

Antibiotic Susceptibilities of AIDS-Associated Mycoplasmas

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Because mycoplasmas may be a cofactor in the progression of human immunodeficiency virus infection to AIDS, their susceptibilities to antibiotics need to be known in the event that appropriate therapy is required. The mycoplasmas studied were a stock culture strain of *Mycoplasma fermentans*, two strains of *M. fermentans* isolated from patients with AIDS, *M. fermentans* var. *incognitus*, *Mycoplasma penetrans*, and *Mycoplasma pirum*. The antibiotics tested were doxycycline, tetracycline, clindamycin, ofloxacin, erythromycin, azithromycin, and clarithromycin at levels consistent with the attainable levels in serum. By the macrodilution metabolic inhibition method, all six mycoplasma strains were susceptible to doxycycline, tetracycline, clindamycin, ofloxacin, azithromycin, and clarithromycin. *M. penetrans* was susceptible to erythromycin. The *M. fermentans* strains and *M. pirum* were resistant to erythromycin. The macrodilution metabolic inhibition method results showed agreement with the Sensititre Gram Positive MIC Panel results for tetracycline, clindamycin, and erythromycin. MICs of clarithromycin for all six mycoplasma isolates tested were low, indicating susceptibility.

Antiviral therapy of patients with AIDS has shown little success. Gougeon and Montagnier (5) have suggested that the answer may be a combination of several treatments, including antiviral agents, antibiotics, and antiapoptotic drugs. The use of antibiotics may be helpful considering recent reports that mycoplasmas may be a cofactor in the progression of human immunodeficiency virus (HIV) infection to AIDS (4, 7, 12-16). Their susceptibilities to antibiotics need to be known if appropriate therapy is to be instituted. Consideration of the characteristics of the mycoplasma infections, the organs targeted, and the antibiotic susceptibilities of the mycoplasmas must be appreciated in the treatment of these patients. The mycoplasmas reported as associated with AIDS are primarily *Mycoplasma fermentans* (3, 20), more recently *Mycoplasma penetrans* (2, 11, 21), and *Mycoplasma pirum* (6).

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Six strains of mycoplasmas, five actual isolates from patients with AIDS and a stock culture strain of *Mycoplasma fermentans* (NIH 713-001-084) from the National Institutes of Health were studied. We isolated two *M. fermentans* strains from the urine of patients attending the AIDS clinic of the Sloan-Kettering Hospital. This study has been reported previously (3). *M. fermentans*, var. *incognitus* and *M. penetrans* GTU 54-6A1 were obtained from Shyh-Ching Lo. *M. pirum* 70-159 was obtained from Joseph Tully of the National Institutes of Health.

Because it contains glucose, the growth medium used for susceptibility testing was SP4 broth. All of the mycoplasmas tested are glucose fermenters and changed the medium, with phenol red as an indicator, from an initial pink (pH 7.5 to 7.8) to yellow (pH 7.0 or less). The titer of each mycoplasma strain was determined by making 10-fold dilutions in SP4 broth and was expressed as color-changing units.

All of the organisms were tested by the macrodilution metabolic inhibition method (18) for susceptibility to doxycycline (1 µg/ml), tetracycline (6 µg/ml), clindamycin (1.6 µg/ml), ofloxacin (2 µg/ml), erythromycin (3 µg/ml), azithromycin (3 µg/ml), and clarithromycin (3 µg/ml). These antibiotics were

chosen because they are the agents of choice and are commonly used to treat other *Mycoplasma* infections in humans. Commercial filter paper disks were the carriers for the antibiotics. The antibiotic was placed into 5 ml of SP4 broth such that the concentration equaled the attainable levels in serum. To each tube, 50 µl of well-mixed *Mycoplasma* broth containing the specific organism was added. For each strain, a control tube containing 5 ml of SP4 broth was inoculated with 50 µl of *Mycoplasma* culture. All tubes were incubated at 35°C for 6 days and were observed daily for a color change. Growth of the mycoplasmas at this concentration, as indicated by a color change of the medium, was considered resistance.

