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Susceptibility of Genital Mycoplasmas to Antimicrobial Agents

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The susceptibility of 11 T-strains, 12 strains of *Mycoplasma hominis*, and a single strain of *M. fermentans* to 15 antimicrobial agents was determined by study of inhibition of metabolic activity in a broth dilution system. All three species were inhibited by tetracycline, chloramphenicol, streptomycin, gentamicin, and kanamycin, and were relatively resistant to cephalothin, cephaloridine, polymyxin, vancomycin, and ampicillin. Three antimicrobial agents had significant differential effects on these species. Erythromycin was more active against T-strains than against *M. hominis* or *M. fermentans*. Lincomycin, clindamycin, and nitrofurantoin had greater activity against *M. hominis* and *M. fermentans* than against T-strains. The activity of the drugs tested was generally uniform over a wide range of inocula. The effect of *p*H and the difference between minimal inhibiting and minimal mycoplasmacidal concentrations of the drugs tested were consistent with expectations based on the effects of these drugs on bacteria.

The role of the human genital mycoplasmas in infection and disease is poorly understood. The T-strains were first reported by Shepard in 1954 (26) and are characterized by their small colonies on agar, urease activity, and their fastidious growth requirements (27). *Mycoplasma hominis* is a better defined species with characteristic colonies and distinct biochemical and antigenic properties (12, 20). Both species have been recovered from urine, vaginal secretions, and cervical exudates of women without apparent disease. The prevalence of such isolations increases with age from childhood through the childbearing age group (4, 5, 7, 8, 15, 32). Both species have been found in urine and urethral scrapings in men (4, 5, 7, 8, 10, 30, 32) without apparent disease. Several investigators have suggested an association of T-strains with nongonococcal urethritis (4, 5, 7, 8, 10, 30, 32), but a specific etiological relationship has been difficult to establish because of the high prevalence of the organisms in control patients (1, 5, 7, 8, 10, 13, 30, 32) and the difficulties in obtaining appropriate control materials and in demonstrating serological evidence of recent infection (7, 23). Kundsin and co-workers (17) isolated a T-strain from fetal membranes which showed extensive inflammatory changes after spontaneous abortion, stimulating further investigations of the pathogenic role of these organisms, although adequate controls were lacking.

M. fermentans is an antigenically distinct human mycoplasma which forms large colonies on agar. Strains have been isolated from cervical exudates, vaginal secretions, and the glans penis, but their frequency of occurrence and role in infection and disease are uncertain (9, 12, 25).

Strains of *M. hominis* have been isolated from the blood of women with post partum sepsis and after obstetric or gynecologic surgery (33, 34); they have also been recovered from the lungs of aborted fetuses and from a stillborn (16, 21). These reports leave unresolved the question of whether the mycoplasma was a primary invader or reached the fetal tissues secondarily. *M. hominis* has been associated with the development of exudative pharyngitis in volunteers (18), but the role of this agent in naturally occurring pharyngitis is undefined.

Knowledge of the susceptibility of these mycoplasmas to antimicrobial agents would be of value if a relationship to disease were to be securely documented. Such information might also be helpful in empirical therapeutic studies designed to elucidate the role of these agents in pathological processes, and for use of antibiotics in tissue cultures.

The information regarding the effect of various

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TABLE I. *Source and characteristics of mycoplasma strains used in antibacterial assay*

