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 pH 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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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 pH 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 L-arginine hydrochloride, 10 mg. The broth was adjusted to pH 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 pH 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 pH 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 pH 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.

**Table 1. Source and characteristics of mycoplasma strains used in antibacterial assay**

| Mycoplasma* | Source | Titer in broth | Days required | Titer used in tests | Mycoplasma | Source | Titer in broth | Days required | Titer used in tests |
|-------------|--------|----------------|---------------|---------------------|-------------|--------|----------------|---------------|---------------------|
| *M. hominis* strains | | | | | | | | | |
| PG21 | NIH | 10^-7 | 3 | 10^-3 | | | | | |
| Cor. | Urine | 10^-9 | 2 | 10^-4 | | | | | |
| Col. | Urine | 10^-8 | 3 | 10^-4 | | | | | |
| Rey. | Urine | 10^-7 | 3 | 10^-3 | | | | | |
| Smi. | Urine | 10^-6 | 3 | 10^-2 | | | | | |
| Lay. | Urine | 10^-9 | 2 | 10^-4 | | | | | |
| Bro. | Urine | 10^-6 | 4 | 10^-2 | | | | | |
| Sim. | Urine | 10^-6 | 3 | 10^-2 | | | | | |
| Bar. | Urine | 10^-8 | 3 | 10^-4 | | | | | |
| Col. | Urine | 10^-8 | 3 | 10^-4 | | | | | |
| *M. fermentans* | | | | | | | | | |
| | Urine | 10^-6 | 6 | 10^-2 | | | | | |
* Except for the NIH strain, these are designated by the patient from whom obtained.
* Bacterial strains 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*.
* Days required for maximal color change to occur in the terminal dilution.
* Obtained from Sarabelle Madoff of the Massachusetts General Hospital.
* Maximum dilution of culture giving multiplication by tube dilution technique.
* Dilution of culture used as inoculum.

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).

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*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 pH 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% N₂ and 10% CO₂. The pH of culture media was determined colorimetrically against a set of PPLO broth standards for which pH had been determined with a glass electrode, and pH 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 mycoplasmalidal concentrations (MCC) of antimicrobial agents, strains of *M. hominis* were diluted in basic broth with 1% arginine at pH 6.0 to yield 10⁴ to 9 × 10⁶ color changing units (CCU) per 0.1 ml. *M. fermentans* was diluted in basic broth with 1% dextrose at pH 7.4 to yield 10⁴ to 9 × 10⁶ CCU, and T-strains were diluted to contain 10⁷ to 1 × 10⁹ CCU in basic broth with 0.05% urea adjusted to pH 6. One CCU was defined as the lowest inoculum of *M. hominis* or T-strain which produced characteristic rises in pH in arginine or urea broth, respectively, or a characteristic decrease in pH 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 pH by using, for comparison, uninoculated wells and wells containing organisms without antimicrobials.

The MCC 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 was 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 simultaneously 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. Tetracycline, lincomycin, erythromycin, gentamicin, vancomycin, and polymyxin were tested on a separate occasion. Nitrofurantoin was tested 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 mycoplasmalidal 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 10⁹ to 10⁶ CCU/ml of the strains of organisms 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)* | Final MIC (median and range)* |
|---------------------|---------------------------------|-------------------------------|
| 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)               |
| Cephalexin          | >1,000 (500-1,000)              | >1,000 (500-1,000)            |
| Ampicillin          | >1,000 (>1,000)                 | >1,000 (>1,000)               |

* 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)* | 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 (<0.2-0.8)                  | 0.4 (0.05-0.8)                |
| 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)             |
| Cephalexin          | 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)               |

* Median values followed by range values, in parentheses, expressed in micrograms per milliliter.

