ANTIMICROBIAL SUSCEPTIBILITY

Comparative susceptibilities of various AIDS-associated and human urogenital tract mycoplasmas and strains of *Mycoplasma pneumoniae* to 10 classes of antimicrobial agent *in vitro*

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The susceptibilities of 40 strains of various *Mycoplasma* species to 10 classes of antimicrobial agents were compared *in vitro* by a broth microdilution method. The strains tested comprised 20 strains of four AIDS-associated species – *M. penetrans* (1 strain), *M. fermentans* (5 strains), *M. pirum* (6 strains) and *M. genitalium* (8 strains) – nine strains of the urogenital tract species *M. hominis* and 11 strains of *M. pneumoniae*. The results demonstrated wide variation in the susceptibilities of the different *Mycoplasma* spp. to different classes of antimicrobial agent. All the mycoplasmas were susceptible or highly susceptible to the fluoroquinolones, with sparfloxacin the most active, and to the diterpine antibiotic tiamulin. *M. pneumoniae* and *M. genitalium* strains were also highly susceptible to the macrolides, particularly azithromycin and had similar antibiotic susceptibility patterns to most other antimicrobial agents. However, all strains of *M. genitalium* were resistant to streptomycin (MIC 250–>500 mg/L) whereas all *M. pneumoniae* isolates, except the MAC strain, were susceptible (MICs 1.25–12.5 mg/L). *M. pirum* isolates varied considerably in their susceptibility to macrolides (MIC range *versus* azithromycin 0.0025–>100 mg/L). *M. fermentans* strains were susceptible to the tetracyclines, lincosamides and mupirocin, but varied in susceptibility to aminoglycosides. Most *M. hominis* strains were susceptible to the tetracyclines and all were susceptible to clindamycin and mupirocin. *M. penetrans* GTU 54 was susceptible to azithromycin, the tetracyclines and lincosamides as well as to the fluoroquinolones and tiamulin.

Introduction

Over the past decade various species of *Mycoplasma* have been cultured from or detected in body fluids or tissues from AIDS patients or HIV-seropositive individuals. Strains of *M. fermentans*, *M. penetrans* and *M. pirum* have all been cultured during such studies [1–6] and *M. genitalium* has been detected by the polymerase chain reaction [5], leading to speculation that they might act as co-factors in the pathogenesis of AIDS.

The highly fastidious urogenital tract species *M. genitalium* has also been isolated in culture from the throats of non-AIDS patients in association with *M.*

pneumoniae [7], although it is unclear whether *M. genitalium* plays any role in respiratory disease. However, as *M. genitalium* and *M. pneumoniae* share many properties – including the presence of an organised tip attachment structure, similar biochemical characteristics, adherence capabilities and serological cross-reactivities – differentiation in the clinical laboratory is difficult [8, 9]. Attempts to detect antibiotics with selective activity against one or other of these species, which could aid in their differentiation, have so far been unsuccessful [8].

The potential importance of mycoplasmas in AIDS, their role in other human infections and their involvement as cell-culture contaminants has led to studies of the antimicrobial susceptibilities of some of these species [8, 10–25]. This report extends those investigations, compares the antimicrobial susceptibilities of *M. genitalium* and *M. pneumoniae* strains and

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includes MIC data on recent isolates of *M. hominis* from human infections.

Materials and methods

Mycoplasma cultures

The type strains *M. fermentans* PG 18 (NCTC 10117), *M. hominis* PG21 (NCTC 10111), *M. pirum* HRC 70-159 (NCTC 11702), *M. pneumoniae* FH (NCTC 10119), *M. genitalium* G37 (NCTC 10195) and *M. penetrans* GTU 54 (ATCC 55252) were obtained either from the National Collection of Type Cultures (NCTC), 61 Colindale Avenue, London or the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852, USA.

