	0.05	≤0.01	0.02	ND	ND	ND	ND
Pristinamycin	0.02	0.1	≤0.01	0.02	ND	ND	ND	ND
Lincomycin	0.5	2	≤0.01	0.1	ND	ND	ND	ND
Clindamycin	0.05	0.05	≤0.02	≤0.02	ND	ND	ND	ND
Tetracycline	≤0.01	≤0.01	≤0.01	≤0.01	2	4	0.5	1
Doxycycline	≤0.01	0.02	≤0.01	≤0.01	1	1	0.2	2
Minocycline	≤0.01	≤0.01	≤0.01	≤0.01	0.2	0.5	0.2	0.5
Nalidixic acid	ND	ND	ND	ND	2	2	4	8
Ofloxacin	0.2	0.2	0.02	0.05	0.05	0.05	0.02	0.05
Ciprofloxacin	0.1	0.2	0.05	0.1	≤0.01	0.2	≤0.01	≤0.01
Lomefloxacin	0.5	1	0.05	0.5	0.05	0.1	0.05	0.05
Sparfloxacin	0.02	0.05	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
Rifampin	≤0.01	0.2	≤0.01	≤0.01	8	8	8	16
Chloramphenicol	1	8	1	2	4	8	4	8
Amikacin	0.5	1	0.2	0.2	1	1	2	2

[a] ND, not determined.

TABLE 2. Activities of various antibiotics against *M. genitalium* and *M. pneumoniae*

Antimicrobial agent	MIC range (μg/ml)	
	M. genitalium	*M. pneumoniae*
Erythromycin	≤0.01	≤0.01
Spiramycin	0.5	0.5
Josamycin	0.02	≤0.01–0.02
Miocamycin	≤0.01	≤0.01
Roxithromycin	≤0.01	≤0.01
Azithromycin	≤0.01	≤0.01
Clarithromycin	≤0.01	≤0.01–0.05
Pristinamycin	≤0.01–0.02	0.02–0.05
Lincomycin	1–8	4–8
Clindamycin	0.2–1	1
Tetracycline	≤0.01–0.05	0.05
Doxycycline	≤0.01–0.05	0.02–0.05
Minocycline	≤0.01–0.02	0.02–0.05
Nalidixic acid	16–32	64–128
Ofloxacin	1–2	0.5–1
Ciprofloxacin	2	1
Lomefloxacin	2–4	2–8
Sparfloxacin	0.05–0.1	0.1
Rifampin	32–64	64–128
Chloramphenicol	0.5–4	4
Amikacin	16	16

species was moderately susceptible to amikacin. In general, *M. genitalium* and *M. pneumoniae* presented the same general patterns of susceptibility to the antibiotics tested.

Our results demonstrate that tetracyclines and macrolides, the antibiotics of choice for the treatment of both genital tract or respiratory mollicute infections, are very active against *M. genitalium*. The organism is also susceptible to those antimicrobial agents (fluoroquinolones and pristinamycin) that have been proposed as therapeutic drugs for infections with *Mycoplasma* species that exhibit resistance to tetracyclines or erythromycin. However, among the antibiotics tested here, we could not detect any significant differential activity that could be used in the preparation of a diagnostic or selective culture medium.

REFERENCES

1. **Baseman, J. B., S. F. Dallo, J. G. Tully, and D. L. Rose.** 1988. Isolation and characterization of *Mycoplasma genitalium* strains from the human respiratory tract. J. Clin. Microbiol. **26**:2266–2269.
2. **Bébéar, C., P. Cantet, H. Renaudin, and C. Quentin.** 1985. Activité comparée de la minocycline et doxycycline sur les mycoplasmes pathogènes pour l'homme. Pathol. Biol. **33**:577–580.
3. **Bébéar, C., H. Renaudin, J. Maugein, B. de Barbeyrac, and M. T. Clerc.** 1990. Pristinamycin and human mycoplasmas: in vitro activity compared with macrolides and lincosamides, in vivo efficacy in *Mycoplasma pneumoniae* experimental infection. Zentralbl. Bakteriol. Parasitenkd. Int. J. Med. Microbiol. **20**(Suppl.):77–82.
4. **Edward, D. G.** 1947. A selective medium for pleuropneumonia-like organisms. J. Gen. Microbiol. **1**:238–243.