The macrodilution metabolic inhibition susceptibility results for *M. fermentans* NIH 713-001-084, the *M. fermentans* strains from patients 5 and 29, *M. fermentans* var. *incognitus*, *M. penetrans* GTU 54-6A1, and *M. pirum* 70-159 to antibiotics showed that all of the mycoplasma strains were susceptible to doxycycline, tetracycline, clindamycin, and ofloxacin at the attainable levels in serum (1, 6, 1.6, and 2 µg/ml, respectively). Table 1 shows the macrodilution metabolic inhibition susceptibility results for the macrolides. All six *Mycoplasma* strains showed susceptibility to azithromycin and clarithromycin. *M. penetrans* was susceptible to erythromycin. All of the other strains were resistant.

The macrodilution metabolic inhibition test results for tetracycline, clindamycin, and erythromycin were compared with the results obtained with the Sensititre Gram Positive MIC Panel. This 96-well microdilution plate is precision dosed with antimicrobial agents at appropriate dilutions and then dried and thereby stabilized. After reconstitution with 50 µl of broth containing the organism, a result that is both qualitative and quantitative is obtained. The preparation of the inoculum was based on a method of testing *Mycoplasma gallisepticum* as reported by Tanner and Wu (19) and in the Sensititre package insert.

All six *Mycoplasma* strains tested were stored frozen at -70°C in SP4 broth. The isolates were removed from the freezer and were thawed at room temperature. A 1-ml aliquot was added to 4 ml of SP4 broth, and the mixture was incubated until the mycoplasma growth just turned the color of the medium. A 2-ml amount of this broth was added to 18 ml of SP4 broth, and the mixture was homogenized by vortexing. Fifty microliters was then inoculated into each well on the

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TABLE 1. Susceptibilities by the macrodilution metabolic inhibition method: of six *Mycoplasma* strains to the macrolides tested at attainable levels in serum^a

Strain	MIC (μg/ml)		
	Erythro- mycin	Azithro- mycin	Clarithro- mycin
<i>M. fermentans</i> NIH 713-001-084	R	S	S
<i>M. fermentans</i> from patient 5	R	S	S
<i>M. fermentans</i> from patient 29	R	S	S
<i>M. fermentans</i> var. <i>incognitus</i>	R	S	S
<i>M. penetrans</i> GTU 54-6A1	S	S	S
<i>M. pirum</i> 70-159	R	S	S

^a All antibiotics were used at a concentration of 3 μg/ml. S, susceptible; R, resistant.

Sensititre plate. The plate was covered with a transparent self-adhesive seal and was incubated at 35°C. The MIC result was the lowest concentration of antibiotic inhibiting a color change at the time that the control well containing the same strain showed a color change. This generally occurred at between 24 and 48 h of incubation.

Table 2 shows the susceptibility results obtained with the Sensititre Gram Positive MIC Panel. The Sensititre Gram Positive MIC Panel results for tetracycline, clindamycin, and erythromycin were in agreement with the macrodilution metabolic inhibition test results. All six mycoplasma strains were susceptible to tetracycline and clindamycin. *M. penetrans* was susceptible to erythromycin. All of the other strains were resistant. The *Mycoplasma* strains were also susceptible to chloramphenicol and ciprofloxacin, which were included on the Sensititre Gram Positive MIC Panel. They were resistant to penicillin, ampicillin, cephalothin, imipenem, vancomycin, rifampin, and trimethoprim-sulfamethoxazole. *M. pirum* was moderately susceptible to gentamicin; the other strains were resistant.

Clarithromycin, a newer macrolide obtained from Abbott Laboratories, was tested for its MICs for the six strains of mycoplasmas by the MIC test. Clarithromycin was received in a powdered form that contained 98% active product. A stock solution was prepared by dissolving clarithromycin in methanol and then adding phosphate buffer (0.1 M; pH 6.5) to make up the appropriate concentration.

The MIC test was performed in nine dilutions in SP4 broth containing 200, 40, 8, 4, 2, 1, 0.5, 0.25, and 0.125 μg of antibiotic per ml. To each tube, 25 μl of well-mixed *Mycoplasma* broth containing the specific organism was added. For each strain, a control tube containing 2.5 ml of SP4 broth was inoculated with 25 μl of *Mycoplasma* culture. All tubes were incubated at 35°C for 6 days and were observed daily for a color change.