organisms and incubated in 90% nitrogen and 10% CO2. After 3 days of incubation, the MIC against 10^4 to 10^6 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 pH, one strain of M. hominis and one T-strain were tested against each drug at pH 6.0 and pH 7.4 in broth titrations. Organisms were used at a concentration of 10^4 to 10^6 CCU in 0.1 ml, and the two pH 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 (cephalexin, cephalexin, 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
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 10⁷ 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 10⁶ CCU of *M. hominis* were inhibited by 6.2 μg/ml of lincomycin, but 10 to 10⁶ 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 four-fold over the range of inocula, except when inocula containing 10⁷ to 10⁸ CCU were used. By using these large inocula, pH 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 10⁶ to 10⁷ 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 pH of the medium was determined for selected T-strains and strains of *M. hominis* at pH 6.0 and 7.4 (Table 6). Control cultures at these pH levels were inoculated with 10⁶ to 10⁷ 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 pH 7.4 than at pH 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. Mycoplasmalidal concentrations of kanamycin, streptomycin, and erythromycin for all three species were equal to or only twofold greater than the MIC. Polymyxin and cephaloridine were mycoplasma 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 Newham 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 4. Susceptibility of Mycoplasma fermentans* 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 single strain was used in the assay.*
| Drug                | T-strain<sup>a</sup> | M. hominis<sup>b</sup> | M. fermentans |
|---------------------|-----------------------|------------------------|--------------|
|                     | 10<sup>-1</sup> | 10<sup>-2</sup> | 10<sup>-3</sup> | 10<sup>-4</sup> | 10<sup>-5</sup> | 10<sup>-3</sup> | 10<sup>-4</sup> | 10<sup>-5</sup> |
| Kanamycin           | >100               | 6.2                   | 3.1          | 1.6          | 1.6          | 12.5         | 12.5         | 12.5         | 12.5         | 12.5         | 12.5         | 6.2           | 62           | 125          |
| Streptomycin        | >100               | 6.2                   | 3.1          | 1.6          | 1.6          | 12.5         | 12.5         | 12.5         | 12.5         | 12.5         | 12.5         | 6.2           | 62           | 125          |
| Chloramphenicol     | >6.2               | 6.2                   | 6.2          | 6.2          | 6.2          | 6.2          | 6.2          | 6.2          | 6.2          | 6.2          | 6.2          | ND<sup>4</sup> | 6.2          | 6.2          |
| Cephalothin         | >1,000             | 1,000                 | 1,000        | 1,000        | 1,000        | 500          | 500          | 500          | 500          | 500          | 500          | 500          | 500          | 500          |
| Cephaloridine       | >1,000             | >1,000                | >1,000       | >1,000       | >1,000       | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        |
| Nitrofurantoin      | 50                 | 50                    | 25           | 25           | 25           | 12.5         | 3.1          | 25           | 25           | 25           | 12.5         | 3.1          | 25           | 25           |
| Tetracycline        | >12.5              | 12.5                  | 12.5         | 12.5         | 12.5         | 1.6          | 1.6          | 0.8          | 0.8          | 0.8          | 0.8          | 0.4          | 0.04         | 0.02         |
| Lincomycin          | >1,000             | >1,000                | >1,000       | >1,000       | >1,000       | 500          | 500          | 500          | 500          | 500          | 500          | 500          | 500          | 500          |
| Erythromycin        | >1,000             | 12.5                  | 12.5         | 12.5         | 12.5         | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        |
| Gentamicin          | 100                | 50                    | 50           | 25           | 25           | 500          | 500          | 500          | 500          | 500          | 500          | 500          | 500          | 500          |
| Polymyxin           | >1,000             | >1,000                | >1,000       | >1,000       | >1,000       | 1,000        | 1,000        | 1,000        | 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        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        | 1,000        |
| Clindamycin         |                    |                      |              |              |              |              |              |              |              |              |              |              |              |              |

<sup>a</sup> T-strains used in these tests were T 960 (tetracycline, lincomycin, and erythromycin) and Sch. (kanamycin); broth at pH 6.0.

<sup>b</sup> PG 21.

<sup>c</sup> Dilution contained 10<sup>-10</sup> color changing units.

<sup>d</sup> 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<sup>a</sup> | M. hominis<sup>b</sup> |
|------------------------|----------------------|------------------------|
|                        | 6.0                  | 7.4                    | 6.0      | 7.4      |
| Streptomycin           | 3.1<sup>c</sup>      | 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      |
| Cephhalothin           | 500                  | 1,000                  | 500      | 1,000    |
| Ampicillin             | >1,000               | >1,000                 | >1,000   | >1,000   |
| Tetracycline           | 0.8                  | 3.1                    | 0.4      | 1.6      |

<sup>a</sup> T 960 employed in all tests except where Can. T-strain was used (cephaloridine, tetracycline, and polymyxin).

