IN-VITRO SENSITIVITY OF STRAINS OF MYCOPLASMAS FROM HUMAN SOURCES TO ANTIBIOTICS AND TO SODIUM AUROTHIOMALATE AND TYLOSIN TARTRATE

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THE sensitivity of mycoplasmas to antibiotics has become a subject of great interest as a result of the demonstration that at least one species is a human pathogen (Chanock, Hayflick and Barile, 1962) and the finding that tissue cultures are frequently infected with strains of these organisms. The present paper describes the results of an investigation of the sensitivity of 99 strains of mycoplasmas including *Mycoplasma pneumoniae*, *M. hominis*, *M. salivarium*, *M. orale* type 1 and *M. fermentans* to a range of antibiotics, and to sodium aurothiomalate and to tylosin tartrate.

MATERIAL AND METHODS

Organisms. The mycoplasmas tested were as follows: *Mycoplasma pneumoniae*, strain Hetter (FH) and one strain isolated from sputum; *M. hominis*, strain H50 obtained from Dr D. G. ff. Edward, Wellcome Research Laboratories, Beckenham, Kent, and one strain isolated from a pharyngeal swab; *M. salivarium*, 84 strains from sputum or a pharyngeal swab from different patients; *M. orale* type 1, 10 strains from sputum or a pharyngeal swab from different patients; *M. fermentans*, one strain obtained from Dr Edward.

Isolation and identification. Strains of mycoplasma were isolated from the respiratory tract by the methods described by Stewart and Chowdray (1968) and were typed by the growth-inhibition test of Clyde (1964).

Sensitivity tests

Sensitivity test medium. To 40 ml Difco PPLO agar base was added 12 ml unheated swine serum, 6 ml of a 25 per cent. low-temperature yeast extract (Marmion, 1967) and 6.5 ml of a solution of the drug (e.g., antibiotic) at ten times the final concentration required. Convenient volumes of the drug-medium mixture were poured into petri dishes 8.5 cm in diameter.

Antibiotics. The antibiotics and their final concentrations in the medium as number of µg per ml were as follows: ampicillin 250, methicillin 250, tetracycline 100, 25, 6.25, 1.5 and 0.4, kanamycin 100, 25, 6.25, 1.5 and 0.4, erythromycin 1000, 250, 62.5, 16 and 4, streptomycin 100, 25, 6.25, 1.5 and 0.4, chloramphenicol 100, 25, 6.25, 1.5 and 0.4, cephaloridine 100, 25, 6.25 and 1.5, lincomycin 100, 25, 6.25, 1.5 and 0.4, sodium aurothiomalate 100, 25, 6.25, 1.5 and 0.4, and tylosin tartrate 1000, 100, 25, 6.25, 1.5 and 0.4.

Inoculum. One loopful of a 5-day-old culture of the mycoplasma in semi-solid agar (9 volumes of Difco PPLO broth and 1 volume of Difco PPLO agar base supplemented as for the sensitivity test medium) was streaked over a quarter of each plate in the series containing the drugs under test.

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Incubation. The plates were incubated at 37°C in air containing 10 per cent. of CO₂ and observed for the presence or absence of growth with a microscope at $\times 40$ magnification after 5, 8 and 11 days.

Controls. As a control of the inoculum size each organism was inoculated on to a plate of sensitivity test medium containing sterile distilled water instead of drug solution. The tests were considered satisfactory only if confluent growth or innumerable discrete colonies developed on the control plate.

Readings. The results have been reported as the minimum inhibitory concentrations, that is, the smallest concentrations of the drugs that completely inhibited growth. Since no change occurred in the end-point after 8 days' incubation, an 8-day reading has been used.