The initial MIC was the lowest concentration of antibiotic

TABLE 3. Susceptibilities of six *Mycoplasma* strains to clarithromycin expressed as initial and final MICs

Strain	MIC (μg/ml)		Titer (CCU/ml) ^a
	Initial	Final	
<i>M. fermentans</i> NIH 713-001-084	≤0.125	0.5	10 ⁴
<i>M. fermentans</i> from patient 5	≤0.125	0.5	≥10 ⁵
<i>M. fermentans</i> from patient 29	≤0.125	0.5	≥10 ⁵
<i>M. fermentans</i> var. <i>incognitus</i>	≤0.125	1.0	10 ³
<i>M. penetrans</i> GTU 54-6A1	≤0.125	≤0.125	10 ⁴
<i>M. pirum</i> 70-159	≤0.125	≤0.125	10 ²

^a CCU, color-changing unit.

that inhibited a color change at the time that the control tube containing the same strain showed a color change. The lowest concentration of antibiotic that completely inhibited a color change after 6 days was the final MIC.

Table 3 shows the MICs of clarithromycin. All six *Mycoplasma* isolates tested were susceptible to clarithromycin. MICs were low, indicating that clarithromycin is highly active against all six *Mycoplasma* strains. The attainable level of clarithromycin in serum is 3.4 μg/ml, with a half-life in serum of 6.5 h following administration of a dose of 500-mg. Final MICs ranged from ≤0.125 to 1.0 μg/ml. The initial MIC was ≤0.125 μg/ml for all of the strains tested.

In patients with AIDS, genetic material specific for *M. fermentans*, var. *incognitus* in tissues such as lymph nodes, spleen, liver, adrenal gland, heart, Kaposi's sarcoma lesions, brain, and placentas (10) as well as kidneys (1) has been found by PCR. Consequently, attainable levels of drug in tissues must be taken into consideration for the eradication of the mycoplasmas from these diverse areas. For this reason, the susceptibilities of *Mycoplasma* isolates from patients to the commonly used antibiotics, if the *Mycoplasma* isolates do prove to have a role in human immunodeficiency virus infections, need to be evaluated in detail.

Although very few strains of *M. fermentans* have been tested for their susceptibilities to antibiotics, our findings are consistent with previous reports from other investigators who have published the antimicrobial susceptibilities of the organism. Renaudin and Bebear (17) reported that sparfloxacin, ofloxacin, and doxycycline are active against the three *M. fermentans* strains tested, but that erythromycin is inactive. Hayes et al. (9) reported that *M. fermentans*, var. *incognitus* is susceptible in vitro to tetracycline, doxycycline, chloramphenicol, clindamycin, lincomycin, and ciprofloxacin but is resistant to erythromycin. In another study, Hayes et al. (8) reported that the 24 *M. fermentans* tested indicated that the antibiotics of potential clinical usefulness for the treatment of *M. fermentans* infections are ciprofloxacin, levofloxacin, clindamycin, lincomycin,

TABLE 2. Susceptibilities of six mycoplasma strains to antibiotics by using the Sensititre Gram Positive MIC Panel

Strain	MIC (μg/ml) [susceptibility] ^a			Titer (CCU/ml) ^b
	Tetracycline	Clindamycin	Erythromycin	
<i>M. fermentans</i> NIH 713-001-084	≤2 (S)	0.25 (S)	>16 (R)	10 ⁵
<i>M. fermentans</i> from patient 5	≤2 (S)	0.25 (S)	>16 (R)	10 ⁵
<i>M. fermentans</i> from patient 29	≤2 (S)	0.25 (S)	>16 (R)	10 ⁵
<i>M. fermentans</i> var. <i>incognitus</i>	≤2 (S)	≤0.125 (S)	>16 (R)	10 ⁴
<i>M. penetrans</i> GTU 54-6A1	≤2 (S)	≤0.125 (S)	0.5 (S)	10 ⁴
<i>M. pirum</i> 70-159	8 (MS)	≤0.125 (S)	8.0 (R)	10 ³

^a S, susceptible; MS, moderately susceptible; R, resistant.

^b CCU, color-changing unit.

doxycycline, tetracycline, and chloramphenicol. Erythromycin, gentamicin, and streptomycin were ineffective in that study.

In conclusion, all six AIDS-associated *Mycoplasma* strains were susceptible to doxycycline, tetracycline, clindamycin, ofloxacin, azithromycin, and clarithromycin. All test methods showed good agreement and indicate that *M. penetrans* is the only *Mycoplasma* strain tested that is susceptible to erythromycin. It is interesting that the AIDS-associated mycoplasmas were susceptible in vitro to the newly developed macrolides clarithromycin and azithromycin but were generally resistant to erythromycin.

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