M. fermentans incognitus was supplied by Dr S-C. Lo (Department of Defense, Armed Forces Institute of Pathology, Washington, DC, USA) and was isolated during transfection studies with DNA extracted from Kaposi's sarcoma tissue from an AIDS patient [1]. *M. fermentans* strain nos. 5 and 29, isolated in cell-free medium from urine sediments of AIDS patients [3], were obtained from Dr R. B. Kundsins (Brigham Women's Hospital, Harvard Medical School, Boston, MA, USA). Professor L. Montagnier (Institute Pasteur, Paris, France) kindly supplied *M. fermentans* strain AOU which was cultured from peripheral blood mononuclear cells (PBMCs) purified from the blood of an AIDS patient [2].

Six recent isolates of *M. hominis* (M443/90, M411/90, M286/93, M329/92, M265/92 and M680/90) from infected hospitalised patients in the UK, and strain H27 isolated from the urethra of a male with acute Reiter's syndrome in 1962 were supplied by Dr D. Pitcher (Central Public Health Laboratory, 61 Colindale Avenue, London). Dr J. Fabbri (Kantonsspital, Basel, Switzerland) supplied *M. hominis* strain Basel isolated from a Swiss patient with pneumonia. Five previously documented strains of *M. pirum* isolated from PBMCs from AIDS patients (COL, BER, RAO, VUC and NOU) [5] were supplied by Dr O. Grau (Institute Pasteur) and strain ICRF (UK cell culture isolate) by Helena M. Windsor (Mycoplasma Experience Ltd, Reigate, Surrey). These strains were tested against a wide selection of antimicrobial agents. Dr J. G. Tully (Mycoplasma Section, NIAID, Frederick Cancer Research Facility, Frederick, MD, USA) provided four additional American (1901672, 1890758, 1902677 and FD870) and one British (MAD-T) lyophilised cell-culture isolates of *M. pirum*, identified and described previously [26]. These strains were used only to confirm the macrolide susceptibilities of *M. pirum* cell-culture isolates.

Low passage isolates of *M. pneumoniae* (strains M 192/77 and M 195/77) from patients with primary atypical pneumonia (PAP) were supplied by Dr R.

Leach (Mycoplasma reference Facility, NCTC, Central Public Health Laboratory, 61 Colindale Avenue, London). Dr D. Pitcher supplied a further five strains of *M. pneumoniae* (M 336/81, M163/95, M 113/95, M 167/95 and M 1150/96) and Dr G. Taylor (Clinical Research Centre, Northwick Park Hospital, Harrow), strain 427a. *M. pneumoniae* strains MAC (ATCC 15492) and 1428 (ATCC 29085) were obtained from the ATCC. All these strains were tested for susceptibility to a wide range of antimicrobial agents. Additional *M. pneumoniae* strains M 289/67, M 129-B7, M 129-B170 and M 36/92 (supplied by Dr D. Pitcher) and M 950/81, M 1598/81, M 2659/82, M 191/83 (obtained from Dr M. Sillis, Virology Department, Public Health Laboratory, Bowthorpe Road, Norwich) and UTMB-10P (ATCC 49894) supplied by the ATCC, were tested only to confirm their susceptibilities to streptomycin. *M. genitalium* strain UTMB-10G (ATCC 49899) which was isolated in mixed culture with *M. pneumoniae* UTMB-10P from the synovial fluid from a patient with PAP and subsequent polyarthritis [27] was obtained, freeze-dried, from the ATCC. *M. genitalium* strains M2288, M2300, M2321 and M2341, cited and identified previously [28], were supplied by Dr J. S. Jensen (Mycoplasma Laboratory, Neisseria Department, Statens Serum Institut, DK 2300, Copenhagen, Denmark) and were isolated by cell culture from urethral swabs from Danish men with urethritis. *M. genitalium* strains M30 and 12959 (a low passage strain of the G37 strain) were isolated from the urethras of male patients with non-gonococcal urethritis by Dr P. M. Furr (St Mary's Hospital, Paddington, London).