TABLE I

Sensitivity of strains of Mycoplasma pneumoniae, M. hominis and M. fermentans to antibiotics and sodium aurothiomalate in vitro

Drug	Minimum inhibitory concentration of drug (μ g per ml) for				
	<i>M. pneumoniae</i>		<i>M. hominis</i>		<i>M. fermentans</i> (one strain)
	strain Hetter (FH)*	strain P1546	strain H50	strain P3485	
Ampicillin	>250	>250	>250	>250	>250
Cephaloridine	100	...	...	...	>100
Chloramphenicol	1.5	0.4	6.25	1.5	1.5
Erythromycin	<4.0	<4.0	250	62.5	1000
Kanamycin	6.25	25	6.25	1.5	6.25
Lincomycin	25	25	...	0.4	0.4
Methicillin	>250	>250	>250	>250	>250
Streptomycin	25	6.25	>100	25	6.25
Tetracycline	1.5	1.5	1.5	0.4	6.25
Sodium aurothiomalate	>100	>100	100	6.25	25

* Mean of 6 tests. ... Not tested.

RESULTS

The results of the sensitivity tests on 2 strains of *Mycoplasma pneumoniae*, 2 strains of *M. hominis* and 1 strain of *M. fermentans* are shown in table I. Table II gives the results of tests on 84 strains of *M. salivarium* and table III the results on 10 strains of *M. orale* type 1.

Both strains of *M. pneumoniae* were sensitive to tetracycline, chloramphenicol and erythromycin. The strains of *M. hominis* and *M. fermentans* were sensitive to tetracycline, chloramphenicol, lincomycin and kanamycin. The degree of resistance of strains of *M. salivarium* was very variable. Many strains were resistant to high concentrations of the substances tested, including tetracycline and tylosin tartrate, although in the case of tylosin the growth on the higher concentrations was not as heavy as on the control plate. The strains of *M. orale* type 1 also showed some variation in the degree of their resistance to some of the drugs, but they were all sensitive to lincomycin and to low concentrations of tylosin tartrate.

DISCUSSION

The only species of *Mycoplasma* of undisputed pathogenicity for man is *Mycoplasma pneumoniae*, which has been shown to be associated with both

TABLE II

Sensitivity of strains of Mycoplasma salivarium to antibiotics, sodium aurothiomalate and tylosin tartrate

Drug	Number of strains tested	Number of strains with minimum inhibitory concentration (μg per ml)					
		0.4	1.5	6.25	25	100	>100
Ampicillin . . .	84	0	0	0	0	0	84*
Cephaloridine . . .	18	0	0	0	0	1	17
Chloramphenicol . . .	84	3	9	44	6	3	19
Erythromycin . . .	84	0	0	0	0	5	79
Kanamycin . . .	84	0	6	48	10	2	18
Lincomycin . . .	33	2	19	11	1	0	0
Methicillin . . .	84	0	0	0	0	0	84*
Streptomycin . . .	84	0	0	2	5	35	42
Tetracycline . . .	84	3	28	27	5	6	15
Sodium aurothiomalate . . .	84	2	4	8	22	26	22
Tylosin tartrate . . .	42	3	10	17	0	4	8

* All strains insensitive to ampicillin and methicillin at 250 μg per ml.

TABLE III

Sensitivity of strains of Mycoplasma orale type 1 to antibiotics, sodium aurothiomalate and tylosin tartrate in vitro

Drug	Number of strains tested	Number of strains with minimum inhibitory concentration (μg per ml)					
		0.4	1.5	6.25	25	100	>100
Ampicillin . . .	10	0	0	0	0	0	10*
Cephaloridine . . .	3	0	0	0	0	0	3
Chloramphenicol . . .	10	0	3	6	1	0	0
Erythromycin . . .	10	0	0	0	0	0	10
Kanamycin . . .	10	0	4	4	1	0	1
Lincomycin . . .	7	2	5	0	0	0	0
Methicillin . . .	10	0	0	0	0	0	10*
Streptomycin . . .	10	0	0	0	0	7	3
Tetracycline . . .	10	0	0	7	3	0	0
Sodium aurothiomalate . . .	10	0	1	3	2	3	1
Tylosin tartrate . . .	4	4	0	0	0	0	0

* All strains insensitive to ampicillin and methicillin at 250 μg per ml.

upper and lower respiratory tract infections (Chanock, Mufson *et al.*, 1962). The organism has been shown to be sensitive *in vitro* to low concentrations of tetracycline, chloramphenicol, erythromycin and streptomycin by Jao and

Finland (1967), and our findings agree with these results. It has been found that respiratory infections associated with *M. pneumoniae* respond more rapidly to tetracycline than to placebo tablets (Chanock *et al.*, 1963) and that clinical improvement also occurs during treatment with erythromycin (Smith, Friedewald and Chanock, 1967). These therapeutic results are in keeping with the results of the in-vitro sensitivity tests.

