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ANTIMICROBIAL AGENTS

Antibiotic susceptibility of *Mycoplasma fermentans* strains from various sources and the development of resistance to aminoglycosides *in vitro*

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Summary. *Mycoplasma fermentans* strains reputedly from human infections or tissue culture cells were much more susceptible to azithromycin than to clarithromycin or erythromycin. Lincomycin, clindamycin and several tetracyclines also exhibited good mycoplasmastatic activity but mycoplasmacidal concentrations were substantially greater than the MICs. Ciprofloxacin was the most active of three fluoroquinolones tested and was mycoplasmacidal at concentrations close to the MIC. Tiamulin and mupirocin were also very active. Synergy with specific *M. fermentans* antiserum plus guinea-pig complement was not observed with any class of antibiotic although the number of viable mycoplasmas was markedly reduced by the combined immunological components. Marked differences in susceptibility to various aminoglycosides were observed. Human strains isolated in cell-free media up to 1967 were aminoglycoside susceptible (MIC range 0·5–25 mg/L) but recent human isolates and strains isolated from tissue culture cells often showed either single or multiple aminoglycoside resistance (MIC > 500 mg/L). Two aminoglycoside-susceptible strains developed resistance to streptomycin or neomycin (> 500 mg/L) within five passages in broth containing streptomycin or neomycin, respectively. Resistance to tobramycin, kanamycin or gentamicin emerged after seven, eight and 14 cycles of exposure to the respective antibiotic. Streptomycin resistance was associated with a five-fold increase in resistance to tobramycin. Neomycin-, kanamycin-, gentamicin- and tobramycin-resistant variants showed mutual cross-resistance but remained susceptible to streptomycin. Induced resistance persisted for at least 17 passages in aminoglycoside-free broth. The use of aminoglycosides in human medicine and the frequent inclusion of some of these drugs in tissue cell cultures to combat bacterial and mycoplasmal contamination might account for the aminoglycoside resistance of recent *M. fermentans* isolates.

Introduction

Mycoplasma fermentans was first isolated in 1950[1] from the genital mucosa of human patients with ulcerative balanitis. These strains were named "G" strains and were found to cause arthritis experimentally in mice. Subsequently *M. fermentans* was isolated from the bone marrows of leukaemic patients[2] and from the joints of patients with rheumatoid arthritis.[3] These strains caused a leukaemia-like disease and death[2,4] and recurrent mild arthritis[5], respectively, in mice but their role in human disease is unresolved. Further attempts to isolate *M. fermentans* from rheumatoid patients have generally been unsuccessful[6–9] and its role in rheumatoid arthritis remains unproven. Since *M. fermentans* is also a contaminant of tissue culture cells[10] and may be refractory to antibiotic treatment, great caution is needed before assigning a pathogenic role to this mycoplasma when cell cultures have been used during the isolation process.

The incognitus strain of *M. fermentans* was isolated from NIH/3T3 cells during transfection studies with DNA extracted from Kaposi's sarcoma tissue from a patient with AIDS.[11] The culture was shown subsequently to be lethal for silvered leaf monkeys.[12] The organism has been demonstrated in the tissues of non-AIDS patients with a lethal "flu-like" illness;[13] it has also been isolated from the respiratory tracts of non-AIDS patients with acute respiratory disease[14] and from children with community-acquired pneumonia.[15] Use of the polymerase chain reaction has indicated a much higher prevalence of *M. fermentans* in human

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immunodeficiency virus 1 (HIV-1) seropositive and seronegative patients than had been supposed previously.[16] *M. fermentans* (incognitus) enhances the cytotoxic effect of HIV-1 on CD_4^+ lymphocytes in culture[17] and this can be reduced significantly by non-toxic doses of various tetracyclines,[18] suggesting a possible co-factor role for *M. fermentans* in the pathogenesis of AIDS.

Information on the antibiotic susceptibility of *M. fermentans* strains is limited.[14,19–22] In the present study the susceptibility to various antimycoplasma drugs of strains of *M. fermentans* isolated over many years was investigated. The possibility of synergy between certain antibiotics and specific *M. fermentans* antibodies in the presence of guinea-pig complement and the development of resistance to aminoglycosides *in vitro* was also studied.