The identities of the *M. fermentans*, *M. hominis*, *M. pneumoniae* and *M. pirum* strains were confirmed serologically by the growth inhibition test [29] with species-specific antisera. Antisera to *M. genitalium* G37 inhibited the reference strain and all the donated *M. genitalium* strains, but showed some cross-reaction with *M. pneumoniae* strains FH and 1428. *M. penetrans* GTU 54, for which no growth inhibiting antiserum was available, was used within three in-vitro passages after recovery from the freeze-dried state. All strains were cultured on inhibitor-free mycoplasma agar and were found to be free from bacterial contamination.

Mycoplasma culture media

A complete, commercially available, quality controlled, liquid medium (Mycoplasma Experience Ltd) containing serum and fresh yeast extract and either glucose 0.1% w/v at pH 7.6 for the glucose fermenting mycoplasmas (*M. fermentans*, *M. penetrans*, *M. pirum*, *M. pneumoniae* and *M. genitalium*) or arginine 0.1% w/v at pH 6.8 for *M. hominis* (arginine cataboliser) and phenol red 0.004%, was used in the cultivation of the mycoplasmas and in MIC tests.

Growth of the glucose-fermenting mycoplasmas was denoted by a change in colour of the medium from pink to orange/yellow and that of *M. hominis* from orange/yellow to pink.

Preparation of stock cultures

All mycoplasma strains were grown aerobically at 36°C in mycoplasma broth in sterile plastic 5-ml screw-capped vials until an acid or alkaline colour shift occurred. After addition of sterile glycerol 5% w/v the cultures were dispensed in 1-ml aliquots and frozen at -70°C. These were used to initiate further broth cultures, which, after the addition of 4 ml of fresh broth medium and incubation until a definite colour change occurred, were titrated in sealed microdilution trays to determine the number of colour changing units (ccu) after a recorded period of incubation. Replication of this procedure allowed the preparation of inocula which were then further diluted in mycoplasma broth to give between 10^3 and 10^5 ccu/ml for use in MIC tests.

Sources and preparation of antimicrobial agents

With the exception of the following, all compounds were supplied by Sigma. Sparfloxacin (technical grade) and azithromycin (technical grade) were supplied by Rhône Poulenc Rorer, Paris, France and Pfizer Central Research, Sandwich, Kent, respectively. Tiamulin hydrogen fumarate was supplied by Sandoz, and mupirocin lithium salt and clarithromycin by Smith Kline Beecham. Sterile solutions of ciprofloxacin (Ciproxin, Bayer) and netilmicin (Netillin, Schering-Plough) were purchased and diluted for use in MIC tests. Most of the compounds were dissolved in de-ionised water to achieve a concentration of 1 mg/ml and were sterilised by membrane filtration (0.2 µm pore size). The aminoglycosides were prepared at a concentration of 4 mg/ml. Erythromycin, clarithromycin and azithromycin were dissolved in aqueous ethanol 10% v/v. Ofloxacin and sparfloxacin were first dissolved in sterile 0.1 N NaOH and were then made up to volume with de-ionised water before sterilisation. Further compound dilutions were prepared in sterile plastic or glass vials at double the required concentration in mycoplasma broth to allow for a 1:1 dilution by the mycoplasma inoculum in MIC tests.

The dilution series of antimicrobial agents used included both 10-fold and doubling dilutions to minimise dilution errors and to reduce excessive compound 'carry over'. The dilution ranges used to test particular mycoplasma-antimicrobial combinations varied and were selected either with prior knowledge of the likely MIC or after siting experiments. Thus, *M. fermentans* strains, some of which have been shown previously to be resistant to aminoglycosides [11], were tested against a range of concentrations for this class of compound from 500 to 0.25 mg/L,

whereas *M. pneumoniae* strains, which are known to be highly sensitive to macrolides [8], were tested against a much lower range, e.g., 1–0.0001 mg/L, as were the *M. genitalium* strains. Other dilution ranges used for various mycoplasma-antimicrobial combinations were 100–0.1 mg/L and 10–0.01 mg/L.