Mycoplasma[a]	Source[b]	Titer in broth[e]	Days required[c]	Titer used in tests[f]	Mycoplasma	Source[b]	Titer in broth	Days required	Titer used in tests[e]
M. hominis strains					T-strains				
PG21	NIH	10^{-7}	3	10^{-3}	2 K 160	M. Shepard	10^{-6}	3	10^{-2}
Cor.	Urine	10^{-9}	2	10^{-4}	T 960	M. Shepard	10^{-6}	2	10^{-2}
Cur.	Urine	10^{-8}	3	10^{-4}	Rod.	Nose	10^{-7}	2	10^{-3}
Mor.	Urine	10^{-7}	3	10^{-3}	Can.	Vagina	10^{-7}	2	10^{-3}
Car.	Urine	10^{-7}	2	10^{-3}	Aub.	Throat	10^{-6}	2	10^{-2}
Rey.	Urine	10^{-7}	3	10^{-3}	Sch.	Nose	10^{-7}	3	10^{-3}
Smi.	Urine	10^{-6}	3	10^{-2}	Cla.	Throat	10^{-6}	2	10^{-2}
Lay.	Urine	10^{-9}	2	10^{-4}	Rec.	Throat	10^{-7}	3	10^{-3}
Bro.	Urine	10^{-6}	4	10^{-2}	Ell.	Throat	10^{-7}	2	10^{-3}
Sim.	Urine	10^{-6}	3	10^{-2}	Smi.	Urine	10^{-6}	2	10^{-2}
Bar.	Urine	10^{-8}	3	10^{-4}	Hom.	Throat	10^{-6}	3	10^{-2}
Col.	Urine	10^{-8}	3	10^{-4}					
M. fermentans[d]	Urine	10^{-6}	6	10^{-2}					

[a] Except for the NIH strain, these are designated by the patient from whom obtained.
[b] Isolations from nose, throat, and vagina were by swabs of newborns. The isolation from the urine was from an adult whose urine contained both T-strains and *M. hominis*.
[c] Days required for maximal color change to occur in the terminal dilution.
[d] Obtained from Sarabelle Madoff of the Massachusetts General Hospital.
[e] Maximum dilution of culture giving multiplication by tube dilution technique.
[f] Dilution of culture used as inoculum.

antimicrobial agents on this group of microorganisms also has implications with respect to the mode or site of action of such drugs. Therefore, the susceptibility of *M. hominis*, *M. fermentans*, and T-strain mycoplasmas to a variety of commonly available drugs was determined, and the effects of varying the *p*H of the media and the size of the inoculum on susceptibility were investigated.

MATERIALS AND METHODS

Because the growth requirements of mycoplasmas are critical, the methods for isolation and testing of sensitivity to antimicrobial drugs are presented in detail. In general, a broth dilution technique similar to that employed by Jao and Finland (14) for testing the susceptibility of *M. pneumoniae* to antibiotics was used. The source and characteristics of the mycoplasma strains used in the assays are outlined in Table 1. All strains were cloned three times.

Since the genital mycoplasmas have different cultural requirements, primary isolations and testing against antimicrobial drugs were performed in different media. In addition, the testing was conducted in both broth and agar under various cultural conditions. Therefore, many different media were used.

The basic broth consisted of 70% Difco PPLO broth, 20% unheated horse serum (generously supplied by the Biological Laboratories of the Massachusetts Department of Public Health), 10% yeast extract, and 0.002% phenol red.

For primary isolation of *M. hominis*, the following were also added to each milliliter of media: nafcillin, 100 μg; polymyxin B, 5 μg; amphotericin B, 5 μg; and 1-arginine hydrochloride, 10 mg. The broth was adjusted to *p*H 7.2. Subcultures from this broth were made in basic broth plus 10 mg of *l*-arginine and 100 μg of penicillin per ml. When an agar medium was desired for propagation of *M. hominis*, Difco PPLO agar was used instead of PPLO broth, with horse serum, yeast extract, and phenol red as in the basic broth, and 100 μg of penicillin G per ml also added (2). Specific identification of strains was made by growth inhibition with hyperimmune serum (3).

For primary isolation of T-strains from urine, the broth was identical to that used to isolate *M. hominis* except that urea (0.5 mg/ml) was used instead of arginine, and the *p*H was adjusted to 6.0. For isolation of T-strains from nose, throat, and vagina of newborn infants, the medium contained cloxacillin instead of nafcillin, colistin sulfate (100 μg/ml) instead of polymyxin B, urea (1.0 mg/ml), and the broth was adjusted to *p*H 6.0. Passage of T-strains was performed in basic broth containing added urea and benzyl penicillin in the concentrations listed above and adjusted to *p*H 6.0. Shepard's A-3 agar medium was used for cultivation of T-strain colonies (*personal communication*). T-strains were identified on the basis of their characteristic morphology, alkaline reaction in urea broth, and inhibition by discs containing 10 μg of erythromycin.