Materials and methods

M. fermentans strains

"G" strains of *M. fermentans* were obtained from the National Collection of Type Cultures (NCTC), 61 Colindale Avenue, London (PG-18: NCTC 10117) and from the American Type Culture Collection, Rockville, MD, USA (ATCC 15474). Both were isolated in cell-free media in 1950 from human cases of ulcerative balanitis. *M. fermentans* strains KL4 and KL8 were obtained from Dr M. H. Williams (Arthur Stanley Institute of Rheumatology, London) in 1972. These were isolated in cell-free media in 1967 from patients with rheumatoid arthritis. *M. fermentans* strains E10 and Z62 were obtained from Dr W. H. Murphy (University of Michigan, School of Medicine, Ann Arbor, MI, USA) and were isolated from bone marrow samples from leukaemic patients between 1961 and 1966 with the aid of tissue culture cells. Strains GIM (human joint isolate) and BRO (human urethral isolate) were obtained from Professor C. Bébéar (University of Bordeaux 11, France) and were isolated in cell-free media in 1992. *M. fermentans* incognitus originally isolated *c.* 1989 was kindly supplied by Dr S.-C. Lo (Department of Defense, Armed Forces Institute of Pathology, Washington DC, 20306-6000, USA). Strains 2059, A6, F3A, C5 and 28 AC were supplied by Dr G. D. Windsor and Helena M. Windsor (Mycoplasma Experience Ltd) and were isolated from tissue culture cells from various European sources between 1982 and 1992.

Mycoplasma media (modified Hayflick's)[23]

Mycoplasma broth medium[24] consisted of sterile Heart Infusion Broth (Difco) 90 ml, heat inactivated (1 h at 56°C) horse serum (Tissue Culture Services Ltd) 20 ml, fresh yeast extract 10 ml, 10% aqueous glucose 10 ml; phenol red and ampicillin were added to give final concentrations of 0·004% and 2·5 mg/ml, respectively. The medium was adjusted to pH 7·8 with sterile 1N NaOH. Solid mycoplasma medium was prepared by adding 1·4 g of purified agar (Oxoid) to 90 ml of heart infusion broth before autoclaving (15 lb/15 min). The remainder of the broth components were added after cooling. Thallium acetate 0·025% was added to the agar medium to combat bacterial and mould contamination.

Preparation of M. fermentans stock cultures

M. fermentans strains were grown aerobically in modified Hayflick's broth. Cultures were incubated at 36°C until an acid colour shift to *c.* pH 6·8 occurred, and were then frozen at −70°C in 2 ml portions. Viable counts were done on thawed samples of each *M. fermentans* strain and identities were checked by the growth inhibition test[25] with specific *M. fermentans* PG-18 antiserum. The bacterial purity of each culture was determined by plating on mycoplasma agar without inhibitors and incubating the plates aerobically and anaerobically at 36°C for at least 4 days.

Sources and preparation of antimicrobial agents

With the exception of clarithromycin, (Klaricid, Abbott), azithromycin (Zithromax, Pfizer), ofloxacin (Tarivid, Hoechst), tiamulin hydrogen fumarate (Sandoz), chloramphenicol (Aldrich) and mupirocin (SmithKline Beecham), all other antimicrobial drugs were obtained from the Sigma Chemical Company. Most of the drugs were dissolved in de-ionised water to achieve a concentration of 1 mg/ml and sterilised by membrane filtration (0·22 μm pore size). Erythromycin, clarithromycin and azithromycin were dissolved or suspended in aqueous ethanol 10% v/v. The fluoroquinolones apart from ciprofloxacin hydrochloride, which is water soluble, were first dissolved in sterile 0·1 N NaOH and were then made up to volume with de-ionised water before sterilisation. Further drug dilutions were prepared at four times the required concentration in modified Hayflick's broth to allow for further dilution by other test components in the MIC tests.