MIC tests

MIC tests were performed in 96-well, microdilution sensititre plates by a modification of the method described by Tanner and Wu [30]. The compound dilutions were mixed with an equal volume (100 µl) of the challenge inocula containing 10^3 – 10^5 ccu/ml in microtitration wells. Each sensititre plate contained uninoculated medium as a sterility control, medium at pH 6.8 or pH 7.6 as end-point controls (depending on whether the pH shift was to an acid or alkaline reaction) and drug-free inoculated growth controls. To confirm the challenge inoculum titres, 100 µl of dilutions of the challenge inoculum (10^1 – 10^5) were added to 100 µl of medium in sensititre wells. The plates were then sealed with adhesive film (Falcon) and were incubated at 36°C until the colour in the growth control well matched that of the end-point control. The incubation periods varied according to the *Mycoplasma* species, the faster growing species taking 2–3 days to cause a colour change and the slower growers such as *M. genitalium* up to 7 days. Initial MICs were recorded when the colour of the growth control matched that of the end-point control, the MIC being the lowest drug concentration to show no change in colour. Final MICs were recorded 7 days later and were the lowest drug concentration in which no colour change was seen. Incubation of the plates containing dilutions of the challenge inocula was continued until colour changes were complete. If MIC end-points were not achieved, or if the mycoplasma challenge fell outside 10^3 – 10^5 ccu/ml, tests were repeated.

Results

The in-vitro susceptibilities of the six mycoplasma species examined to 10 classes of antimicrobial agents are shown in Table 1. Sparfloxacin and the veterinary antibiotic tiamulin showed the broadest spectra of anti-mycoplasma activities with MICs of sparfloxacin ranging from 0.0025–0.1 mg/L against *M. pneumoniae* to 0.1–0.5 mg/L against strains of *M. genitalium*. MICs for tiamulin ranged from 0.0025–0.01 mg/L against *M. genitalium* to 0.025–0.1 mg/L against *M. fermentans*.

The macrolides were particularly active *in vitro* against *M. genitalium* and *M. pneumoniae* strains, azithromycin being the most active of this class of compound (initial MICs against *M. genitalium* 0.0005–0.001 mg/L and against *M. pneumoniae* 0.001–0.005 mg/L). The macrolides were relatively

Table 1. In-vitro susceptibilities of AIDS-associated mycoplasmas and *M. pneumoniae* and *M. hominis* strains to 10 classes of antimicrobial agents (MIC* mg/L)