M. fermentans was assayed in basic broth, as for *M. hominis*, but 1% dextrose was substituted for *l*-arginine and the medium was adjusted to *p*H 7.4.

Broth cultures were incubated aerobically and agar cultures anaerobically at 37 C. For anaerobic incubation, jars containing the agar plates were evacuated in three successive cycles to < 3 psi and refilled each time with a mixture of 90% N_2 and 10% CO_2. The *p*H of culture media was determined colorimetrically against a set of PPLO broth standards for which *p*H had been determined with a glass electrode, and *p*H was adjusted by using 1.5 or 1.0 N HCl. All dilutions were made with separate pipettes between tubes.

The antimicrobial agents used were as follows:

reference standard powders of kanamycin sulfate, streptomycin sulfate, chloramphenicol, cephalothin, ampicillin trihydrate, cephaloridine, tetracycline hydrochloride, lincomycin hydrochloride, clindamycin hydrochloride, erythromycin base, gentamicin sulfate, polymyxin B sulfate, vancomycin, and nalidixic acid, and nitrofurantoin clinical powder for intravenous use. Kanamycin, streptomycin, chloramphenicol, and tetracycline were diluted in distilled water to produce solutions containing 2,000 μg/ml. Solutions of erythromycin base were made by solution in 2.1 ml of methanol with subsequent addition of 7.9 ml of PPLO broth to yield 2,000 μg of antibiotic per ml. Solutions of the remaining antibiotics were prepared to yield 2,000 μg of antibiotic per ml of PPLO broth. These and all subsequent dilutions were made in broth containing only 70% Difco PPLO broth, 20% unheated horse serum, 10% yeast extract, and 0.002% phenol red. Stock solutions of antimicrobial agents were used on the day of preparation or were stored at -20 C and used within 1 month of preparation.

For determination of minimal inhibitory (MIC) and minimal mycoplasmacidal concentrations (MCC) of antimicrobial agents, strains of *M. hominis* were diluted in basic broth with 1% arginine at *p*H 6.0 to yield 10^4 to 9×10^4 color changing units (CCU) per 0.1 ml, *M. fermentans* was diluted in basic broth with 1% dextrose at *p*H 7.4 to yeild 10^4 to 9×10^4 CCU, and T-strains were diluted to contain 10^4 to 9×10^4 CCU in basic broth with 0.05% urea adjusted to *p*H 6.0. One CCU was defined as the lowest inoculum of *M. hominis* or T-strain which produced characteristic rises in *p*H in arginine or urea broth, respectively, or a characteristic decrease in *p*H in glucose broth in the case of *M. fermentans*.

Dilutions of antibiotics were made on the day each antibiotic was tested. Tests were performed in clear polystyrene plastic tubes or in "Microtiter" plates (Microbiological Associates Inc., Bethesda, Md.), which were used to conserve media and to facilitate the testing of large numbers of specimens simultaneously. In all tests, equal volumes of solutions of antimicrobial and of media containing mycoplasma were used. When the tests were performed in tubes, 0.5 ml of medium containing mycoplasma was added to 0.5 ml of dilution of antibiotic in each tube and the tubes were sealed with tight-fitting plastic caps. When the tests were performed in methyl-methacrylate "Microtiter" plates, 0.1 ml of the mycoplasmal suspension was added by pipette to 0.1 ml of dilution of antibiotic, which had previously been introduced by pipette. The plates were sealed with plastic tape after an initial warming period of 30 min at 37 C. Daily observations were made for change in *p*H by using, for comparison, uninoculated wells and wells containing organisms without antimicrobials.

The MIC was defined as the lowest concentration of drug inhibiting color change in broth by the test organism and was determined two times for each strain tested. The "initial MIC" was determined when control wells containing organisms in broth without antibiotics first showed color change. The "final MIC" was determined when metabolic change was fully expressed in broth containing dilutions of antibiotic and the end point did not change for at least two consecutive daily readings.

Growth of both T-strains and *M. hominis* in subcultures from wells with minimal color change established in numerous trials that viable organisms were present, even when minimal color change was observed in cultures containing drugs.