Immunological reagents

M. fermentans PG-18 antiserum, raised in rabbits, was kindly supplied by Dr G. D. Windsor (Mycoplasma Experience Ltd). This antiserum showed specific activity against *M. fermentans* in the growth inhibition test with a 3-mm zone of inhibition against the homologous strain (PG-18), and an antibody titre of 32 in the metabolic inhibition test[26] after heat inactivation (30 min at 56°C). Normal rabbit serum (Sigma) was also heat inactivated before use. Lyophilised guinea-pig complement (ICN Biomedicals Inc.) was reconstituted according to the manufacturer's instructions. Dilutions of these reagents were prepared in Hayflick's broth for use in the minimal

inhibitory and minimum mycoplasmacidal concentration (MIC and MMC) tests. In preliminary studies, the anti-mycoplasma titres against *M. fermentans* PG-18 in the metabolic inhibition test were: normal rabbit serum < 4; guinea-pig complement, 8–16. The dilutions of *M. fermentans* PG-18 antiserum and guinea-pig complement that just allowed mycoplasmal growth when used in combination were determined in checkerboard tests and were found to be 1 in 128 and 1 in 64, respectively. These concentrations of antiserum and complement were used in antibiotic synergy studies.

MIC and MMC tests

MIC tests were performed in 96-well microtitration plates (Costar UK Ltd) by a modification of the broth dilution method.[27] Antimicrobial agents were diluted in Hayflick's broth (pH 7·8) and 0·05 ml of each drug dilution was added to separate wells. After addition of 0·1 ml of Hayflick's broth, each well was inoculated with 0·05 ml of broth containing between 10^3 and 10^6 cfu/ml of the appropriate *M. fermentans* strain. A drug-free, growth control, a sterility control and an end-point (pH 6·8) control were included with each plate. Plates were sealed with adhesive tape and incubated aerobically at 36°C. Initial MICs were recorded when the colour of the growth control matched that of the end-point control, a $\geqslant 50\%$ colour change denoting the MIC. Final MICs were recorded 7 days later and were the lowest drug concentration in which no colour change was seen. MMCs were determined at the time of recording the initial MICs, by transferring 2 μl from each well to Hayflick's agar with a 96-pin hand replicator (Intek Services Ltd, Horley, Surrey) and incubating the plates either aerobically or in a CO_2-enriched atmosphere (Gaspak, BBL) at 36°C. MMCs were defined as the lowest concentration to inhibit mycoplasmal growth completely as judged by microscopy after incubation for at least 4 days. Antibiotic susceptibility tests in the presence of a combination of specific *M. fermentans* PG-18 antiserum and guinea-pig complement diluted to achieve concentrations that were just subinhibitory to *M. fermentans* PG-18 (see above) were performed on certain classes of drug to detect possible synergy. In these tests, 0·05 ml of *M. fermentans* PG-18 antiserum diluted 1 in 32 in Hayflick's broth and 0·05 ml of guinea-pig serum (diluted 1 in 16) were substituted for the 0·1 ml of Hayflick's broth in the MIC tests to give final dilutions of 1 in 128 and 1 in 64, respectively. MMC tests in the presence of antiserum and complement were performed as described above for tests without these immune components.

Induction of aminoglycoside resistance in M. fermentans

Development of resistance and cross-resistance to aminoglycosides in *M. fermentans* strains PG-18 and KL4 (aminoglycoside-sensitive) were studied *in vitro*. Tubes containing 1-ml volumes of streptomycin, kanamycin, gentamicin, neomycin or tobramycin in a range of concentrations covering their respective MICs were inoculated with 0·2 ml of freshly thawed (from −70°C) cultures diluted to contain *c.* 10^4 cfu/ml and incubated at 36°C. A 0·2-ml volume of culture from the highest drug concentration showing an acid colour change was transferred to a fresh series of tubes of drug-containing broth. Such transfers were repeated at 4-day intervals, after which MICs of aminoglycosides for mycoplasmas growing in the highest concentration of drug were compared with those of the parent cultures (PG-18 or KL4). The identities and purities of all resistant variants were checked by testing inhibition of growth by specific antiserum and by plating cultures on inhibitor-free mycoplasma agar.

The stability of the induced aminoglycoside resistance was determined in each case by repeated passage of the resistant variants in aminoglycoside-free broth.