Antimicrobial agent	AIDS - associated											
	<i>M. penetrans</i> (GTU 54)			<i>M. fermentans</i> (n = 5, range)			<i>M. pirum</i> (n = 6, range)			<i>M. genitalium</i> (n = 8, range)		
	Initial†	Final†	Initial	Initial	Final	Initial	Initial	Final	Initial	Final	Initial	Final
Aminoglycosides												
Netilmicin	125	>500	2.5-500	125-500	>500	12.5-25	10-100	25-125	12.5-25	25-125	0.125-1.25	1.25-12.5
Gentamicin	50	125	2.5-500	50-500	125-500	10-25	10-25	50-100	2.5-12.5	6.25-62.5	2.5-12.5	5-50
Kanamycin	125	500	5-500	500-500	25-500	10-25	10-25	25	12.5-25	25-125	1.25-12.5	12.5-250
Neomycin	125	250	2.5-500	125-500	25-500	12.5-50	12.5-50	12.5-125	25-125	25-500	25-12.5	50-250
Tobramycin	25	250	50-250	125-250	25-500	25-100	25-100	50-100	25-62.5	62.5-125	2.5-125	12.5-50
Streptomycin	12.5	25	5-500	12.5-500	25-500	250-500	250-500	250-500	2.5-125	5-500	25-50	25-500
Aminocyclitol												
Spectinomycin	5	50	0.5-5	2.5-5	2.5-10	1-5	2.5-25	2.5-25	1-2.5	2.5-10	1-2.5	1-10
Macrolides												
Erythromycin	1	1	10-100	50-100	2.5-100	0.005-0.1	0.005-0.1	0.01-0.1	0.01-0.025	0.025-0.1	50-100	>100
Clarithromycin	0.5	0.5	2.5-25	50-100	0.1-100	0.005-0.025	0.005-0.025	0.01-0.025	0.01-0.025	0.025-0.1	25-100	50-100
Spiramycin	2.5	50	0.1-5	0.5-100	5-100	0.001-0.1	0.001-0.1	0.001-0.25	0.05-0.25	0.25-0.5	5-100	25-100
Azithromycin	0.025	0.1	0.1-10	0.0025-100	0.025-100	0.0005-0.001	0.0005-0.001	0.0005-0.005	0.001-0.005	0.0025-0.01	5-50	50-100
Fluoroquinolones												
Ciprofloxacin	0.1	0.25	0.025-0.05	0.05-0.25	0.5-1	0.25-10	0.25-10	0.5-25	0.025-1	0.025-2.5	0.05-0.5	0.05-2.5
Oxofloxacin	0.05	0.25	0.05-0.1	0.1-0.5	0.25-0.5	1-10	1-10	1-25	0.025-2.5	0.025-2.5	0.005-0.025	0.05-1
Sparfloxacin	0.05	0.05	0.005	0.025-0.1	0.05-0.25	0.1-0.5	0.1-0.5	0.25-1	0.0025-0.1	0.01-0.5	0.005-0.025	0.005-0.1
Tetracyclines												
Tetracycline HCl	0.25	2.5	0.05-0.25	0.5-1	0.5-10	0.25-2.5	0.25-2.5	2.5-10	0.05-0.25	0.5-5	0.025-2.5	0.1-100
Doxycycline HCl	0.25	1	0.1-0.25	0.5-1	0.5-10	0.25-2.5	0.25-2.5	1-10	0.05-0.25	1-5	0.005-2.5	0.05-25
Minocycline HCl	0.5	2.5	0.05-0.1	0.5-1	1-5	0.25-2.5	0.25-2.5	1-10	0.1-0.5	2.5-10	0.005-2.5	0.05-25
Lincosamides												
Lincomycin	0.25	50	0.05-0.5	0.5-5	25-50	2.5-50	2.5-50	10-25	5-10	25	0.1-1	1-10
Clindamycin	0.1	0.25	0.01-0.5	0.25-1	25-50	2.5-5	2.5-5	2.5-10	1-5	5-25	0.01-0.25	0.05-1
β-lactams												
Ampicillin	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
Others												
Chloramphenicol	2.5	2.5	0.5-2.5	0.5-25	0.5-25	0.5-5	5-25	10-25	2.5-10	5-25	0.5-5	5-25
Tiamulin	0.01	0.05	0.025-0.1	0.1	0.1	0.0025-0.01	0.0025-0.01	0.005-0.01	0.005-0.05	0.025-0.1	0.0025-0.025	0.0025-0.1
Mupirocin	0.25	5	0.01-0.025	0.25	10-25	0.25-1	0.25-1	0.5-2.5	0.5-1	2.5-5	0.025	0.025-0.5

*Tanner and Wu method [30].

†Initial MICs were recorded when the colour change of the growth control matched that of the end-point control well; final MICs were recorded 7 days later.

inactive against the other *Mycoplasma* species tested; some strains of *M. pirum*, *M. hominis* and *M. fermentans* were resistant to ≥ 100 mg/L of these agents. *M. pirum* strains isolated from PBMCs purified from the blood of French AIDS patients were highly resistant to erythromycin, clarithromycin, spiramycin and azithromycin, whereas the type strain and another tissue culture cell isolate (ICRF) were sensitive. MIC tests on additional American and British *M. pirum* strains isolated from contaminated tissue culture cells revealed wide variation in susceptibility to macrolides (Table 2).