<sup>b</sup> PG 21 employed except where Cor. M. hominis was used (erythromycin and nitrofurantoin).

<sup>c</sup> Final MIC, expressed in micrograms per milliliter.

TABLE 7. Minimal inhibitory (MIC) and minimal mycoplasmacidal concentrations (MCC) of antimicrobials for selected genital mycoplasmas<sup>a</sup>

| Antimicrobial agent     | T-Strain<sup>b</sup> | M. hominis<sup>c</sup> | M. fermentans |
|------------------------|----------------------|------------------------|---------------|
|                        | MIC<sup>d</sup>      | MCC                    | MIC<sup>d</sup> | MCC     | MIC<sup>d</sup> | 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     | 0.16            | 0.16    |
| Clindamycin            | 25                   | 25                     | 25            | 25      | 50              | 100     |
| Kanamycin              | 3.1                  | 3.1                    | 50            | 100     | 250             | 500     |
| Streptomycin           | 1,000                | >1,000                 | 500           | >1,000  | 500             | 500     |
| Cephyhalothin          | 1,000                | 1,000                  | 1,000         | 1,000   | 1,000           | 1,000   |
| Ampicillin             | >1,000               | >1,000                 | >1,000        | >1,000  | 250             | 500     |
| Cephaloridine          | 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     |

<sup>a</sup> Values are expressed in micrograms per milliliter.

<sup>b</sup> T 960 used in all tests except for those employing Can. T-strain (nitrofurantoin) and Sch. T-strain (polymyxin).

<sup>c</sup> PG 21 used in all tests except for that employing Car. M. hominis strain (nitrofurantoin).

<sup>d</sup> 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. fer-
mentans 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 compara-
tive 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 sen-
tive than T-strains to nitrofurantoin.

Particularly striking are the differences in ac-
tivity of lincomycin, clindamycin, and erythromy-
icin. M. fermentans and M. hominis were sen-
tive to low concentrations of lincomycin but
resistant to erythromycin, whereas T-strains were
resistant to all but high concentrations of linco-
mycin, 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. fer-
mentans 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 suscepti-
bility 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 tech-
niques are not described in detail. However,
differences in pH, time of incubation, nature of the
culture medium, size of inoculum, definition of end
points for inhibition or killing, and, per-
haps, differences in the activity of strains tested
may account for the variations observed.

The susceptibility of the mycoplasmas at pH
6.0 and 7.4 followed expectation based on the ac-
tivity 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 mycoplasmasstatic agent. Effects of chlor-
tetracycline with in vitro testing against myco-
plasma (22), for example, might be difficult to in-
terpret because of this agent's rapid deterioration
in broth media.