Results

The in-vitro susceptibilities of 14 strains of *M. fermentans* from various sources to 15 classes of antimicrobial agent are shown in table I. Five classes of agent showed good activity: tetracyclines (initial MIC range < 0·025–0·5 mg/L); lincosamides (0·025–0·25 mg/L); fluoroquinolones (0·025–0·25 mg/L); tiamulin hydrogen fumarate (0·005–0·05 mg/L); and mupirocin (< 0·0025–0·01 mg/L). Azithromycin (initial MIC range 0·0025–0·05 mg/L) was strikingly more active than erythromycin (MIC 0·05–50 mg/L) or clarithromycin (MIC 1–> 10 mg/L). In terms of mycoplasmacidal activity, the fluoroquinolones, in particular ciprofloxacin (MMC90 0·1 mg/L), the diterpine tiamulin (MMC90 0·1 mg/L), mupirocin (MMC90 0·025 mg/L) and azithromycin (MMC90 0·25 mg/L), were the most effective agents. The tetracyclines and lincosamides were predominantly mycoplasmastatic agents against *M. fermentans*, the cidal concentration being up to 100-fold or up to 40-fold higher, respectively, than the initial MICs. As expected, all strains of *M. fermentans* were resistant to ampicillin (MIC > 2500 mg/L).

Synergy was not observed between any class of antimicrobial agent and specific *M. fermentans* PG-18 antiserum plus guinea-pig complement, although the colour changes denoting the end-point of MIC tests containing these reagents was markedly retarded (table II). Subculture of these tests on mycoplasma agar at the time of recording the initial MICs showed this delay to be associated with large reductions in mycoplasmas (< 50 cfu/2-μl sample) from wells containing specific antiserum and complement compared with confluent mycoplasmal growth from the control wells.

Marked differences were observed in the susceptibility of *M. fermentans* strains to the aminoglycosides

Table I. In-vitro suceptibilities of 14 *M. fermentans* strains to 15 classes of antimicrobial agent*

Antimicrobial agent	Initial MIC†			Final MIC†			MMC		
	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MMC50	MMC90
Macrolides									
Erythromycin	0·5–50	5	50	10–> 50	10	> 50	2·5–> 50	10	50
Clarithromycin	1–> 10	2·5	5	5–25	10	10	2·5–> 10	10	10
Azithromycin	0·0025–0·05	0·01	0·25	0·025–0·5	0·05	0·5	0·01–0·25	0·05	0·25
Aminoglycosides									
Streptomycin SO_4	2·5–> 500	> 500	> 500	5–> 500	> 500	> 500	5–> 500	> 500	> 500
Neomycin SO_4	1–> 500	5	> 500	2·5–> 500	50	> 500	10–> 500	25	> 500
Gentamicin SO_4	0·25–> 500	2·5	> 500	2·5–> 500	5	> 500	2·5–> 500	10	> 500
Kanamycin SO_4	1–> 500	5	> 500	2·5–> 500	10	> 500	2·5–> 500	10	> 500
Tobramycin SO_4	5–> 500	25	> 500	5–> 500	50	> 500	10–> 500	50	> 500
Aminocyclitol									
Spectinomycin	1–2·5	1	2·5	2·5–10	2·5	> 10	2·5> 10	2·5	10
Tetracyclines									
Tetracycline HCl	0·1–1	0·25	1	1–10	1	5	0·5–10	1	10
Doxycycline HCl	0·05–1	0·25	0·25	0·25–10	1	5	0·1–10	0·5	5
Minocycline HCl	< 0·025–0·5	0·05	0·25	0·5–10	0·5	5	0·25–10	1	10
Lincosamides									
Lincomycin HCl	0·025–0·25	0·05	0·25	0·1–> 0·5	0·25	> 0·5	0·1–1	0·1	1
Clindamycin HCl	0·01–0·25	0·025	0·25	0·025–> 0·5	0·1	0·25	0·05–1	0·1	1
Fluoroquinolones									
Ciprofloxacin HCl	0·025–0·05	0·025	0·05	0·05–0·25	0·1	0·1	0·025–0·25	0·05	0·1
Ofloxacin	0·05–0·25	0·1	0·25	0·1–> 0·5	0·25	0·5	0·05–0·5	0·1	0·25
Norfloxacin	0·1–1	0·25	1	0·5–> 1	1	1	0·5–1	1	1
Diterpines									
Tiamulin	0·005–0·05	0·01	0·05	0·025–0·25	0·05	0·1	0·025–0·5	0·1	0·1
β-Lactam antibiotics									
Ampicillin	> 2500	> 2500	> 2500	> 2500	> 2500	> 2500	> 2500	> 2500	> 2500
Others									
Chloramphenicol	0·5–10	1	5	2·5–> 10	5	10	2·5–> 10	10	> 10
Nitrofurantoin	0·1–2·5	0·5	2·5	2·5–> 10	5	10	0·25–> 10	0·5	2·5
Rifampicin	25–> 50	50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
Puromycin	0·025–0·25	0·1	0·1	0·1–2·5	0·25	0·5	0·25–5	0·25	2·5
Mupirocin (lithium salt)	< 0·0025–0·01	0·01	0·025	< 0·0025–0·25	0·05	0·05	0·01–0·05	0·025	0·025
Novobiocin (Na salt)	2·5–10	2·5	5	1–25	10	25	1–25	10	10
Fusidic acid (Na salt)	2·5–25	10	25	2·5–50	50	50	10–50	50	> 50