The tetracyclines showed good to moderately good in-vitro activity against *M. penetrans* GTU-54 (initial MIC range 0.25–0.5 mg/L), *M. fermentans* (initial MIC range 0.05–0.25 gm/L), *M. pneumoniae* (initial MIC range 0.05–0.5 mg/L) and *M. hominis* (initial MIC range 0.005–25 mg/L), but were slightly less effective against *M. genitalium* (initial MIC range 0.25–2.5 mg/L) (Table 1). *M. hominis* strain Basel was moderately resistant to tetracycline HCl (MIC 25 mg/L).

The aminoglycosides were mainly inactive *in vitro* against the majority of the AIDS-associated and urogenital tract mycoplasmas tested. Some strains of *M. fermentans*, *M. pirum* and *M. penetrans* showed overall aminoglycoside resistance of 125–>500 mg/L. The type strain of *M. fermentans* (PG-18) and two strains of *M. fermentans* isolated from urine deposits from AIDS patients without the use of cell cultures were – with the exception of tobramycin – aminoglycoside-sensitive; two other AIDS isolates (*M. fermentans* strains incognitus and AOU), obtained from cultured cells, were aminoglycoside resistant (Table 3). All strains of *M. genitalium* were highly resistant to streptomycin (initial MIC range 250–>500 mg/L) irrespective of whether cell cultures were used in the isolation process, whereas with the exception of *M. pneumoniae* strain MAC (initial MIC 125 mg/L), all the *M. pneumoniae* strains were streptomycin-sensitive (initial MIC range 2.5–12.5 mg/L). MIC tests on a further nine *M. pneumoniae* strains, including strain UTMB-10P which was isolated in mixed culture with *M. genitalium* UTMB-10G from an arthritic joint of a patient with PAP, substantiated these findings (Table 4).

Clindamycin showed good in-vitro activity against *M. hominis* (initial MICs 0.01–0.25 mg/L) and *M. fermentans* strains (initial MICs 0.01–0.5 mg/L) (Table 1). Final MICs of most of the compounds examined were generally 2–10-fold higher than the initial MICs, suggesting that most classes of antimicrobial agent were predominantly mycoplasmastatic. However, the degree of difference between the initial MICs and the final MICs varied from compound to compound, both within particular classes of antibiotic and between the different antibiotic classes themselves

Table 2. In-vitro susceptibilities of AIDS-associated and cell culture isolates of *M. pirum* to four macrolides: MIC* (mg/L) versus *M. pirum* strains

Macrolide	Type strain HRC 70-159 (NCTC 11702)		AIDS-associated French isolates (n = 5 [†] , range)				American cell culture isolates				British cell culture isolates			
			1901672		1890758		1902677		FD870		MAD-T		ICRF	
	Initial [‡]	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Erythromycin	2.5	10	>100	>100	>100	>100	>100	>100	1	1	≤0.01	≤0.01	2.5	10
Clarithromycin	0.5	1	50–>100	>100	>100	>100	>100	>100	0.05	0.05	≤0.01	≤0.01	0.25	0.5
Spiramycin	5	25	≥100	>100	25	100	10	50	0.25	2.5	0.1	0.5	5	100
Azithromycin	0.005	0.025	50–>100	>100	>100	>100	100	>100	≤0.01	≤0.01	≤0.01	<0.01	≤0.01	0.1

*Tanner and Wu method [30].
[†]Strains COL, NOU, RAO, VUC, BER.
[‡]Initial MICs were recorded when the colour change of the growth control matched that of the end-point control well; final MICs were recorded 7 days later.