Mufson *et al.* (1967) found evidence of infection with *M. hominis* in 3 per cent. of patients with pneumonia as compared with only 0.3 per cent. of patients without respiratory disease. From the results of our sensitivity tests and those of Taylor-Robinson (1967), tetracycline appears to be the drug of choice for *M. hominis* as well as *M. pneumoniae* infections. Our 2 strains of *M. hominis* were sensitive only to high concentrations of erythromycin.

It is possible that T-strain mycoplasmas may be associated with non-specific urethritis (Shepard, 1956). These organisms were shown by Shepard, Lunceford and Baker (1965) to be sensitive to erythromycin as well as to tetracycline and streptomycin. Shipley, Bowman and O'Connor (1968) recently confirmed that T-strains are sensitive to erythromycin and tetracycline, but reported that all of 5 strains were resistant to 5 and 10 μ g streptomycin per ml.

In agreement with other reports, all the mycoplasmas tested were resistant to ampicillin and methicillin. None of the strains was sensitive to less than 100 μ g cephaloridine per ml. This finding is in agreement with that of Fallon and Hutchinson (1967).

It has previously been shown (Findlay, MacKenzie and MacCallum, 1940; Goodburn and Marmion, 1962*a* and *b*) that strains of mycoplasma are sensitive to gold salts in mice, hamsters and chick embryos. It is difficult to explain why many strains in the present series and in that of Robinson, Wichelhausen and Brown (1952) were resistant to sodium aurothiomalate *in vitro*. The in-vitro results may have been influenced by the size of inoculum, since this was shown by Davidson and Thomas (1966) to affect the minimum inhibitory concentration.

The presence of mycoplasmas in tissue cultures has proved troublesome to virologists because it reduces the sensitivity of cell lines to viruses (Rouse, Bonifas and Schlesinger, 1963). The eradication of the mycoplasmas from the cultures has often proved difficult. A number of antibiotics and sodium aurothiomalate have been used. The failure to free the tissue cultures from mycoplasmas may in many instances have been due to resistance of the infecting mycoplasma to the drug used. The results of sensitivity tests in the present series and the reports of Friend, Patuleia and Nelson (1966) and Cross, Goodman and Shaw (1967) suggest that tylosin may prove to be the most useful of the agents so far available, although a few strains of mycoplasma seem to contain a small percentage of organisms resistant to it.

SUMMARY

The results of in-vitro sensitivity tests of strains of *Mycoplasma pneumoniae*, *M. hominis*, *M. fermentans*, *M. salivarium* and *M. orale* type 1 to ampicillin, cephaloridine, chloramphenicol, erythromycin, kanamycin, lincomycin, methicillin, streptomycin, tetracycline, sodium aurothiomalate and tylosin tartrate

are reported. They confirm that *M. pneumoniae* and *M. hominis* are sensitive to tetracycline and chloramphenicol, but show that a number of strains of *M. salivarium* and *M. orale* type 1 are resistant to these drugs. Some of these strains were also resistant to kanamycin, but most were sensitive to tylosin tartrate.

All the mycoplasmas were resistant to ampicillin and methicillin, and to cephaloridine at concentrations of 25–100 μg per ml. Strains of *M. pneumoniae* were sensitive to erythromycin, but most of the other strains were resistant. The mycoplasmas were very variable in their sensitivity to sodium aurothiomalate.

It is suggested that tylosin tartrate is the agent most likely to prove useful for eradicating mycoplasmas from contaminated tissue cultures.

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