After the sensitivity of representative strains had been determined by the tube dilution technique in preliminary tests, each antimicrobial drug was tested simultaneously against the 12 strains of *M. hominis* and 11 T-strains. Dilution of each antimicrobial were made on the day of the test and used against all T- and *M. hominis* strains, which were added in the concentrations stated above. With the use of the microtitration technique, it was possible to test dilutions of six antimicrobials simultaneuosly in eight concentrations against the 23 mycoplasma strains. Readings were made regularly over a period of at least 5 days. In these experiments kanamycin, streptomycin, chloramphenicol, cephalothin, ampicillin, and cephaloridine were tested at one time against all of the 23 strains. Tetracyline, lincomycin, erythromycin, gentamicin, vancomycin, and polymyxin were tested on a separate occasion. Nitrofurantoin was tested on another day in the "Microtiter" system, as were occasional strains for which end points were not reached in initial tests. Nalidixic acid was used in tests of one T-strain and one *M. hominis* strain. Antimicrobials were tested on a separate occasion for activity against *M. fermentans*, and readings were made regularly for 14 days.

To determine the MCC, broth tubes were subcultured after 24 hr of incubation in the cases of *M. hominis* and the T-strains, and at 7 days in the case of *M. fermentans*. An inoculum of 0.1 ml was added to 2 ml of drug-free broth supplemented only with arginine, dextrose, or urea. Thus, a 20-fold dilution of remaining drug was achieved, allowing subculture of surviving organisms from tubes containing antimicrobial solutions in excess of the MIC. Since residual antibiotic is carried over in this test and the dilution is 20-fold, the MCC cannot be determined accurately by this technique when the concentration of antimicrobial in the original mixture is more than 20-fold higher than the MIC.

To study the effect of inoculum size on the antimycoplasmal action of the drugs, serial 10-fold dilutions of organisms were incubated in replicate titrations with serial dilutions of antimicrobial agents with "Microtiter" plates. Parallel control titrations of organisms were incubated simultaneously. In these tests, 0.1 ml of organism was combined with 0.1 ml of drug and incubated in sealed "Microtiter" plates.

To study the comparative effect of different media on the action of a mycoplasmacidal drug, MIC values were determined simultaneously by the microtitration technique in a tube dilution system, and in agar. Polystyrene tubes containing 0.5 ml with 10^4 to 10^5 CCU/ml of the strains of organism were incubated with 0.5 ml of twofold dilutions of tetracycline. "Microtiter" plates were inoculated with antibiotic and with organisms in similar fashion. PPLO agar plates containing known concentrations of tetracycline were inoculated with 0.05-ml drops of the same dilution of

TABLE 2. *Susceptibility of 11 T-strains to 14 antimicrobial agents in vitro (MIC in broth at pH 6.0)*

Antimicrobial agent	Initial MIC (median and range)[a]	Final MIC (median and range)[a]
Tetracycline	0.4 (0.05–0.8)	0.8 (0.4–1.6)
Erythromycin	1.6 (0.8–1.6)	12.5 (6.2–25)
Chloramphenicol	1.6 (0.4–3.1)	1.6 (0.4–3.1)
Streptomycin	1.6 (0.4–3.1)	1.6 (0.8–3.1)
Kanamycin	3.1 (1.6–12.5)	6.2 (3.1–25)
Gentamicin	6.2 (3.1–12.5)	6.2 (3.1–25)
Clindamycin	12.5 (<6.2–50)	50 (25–50)
Lincomycin	200 (25–500)	500 (100–>1,000)
Polymyxin	500 (12.5–>1,000)	1,000 (400–>1,000)
Cephalothin	1,000 (200–1,000)	1,000 (200–1,000)
Nitrofurantoin	>1,000 (12.5–>1,000)	>1,000 (200–>1,000)
Vancomycin	>1,000 (500–>1,000)	>1,000 (>1,000)
Cephaloridine	>1,000 (500–>1,000)	>1,000 (500–>1,000)
Ampicillin	>1,000 (>1,000)	>1,000 (>1,000)

[a] Median values followed by range values in parentheses expressed in micrograms per milliliter.