Table 3. The susceptibilities of four AIDS-associated strains of *M. fermentans* to six aminoglycoside antibiotics compared with that of the type strain PG18 (MIC* mg/L)

Aminoglycoside	Isolated in cell-free media						Isolated with cultured cells			
	Type strain PG-18		Strain 5 from AIDS urine		Strain 29 from AIDS urine		AIDS Kaposi's sarcoma DNA incognitus		AIDS PBM cells [†] AOU	
	Initial [‡]	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Netilmicin	2.5	50	12.5	125	12.5	125	>500	>500	>500	>500
Gentamicin	2.5	12.5	12.5	25	12.5	25	>500	>500	>500	>500
Kanamycin	5	25	12.5	25	12.5	25	>500	>500	>500	>500
Neomycin	25	50	50	125	50	125	>500	>500	>500	>500
Tobramycin	50	125	125	250	125	250	250	250	125	250
Streptomycin	5	12.5	12.5	>500	12.5	>500	>500	>500	500	>500

*Tanner and Wu method [30].
[†]PBMC cultures from the blood of an AIDS patient.
[‡]Initial MICs were recorded when the colour change of the growth control matched that of the end-point control well; final MICs were recorded 7 days later.

Table 4. In-vitro susceptibilities of 11 *M. pneumoniae* and two *M. genitalium* strains to streptomycin

Strain	MIC* (mg/L)	
	Initial [†]	Final
<i>M. pneumoniae</i>		
Type strain FH (NCTC 10119)	12.5 [‡]	25 [‡]
MAC (ATCC 15492)	125.0 [‡]	500 [‡]
Additional strains		
M129-B7 (ATCC 29342)	1.25	2.5
M129-B170 (ATCC 29343)	2.5	12.5
M289/67	2.5	12.5
M950/81	2.5	5
M1598/82	2.5	5
M191/83	2.5	5
M36/92	1.25	5
M2659/82	2.5	5
UTMB-10P (ATCC 49894)	2.5 [‡]	12.5 [‡]
Range (additional strains)	1.25–2.5	2.5–12.5
<i>M. genitalium</i>		
Type strain G37 (NCTC 10195)	>500 [‡]	>500 [‡]
UTMB-10G (ATCC 49899)	≥500 [‡]	≥500 [‡]

*Tanner and Wu method [30].
[†]Initial MICs were recorded when the colour change of the growth control matched that of the end-point control well; final MICs were recorded 7 days later.
[‡]Results of duplicate tests.

and was influenced by the *Mycoplasma* sp. under investigation. The fluoroquinolones, which are known to be mycoplasmacidal *in vitro* [31], had final MIC values only slightly higher than the initial MICs.

Discussion

Although *M. pneumoniae*, *M. fermentans* and *M. hominis* have been studied extensively, there are few reports on the antimicrobial susceptibilites of *M. penetrans*, *M. pirum* and *M. genitalium*. Hayes *et al.* examined nine strains of *M. penetrans* isolated from either the urine or semen of AIDS patient [12], and Poulin *et al.* investigated the susceptibilities of the type strains of *M. pirum* (HRC 70-159) and *M. penetrans* (GTU-54) together with certain strains of *M. fermentans* [23]. The antimicrobial susceptibilities of seven

M. genitalium strains have also been reported recently [8].

The results reported in the current study are in general agreement with those of previous studies in which the same species were investigated, although different MIC methods were used by different researchers. Those who used freshly thawed inocula [11–13] or cultures in the lag phase [17–22] tended to obtain somewhat lower MICs for some compounds than those reported in the current study, in which mycoplasmas in the logarithmic growth phase were used. Those studies that used agar dilution methods obtained MICs similar to or slightly higher than those recorded in the present study [8, 18–21, 24].

The current results clearly demonstrate the wide variation in susceptibilities of AIDS-associated and human urogenital tract and respiratory mycoplasmas to different classes of antibiotic. Sparfloxacin had excellent in-vitro activity against all the *Mycoplasma* spp. tested and, therefore, has significant potential for treating mycoplasma infections in AIDS patients and infections caused by urogenital tract mycoplasmas and *M. pneumoniae* in non-AIDS patients. Tiamulin also had a broad spectrum of good anti-mycoplasmal activity *in vitro*, but is not licensed for human use. However, both these compounds might offer alternative means of eradicating human mycoplasmas from tissue culture cells if used under controlled conditions. Ciprofloxacin has already been recommended for this purpose [32].