5. **Furr, P. M., and D. Taylor-Robinson.** 1984. Microimmunofluorescence technique for detection of antibody to *Mycoplasma genitalium*. J. Clin. Pathol. **37**:1072–1074.
6. **Gadeau, A. P., C. Mouches, and J. M. Bové.** 1986. Probable insensitivity of mollicutes to rifampin and characterization of spiroplasmal DNA-dependent RNA polymerase. J. Bacteriol. **166**:824–828.
7. **Hooton, T. M., M. C. Roberts, P. L. Roberts, K. K. Holmes, W. E. Stamm, and G. E. Kenny.** 1988. Prevalence of *Mycoplasma genitalium* determined by DNA probe in men with urethritis. Lancet **i**:266–268.
8. **Hyman, H. C., D. Yogev, and S. Razin.** 1987. DNA probes for detection and identification of *Mycoplasma pneumoniae* and *Mycoplasma genitalium*. J. Clin. Microbiol. **25**:726–728.
9. **Jensen, J. S., S. A. Uldum, J. Sondergard-Andersen, J. Vuust, and K. Lind.** 1991. Polymerase chain reaction for detection of *Mycoplasma genitalium* in clinical samples. J. Clin. Microbiol. **29**:46–50.
10. **Kenny, G. E., and F. D. Cartwright.** 1991. Susceptibility of *Mycoplasma pneumoniae* to several new quinolones, tetracyclines, and erythromycin. Antimicrob. Agents Chemother. **35**: 587–589.
11. **Lind, K., B. O. Lindhardt, H. J. Schutten, J. Blom, and C. Christiansen.** 1984. Serological cross-reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. J. Clin. Microbiol. **20**:1036–1043.
12. **Palmer, H. M., C. B. Gibroy, P. M. Furr, and D. Taylor-Robinson.** 1991. Development and evaluation of the polymerase chain reaction to detect *Mycoplasma genitalium*. FEMS Microbiol. Lett. **77**:199–204.
13. **Renaudin, H., J. G. Tully, and C. Bébéar.** 1991. In vitro activity of MLS antibiotics, cyclines, fluoroquinolones, and other antibiotics against *Mycoplasma genitalium*, abstr. G-1, p. 133. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
14. **Risi, G. F., Jr., D. H. Martin, J. A. Silberman, and J. C. Cohen.** 1988. A DNA probe for detecting *Mycoplasma genitalium* in clinical specimens. Mol. Cell. Probe **2**:327–335.
15. **Samra, Z., M. Borin, Y. Bukowsky, Y. Lipshitz, and D. Sampolinsky.** 1988. Non-occurrence of *Mycoplasma genitalium* in clinical specimens. Eur. J. Clin. Microbiol. **7**:49–51.
16. **Taylor-Robinson, D., P. M. Furr, and C. M. Hetherington.** 1982. The pathogenicity of a newly discovered human mycoplasma (strain G 37) for the genital tract of marmosets. J. Hyg. **89**:449–455.
17. **Taylor-Robinson, D., J. G. Tully, P. M. Furr, R. M. Cole, D. L. Rose, and N. F. Hanna.** 1981. Urogenital mycoplasma infections of man: a review with observations on a recently discovered mycoplasma. Isr. J. Med. Sci. **17**:524–530.
18. **Tully, J. G., and D. L. Rose.** Unpublished data.
19. **Tully, J. G., D. Taylor-Robinson, R. M. Cole, and D. L. Rose.** 1981. A newly discovered mycoplasma in the human urogenital tract. Lancet **i**:1288–1291.
20. **Tully, J. G., D. Taylor-Robinson, D. L. Rose, P. M. Furr, C. E. Graham, and M. F. Barile.** 1986. Urogenital challenge of primate species with *Mycoplasma genitalium* and characteristics of infection induced in chimpanzees. J. Infect. Dis. **153**:1046–1054.
21. **Waites, K. B., L. B. Duffy, T. Schmid, D. Crabb, M. S. Pate, and G. H. Cassell.** 1991. In vitro susceptibilities of *Mycoplasma pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* to sparfloxacin. Antimicrob. Agents Chemother. **35**:1181–1185.