TABLE 3. *Susceptibility of 12 strains of Mycoplasma hominis to 14 antimicrobial agents in vitro (MIC in broth at pH 6.0)*

Antimicrobial agent	Initial MIC (median and range)[a]	Final MIC (median and range)
Clindamycin	<0.01 (<0.01–0.4)	0.4 (0.2–3.1)
Tetracycline	0.05 (0.02–0.1)	0.2 (0.1–0.4)
Chloramphenicol	0.4 (<.02–0.8)	0.4 (.05–0.8)
Lincomycin	0.8 (<0.4–1.6)	3.1 (1.6–6.2)
Streptomycin	0.8 (0.4–3.1)	25 (12.5–200)
Gentamicin	12.5 (1.6–12.5)	12.5 (6.2–25)
Kanamycin	12.5 (1.6–12.5)	12.5 (3.1–12.5)
Nitrofurantoin	25 (<6.2–50)	25 (6.2–50)
Cephalothin	500 (500)	500 (500)
Vancomycin	500 (500–1,000)	>1,000 (500–>1,000)
Erythromycin	500 (100–1,000)	1,000 (500–1,000)
Cephaloridine	1,000 (500–1,000)	1,000 (500–1,000)
Polymyxin	1,000 (12.5–>1,000)	>1,000 (500–>1,000)
Ampicillin	>1,000 (>1,000)	>1,000 (>1,000)

[a] Median values followed by range values, in parentheses, expressed in micrograms per milliliter.

organisms and incubated in 90% nitrogen and 10% CO_2. After 3 days of incubation, the MIC against 10^4 to 10^5 CCU was 0.4 μg/ml, and after 4 days, it was 0.8 μg/ml with the use of the microtitration technique. The MIC in tube dilution was 0.4 μg/ml after 3 and 4 days of incubation, and the MIC on agar, as determined by the growth of colonies within 4 days, was 0.2 μg/ml. Thus, the three methods agreed within a single dilution.

To study the effect of *p*H, one strain of *M. hominis* and one T-strain were tested against each drug at *p*H 6.0 and *p*H 7.4 in broth titrations. Organisms were used at a concentration of 10^4 to 10^5 CCU in 0.1 ml, and the two *p*H levels were tested simultaneously for each drug.

RESULTS

The mean and the range of the MIC values of 14 drugs against 12 strains of *M. hominis*, 11 T-strains, and a single strain of *M. fermentans* are summarized in Tables 2 to 4, respectively. For each antibiotic, *M. hominis*, and T-strains were susceptible within a narrow range of concentration of drug, and no strains were encountered that deviated widely from the mean for that drug. The antimicrobials fell into three general groups: one group (tetracycline, chloramphenicol, gentamicin, streptomycin, and kanamycin) was relatively active against all three mycoplasma species; a second group (cephalothin, cephaloridine, polymyxin, vancomycin, and ampicillin) was uniformly ineffective; and a third group (lincomycin, clindamycin, nitrofurantoin, and erythromycin) showed differential effects among the mycoplasmas. Erythromycin inhibited T-strains at levels of 0.8 to 1.6 μg/ml (initial MIC) and gave final MIC values of 6.2 to 25 μg/ml. *M. hominis* was inhibited by erythromycin only at 100 to 1,000 μg/ml (initial MIC) and 500 to 1,000 μg/ml (final

TABLE 4. *Susceptibility of Mycoplasma fermentans*[a] *to 13 antimicrobial agents in vitro (MIC in broth at pH 7.4)*

Antimicrobial agent	Initial MIC (µg/ml)	Final MIC (µg/ml)
Clindamycin	0.01	0.16
Lincomycin	0.02	0.16
Chloramphenicol	1.6	6.2
Tetracycline	1.6	12.5
Streptomycin	3.9	250
Gentamicin	6.2	25
Kanamycin	6.2	50
Erythromycin	15.6	125
Cephaloridine	250	250
Cephalothin	250	500
Polymyxin	500	1,000
Ampicillin	1,000	1,000
Vancomycin	1,000	1,000
Nitrofurantoin	0.25	125

[a] A single strain was used in the assay.