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

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LITERATURE CITED

1. Chanock, R. M. 1965. Mycoplasma infections of man. N.
Engl. J. Med. 273:1199–1206, 1257–1264.
2. Chanock, R. M., L. Hayflick, and M. F. Barile. 1962. Growth
on artificial medium of an agent associated with atypical
pneumonia and its identification as a PPLO. Proc. Nat.
Acad. Sci. U.S.A. 48:41–49.
3. Clyde, W. A., Jr. 1964. Mycoplasma species identification
based upon growth inhibition by specific antisera. J. Im-
munol. 92:958–965.
4. Csonka, G. W., R. E. O. Williams, and J. Corse. 1966. T-strain
mycoplasma in non-gonococcal urethritis. Lancet 1:1292–
1295.
5. Csonka, G. W., R. E. O. Williams, and J. Corse. 1967.
T-strain mycoplasma in nongonococcal urethritis. Ann.
N.Y. Acad. Sci. 143:794–798.
6. Ford, D. K. 1962. Culture of human genital "T-strain"
pleuropneumonia-like organisms. J. Bacteriol. 84:1028–
1034.
7. Ford, D. K. 1967. Relationships between mycoplasma and
the etiology of non-gonococcal urethritis and Reiter's
syndrome. Ann. N.Y. Acad. Sci. 143:501–504.
8. Ford, D. K., and M. DuVernet. 1963. Genital strains of
human pleuropneumonia-like organisms. Brit. J. Vener.
Dis. 39:18–20.
9. Ford, D. K., and M. DuVernet. 1966. Antigenic types of
"large colony" human genital mycoplasmas. J. Bacteriol.
91:899.
10. Ford, D. K., G. Rasmussen, and J. Minken. 1968. T-strain
pleuropneumonia-like organisms as one cause of non-
gonococcal urethritis. Brit. J. Vener. Dis. 38:22–25.
11. Garrod, L. P., and F. O'Grady. 1968. Antibiotics and chemo-
therapy, 2nd ed., p. 475. The Williams and Wilkins Co.,
Baltimore.
12. Hayflick, L., and R. M. Chanock. 1965. Mycoplasma species
of man. Bacteriol. Rev. 29:185–221.
13. Ingham, H. R., W. V. MacFarlane, J. H. Hale, J. B. Selkon,
and A. A. Coid. 1966. Controlled study of the prevalence
of T-strain mycoplasmata in males with non-gonococcal
urethritis. Brit. J. Vener. Dis. 42:269–271.
14. Jao, R. L., and M. Finland. 1967. Susceptibility of Myco-
plasma pneumoniae to 21 antibiotics in vitro. Amer. J.
Med. Sci. 253:639–650.
15. Jones, D. M. 1967. Mycoplasma hominis in pregnancy. J.
Clin. Pathol. (London) 20:633–635.
16. Jones, D. M. 1967. Mycoplasma hominis in abortion. Brit.
Med. J. 1:338–340.
17. Kundin, R. B., S. G. Driscoll, and P. M. L. Ming. 1967.
24. Taylor-Robinson, D., R. H. Purcell, and R. M. Chanock. 1965. Exudative pharyngitis following experimental Mycoplasma hominis type 1 infection. Amer. Med. Ass. 192:1146-1152.

25. Purcell, E. M., S. S. Wright, T. W. Mou, and M. Finland. 1954. Blood levels and urinary excretion in normal subjects after ingestion of tetracycline analogues. Proc. Soc. Exp. Biol. Med. 85:61-65.

26. Shephard, M. C. 1954. The recovery of pleuropneumonia-like organisms from negro men with and without nongonococcal urethritis. Amer. J. Syphilis 38:113-124.

27. Shephard, M. C. 1967. Cultivation and properties of T-strains of mycoplasma associated with nongonococcal urethritis. Ann. N.Y. Acad. Sci. 143:505-514.

28. Shephard, M. C., C. E. Alexander, Jr., C. D. Lunceford, and P. E. Campbell. 1964. Possible role of T-strain mycoplasma in nongonococcal urethritis. J. Amer. Med. Ass. 188:729-735.

29. Shephard, M. C., C. D. Lunceford, and R. L. Baker. 1966. T-strain mycoplasma: selective inhibition by erythromycin in vitro. Brit. J. Vener. Dis. 42:21-24.

30. Shipley, A., S. J. Bowman, and J. J. O'Connor. 1968. T-strain mycoplasmas in non-specific urethritis. Med. J. Aust. 1:794-796.

31. Taylor-Robinson, D. 1967. Mycoplasma of various hosts and their antibiotic sensitivities. Postgrad. Med. J. 43:suppl. (March):100-104.

32. Taylor-Robinson, D., and R. H. Purcell. 1966. Mycoplasmas of the human urogenital tract and oropharynx and their possible role in disease: a review with some recent observations. Proc. Roy. Soc. Med. 59:1112-1116.

33. Tulley, J. G., M. S. Brown, J. N. Sheagren, V. M. Young, and S. M. Wolfe. 1965. Septicemia due to Mycoplasma hominis type 1. N. Eng. J. Med. 273:648-650.

34. Tulley, J. G., and G. Smith. 1968. Post-partum septicemia with Mycoplasma hominis. J. Amer. Med. Ass. 204:827-828.