The excellent in-vitro anti-mycoplasmal activities recorded here for the macrolides, particularly azithromycin, against *M. genitalium* and *M. pneumoniae* strains have also been observed by others [8], as has the similarity between the antimicrobial susceptibility profiles of these two *Mycoplasma* spp. [8]. However, the marked difference in their susceptibility to streptomycin has not been reported previously. In the current study, all the *M. genitalium* strains were highly

resistant to streptomycin, while all strains of *M. pneumoniae*, with the exception of the MAC strain, were susceptible. In particular, *M. pneumoniae* UTMB-10P and *M. genitalium* UTMB-10G, which were isolated together from synovial fluid of the same patient, were streptomycin-sensitive and streptomycin-resistant, respectively. It seems likely that streptomycin resistance in the MAC strain may have resulted from its reported passage in chick embryos containing streptomycin 50 mg/L during its primary isolation [33, 34] as repeated passage in the presence of streptomycin *in vitro* has been shown to induce streptomycin resistance in *M. pneumoniae* [35]. Streptomycin resistance (≥ 100 mg/L) has also been reported in some Japanese *M. pneumoniae* strains isolated between 1965 and 1975 [36], when streptomycin was still in use for the treatment of tuberculosis and other respiratory infections. However, now that streptomycin has been superseded by more active and safer antibiotics and is used only infrequently, streptomycin resistance would not be expected to be present in current *M. pneumoniae* isolates. Gentamicin and the other two deoxystreptamine-containing aminoglycosides do not induce cross-resistance to streptomycin (streptidine-containing aminoglycoside [11, 37]). Therefore, it would seem that streptomycin resistance could be used as a marker for differentiating *M. genitalium* from recent isolates of *M. pneumoniae* and that inclusion of streptomycin at concentrations inhibitory to *M. pneumoniae* might form the basis for developing a selective medium for *M. genitalium* in respiratory infections.

Until recently *M. pirum* had only been isolated from mycoplasma-contaminated cell cultures and its natural host was unknown. However, the recent report by De Barbeyrac *et al.* [6] that *M. pirum* had been cultured from the throats and urine of AIDS patients without the use of cell cultures seems to confirm that *M. pirum* is a human *Mycoplasma* sp. The observation in the present study that the type strain HRC 70-159, the two British cell culture isolates MAD-T and ICRF and the American cell culture isolate FD 870 are macrolide-sensitive, while the other American cell culture strains are resistant, suggests that this class of antibiotic has been used in some laboratories to combat bacterial or mycoplasmal contamination in tissue culture cell lines and, therefore, may be responsible for inducing macrolide resistance in certain strains of *M. pirum*. The reason for the resistance of the French AIDS-associated *M. pirum* strains is unclear, as macrolides were not included in the media used to cultivate the PBMCs from the blood of the AIDS patients (O. Grau, personal communication).

The complete resistance of the AIDS-associated *M. fermentans* strain incognitus and *M. fermentans* cell culture isolates to aminoglycosides and the aminoglycoside susceptibility of *M. fermentans* strains isolated from patients without the use of eukaryotic cells has

been reported previously [11, 38]. This has been used as an argument for concluding that the incognitus strain was probably a cell culture contaminant. The current observation that *M. fermentans* AOU, isolated from PBMCs purified from the blood of an AIDS patient, was also aminoglycoside-resistant, while strains 5 and 29, recovered from the urine of AIDS patients in cell-free medium, were aminoglycoside-sensitive, suggests that strain AOU also probably originated from cultured cells rather than from the AIDS patient. This suggestion is supported by a recent observation that the AOU strain is closely related to a Japanese mouse cell culture isolate (F-7) when compared by the newly developed pyrolysis mass spectrometry method for *M. fermentans* [39].

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