MIC). The strain of *M. fermentans* was intermediate with an initial MIC of 7.8 µg/ml and a final MIC of 125 µg/ml. In contrast, T-strains were markedly less susceptible than *M. hominis* and *M. fermentans* to lincomycin and clindamycin. T-strains were inhibited by lincomycin at 25 to 500 µg/ml (initial MIC) and 100 to >1,000 µg/ml (final MIC), whereas *M. hominis* was inhibited by <0.4 to 1.6 µg/ml (final MIC) and *M. fermentans* was inhibited by 0.02 µg/ml (initial MIC) and 0.16 µg/ml (final MIC). All three strains were more effectively inhibited by clindamycin than by lincomycin.

The effects of inoculum size are summarized in Table 5. The action of nitrofurantoin and lincomycin was most affected by the size of the inoculum. In the case of *M. hominis*, the MIC increased 16-fold for nitrofurantoin and 64-fold for lincomycin, when the inoculum was increased through the tested range up to 10^7 CCU. Nevertheless, the differential effect of lincomycin toward *M. hominis* and T-strains persisted over a wide range of inocula, so that concentrations of up to 10^6 CCU of *M. hominis* were inhibited by 6.2 µg/ml of lincomycin, but 10 to 10^2 CCU of T-strains grew well at 1,000 µg/ml. The other drugs tested showed little inoculum effect, the MIC varying by only two- or fourfold over the range of inocula, except when inocula containing 10^7 to 10^8 CCU were used. By using these large inocula, *p*H rises were observed at the highest concentrations of some antibiotics and end points could not be determined. Subcultures from tubes containing large inocula were not performed.

T-strains showed little or no inoculum effect against kanamycin, tetracycline, lincomycin, and erythromycin except when inocula containing 10^5 to 10^6 CCU/0.1 ml were used. Similarly, only minimal inoculum effect was apparent in the assays of *M. fermentans* and chloramphenicol, tetracycline, kanamycin, streptomycin, erythromycin, lincomycin, and clindamycin.

As might be expected, color changes were slower to develop in the presence of drug compared to the controls without drug; it is for this reason that incubation of the tests of drug susceptibility must be prolonged until no further color changes occur.

The effect of change of *p*H of the medium was determined for selected T-strains and strains of *M. hominis* at *p*H 6.0 and 7.4 (Table 6). Control cultures at these *p*H levels were inoculated with 10^4 to 10^5 CCU and showed color changes for all organisms. Tetracycline showed fourfold greater activity in acid than in alkaline medium against both types of mycoplasma. Kanamycin, streptomycin, erythromycin, lincomycin, and gentamicin showed fourfold or more greater activity at *p*H 7.4 than at *p*H 6.0 against T-strains and *M. hominis*, except for kanamycin against the T-strain and streptomycin against the *M. hominis* strain tested.

The comparison of MIC and MCC for one strain of each species is summarized in Table 7. Mycoplasmacidal concentrations of kanamycin, streptomycin, and erythromycin for all three species were equal to or only twofold greater than the MIC. Polymyxin and cephaloridine were mycoplasmacidal for a T-strain at the MIC. Nitrofurantoin was bactericidal for *M. hominis* at twice the MIC. Drugs which proved to be inhibitory but not bactericidal at many times the minimal inhibiting concentration were tetracycline, chloramphenicol and nalidixic acid, against both *M. hominis* and T-strains. Lincomycin was inhibitory but not bactericidal against *M. hominis*.

DISCUSSION

Prior studies have documented the susceptibility of the genital mycoplasmas to several antimicrobial agents (6, 19, 24, 29, 30, 31). Early work on mycoplasmas of human origin was hampered by inadequate development, at the time, of methods for distinguishing the species of mycoplasma. However, it is likely that the classical genital mycoplasmas were *M. hominis*. These early studies, reviewed by Newnham and Chu (19), and later studies (29, 31), in which *M. hominis* was specifically identified, revealed uniform sensitivity to tetracycline and resistance to erythromycin. Shepard et al. showed that T-strains, in contrast to *M. hominis*, were sensitive to erythromycin and to the tetracyclines (29). Clinical observations corroborated the in vitro results.

TABLE 5. *Effect of inoculum size on the susceptibility of genital mycoplasmas to antimicrobial agents (final MIC in broth, μg/ml)*

Drug	Inoculum (dilution of culture)																	
	T-strain[a]						*M. hominis*[b]							*M. fermentans*				
	10^{-}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}[c]	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}[c]	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}[c]
Kanamycin	>100	6.2	3.1	1.6	1.6	1.6	25	25	12.5	12.5	12.5	12.5	6.2	125	125	125	62	125
Streptomycin							100	50	50	50	50	25	25					
Chloramphenicol							>6.2	6.2	6.2	6.2	6.2	6.2	ND[d]	6.2	6.2	6.2	6.2	6.2
Cephalothin							>1,000	1,000	1,000	500	500	500	500					
Cephaloridine							>1,000	>1,000	>1,000	1,000	1,000	500	500					
Nitrofurantoin							50	50	25	25	25	12.5	3.1					
Tetracycline	>12.5	12.5	12.5	12.5	12.5	12.5	1.6	1.6	1.6	0.8	0.8	0.8	0.4	25	25	25	12.5	6.2
Lincomycin	>1,000	>1,000	>1,000	1,000	1,000	1,000	50	6.2	3.1	3.1	3.1	0.8	0.1	0.04	0.08	0.02	0.02	0.01
Erythromycin	>1,000	12.5	12.5	12.5	12.5	12.5	>1,000	500	500	500	500	500	500	500	500	250	125	125
Gentamicin							100	50	50	25	25	25		16	31	16	16	16
Polymyxin							>1,000	>1,000	>1,000	>1,000	1,000	1,000						
Vancomycin							>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	1,000					
Clindamycin														0.04	0.04	0.02	0.02	0.02

[a] T-strains used in these tests were T 960 (tetracycline, lincomycin, and erythromycin) and Sch. (kanamycin); broth at *p*H 6.0.
[b] PG 21.
[c] Dilution contained 10–10^2 color changing units.
[d] Not determined.

TABLE 6. *Minimal inhibitory concentration (MIC) of antimicrobial agents versus selected strains of Mycoplasma hominis and T-strains at pH 6.0 and 7.4*

Antimicrobial agent	T-strain[a]		M. hominis[b]	
	6.0	7.4	6.0	7.4
Streptomycin	3.1[c]	0.4	100	50
Gentamicin	6.2	0.8	25	3.1
Erythromycin	25	0.4	>1,000	>1,000
Lincomycin	>1,000	100	1.6	1.6
Polymyxin	>1,000	200	>1,000	>1,000
Kanamycin	12.5	6.2	12.5	3.1
Chloramphenicol	6.2	6.2	1.6	3.1
Cephaloridine	500	500	1,000	>1,000
Vancomycin	>1,000	>1,000	>100	>1,000
Nitrofurantoin	200	200	100	200
Cephalothin	500	1,000	500	1,000
Ampicillin	1,000	>1,000	>1,000	>1,000
Tetracycline	0.8	3.1	0.4	1.6

[a] T 960 employed in all tests except where Can. T-strain was used (cephaloridine, tetracycline, and polymyxin).
[b] PG 21 employed except where Cor. *M. hominis* was used (erythromycin and nitrofurantoin).
[c] Final MIC, expressed in micrograms per milliliter.

TABLE 7. *Minimal inhibitory (MIC) and minimal mycoplasmacidal concentrations (MCC) of antimicrobials for selected genital mycoplasmas*[a]

Antimicrobial agent	T-Strain[b]		M. hominis[c]		M. fermentans	
	MIC[d]	MCC	MIC[d]	MCC	MIC[d]	MCC
Erythromycin	12.5	50	500	1,000	125	500
Lincomycin	>1,000	>1,000	3.1	500	0.16	0.16
Chloramphenicol	6.2	100	12.5	100	6.2	25
Tetracycline	1.6	>50	0.8	>100	12.5	25
Nalidixic acid	≤6.2	1,000	25	500		
Clindamycin					0.16	0.16
Kanamycin	25	25	25	25	50	100
Streptomycin	3.1	3.1	50	100	250	500
Cephalothin	1,000	>1,000	500	>1,000	500	500
Ampicillin	>1,000	>1,000	>1,000	>1,000	1,000	1,000
Cephaloridine	1,000	1,000	1,000	>1,000	250	500
Gentamicin	6.2	12.5	6.2	25	25	25
Polymyxin	500	500	>1,000	>1,000	1,000	1,000
Vancomycin	>1,000	>1,000	>1,000	>1,000	1,000	1,000
Nitrofurantoin	1,000	>1,000	25	50	125	125

[a] Values are expressed in micrograms per milliliter.
[b] T 960 used in all tests except for those employing Can. T-strain (nitrofurantoin) and Sch. T-strain (polymyxin).
[c] PG 21 used in all tests except for that employing Car. *M. hominis* strain (nitrofurantoin).
[d] Only the final MIC is given.

Erythromycin failed to eradicate the classical mycoplasmas from cervical cultures (24). Shepard et al. (28) and Csonka et al. (4) demonstrated eradication of T-strains from genital exudates in men and women with nongonococcal urethritis treated with tetracycline. No untreated controls were reported, but the treatment was accompanied by disappearance of the mycoplasma from the urine and urethra. Shepard also associated clinical failure with persistence, and relapse with reappearance of the T-strains (28). Shipley et al. noted resistance of T-strains to 200 μg/ml of lincomycin (30).

In the present study, the 12 strains of *M. homi-*

nis, 11 T-strains, and the single strain of *M. fermentans* showed narrow ranges of sensitivity for the antimicrobial agents tested. All three species were similar in their susceptibility to tetracycline and chloramphenicol and to the aminoglycoside drugs, kanamycin, gentamicin, and streptomycin, although *M. hominis* was less sensitive than the T-strains to streptomycin, and all were comparable in their resistance to ampicillin, cephalothin, cephaloridine, vancomycin, and polymyxin. It is of interest that strains of these organisms are susceptible to cephalosporin antibiotics despite their lack of cell wall. *M. hominis* was more sensitive than T-strains to nitrofurantoin.

Particularly striking are the differences in activity of lincomycin, clindamycin, and erythromycin. *M. fermentans* and *M. hominis* were sensitive to low concentrations of lincomycin but resistant to erythromycin, whereas T-strains were resistant to all but high concentrations of lincomycin, and sensitive to erythromycin. The three species were likewise differentially susceptible to clindamycin, which was approximately 100-fold more active against *M. hominis* and *M. fermentans* than against T-strains.

Should these differences persist when larger numbers of strains have been tested, the effects of using these antibiotics in differential media may be of value in separating the organisms from one another, particularly in specimens where mixtures of *M. hominis* and T-strains are encountered.

In these experiments, antimicrobial activity was consistent over a wide range of inocula. The range of susceptibility of the genital mycoplasmas was generally narrow for a given antimicrobial agent. Results of the assays would be expected to vary with the differences in the test system used, as is observed in the testing of bacterial susceptibility to antimicrobial agents (11). The magnitude of sensitivity of T-strains to erythromycin differs from that observed in previously published data, the final MIC being higher than was observed in two previous studies (29, 30), but not higher than levels observed in a third (31). It is difficult to compare all of these studies because the techniques are not described in detail. However, differences in *p*H, time of incubation, nature of the culture medium, size of inoculum, definition of end points for inhibition or killing, and, perhaps, differences in the activity of strains tested may account for the variations observed.

The susceptibility of the mycoplasmas at *p*H 6.0 and 7.4 followed expectation based on the activity of the drugs used against bacteria. Because the observations were made over a long period of incubation, deterioration of the drug may play a role when late metabolic changes are observed with a mycoplasmastatic agent. Effects of chlortetracycline with in vitro testing against mycoplasma (22), for example, might be difficult to interpret because of this agent's rapid deterioration in broth media.

The effect of different methods of testing was not great. "Microtiter" plates gave results comparable to those obtained in polystyrene tubes and are convenient when large numbers of determinations are carried out.

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