MIC50 and MIC90, concentration inhibiting 50% and 90% of the strains.

* Concentration is given in mg/L.

† Initial MICs were recorded when the colour of the growth control well matched that of the end-point control (pH 6·8); final MICs were recorded 7 days later.

Table II. Antibiotic susceptibilities of *M. fermentans* PG-18 in the absence or presence of specific antiserum and guinea pig complement

Antimicrobial agent	MIC (mg/L)		
	Without antiserum or complement*	With antiserum and complement	
		3 days	7 days
Erythromycin	1	< 0·25	1
Azithromycin	0·0025	< 0·0025	0·0025
Streptomycin	2·5	< 0·25	2·5
Gentamicin	1	< 0·25	0·5
Doxycycline	0·25	< 0·025	0·25
Ciprofloxacin	0·025	< 0·025	0·025
Lincomycin	0·05	< 0·0025	0·1
Mupirocin	0·025	< 0·025	0·025

Table III. MICs (mg/L)* of five aminoglycoside antibiotics for various *M. fermentans* strains

Aminoglycoside	Human isolates 1952–1967		Human isolates 1989–1992		Cell culture isolates 1982–1992 (cell-free media; n = 5)
	cell-free media (n = 4)	cell cultures (n = 2)	cell-free media (n = 2)	cell cultures (n = 1)†	
Streptomycin	2·5	> 500	> 500	> 500	> 500
Neomycin	2·5–5	0·5–5	5	> 500	> 500
Kanamycin	2·5	0·25–1	1	> 500	> 500
Gentamicin	0·5–1	0·25–5	2·5	> 500	> 500
Tobramycin	5–25	5	10–25	> 500	250–> 500

* Initial MICs, recorded when the colour of the infected control well = end point control (pH 6·8).
† Incognitus strain.

Table IV. Development of resistance and cross-resistance to aminoglycosides in *M. fermentans* KL4 *in vitro*

Aminoglycoside	MIC (mg/L) for variants isolated from broth containing					
	Strep 500 mg/L (5th passage)	Neo 500 mg/L (5th passage)	Kan 200 mg/L (8th passage)	Tob 500 mg/L (7th passage)	Gent 500 mg/L (14th passage)	Parent strain
Streptomycin	> 500	2·5	5	5	5	2·5
Kanamycin	10	> 500	250	> 500	500	5
Gentamicin	5	> 500	> 500	> 500	> 500	2·5
Neomycin	25	> 500	250	> 500	500	25
Tobramycin	250	> 500	250	> 500	125	50

Strep, streptomycin; Neo, neomycin; Kan, kanamycin; Tob, tobramycin; Gent, gentamicin. (Similar results were obtained with *M. fermentans* PG-18.)

(table III). The G strains isolated in 1950 and rheumatoid strains (KL4 and KL8) isolated around 1967 in cell-free medium, were susceptible to all the aminoglycosides tested. The bone marrow strains (E10 and Z62) which were isolated during the 1960s with the aid of cell cultures containing penicillin and streptomycin to suppress bacterial contamination were resistant to > 500 mg of streptomycin/L but susceptible to the other aminoglycosides. Strains GIM (joint isolate) and BRO (urethral isolate) isolated in 1992 without the use of cell cultures were also more resistant to streptomycin than to other aminoglycosides whereas *M. fermentans* incognitus and all of the cell-culture isolates were resistant to all the aminoglycosides. All strains were susceptible to the aminocyclitol, spectinomycin (initial MIC range 1–2·5 mg/L).

Resistance to streptomycin and neomycin (MIC > 500 mg/L) emerged within five passages in broth containing the appropriate aminoglycoside (table IV). Resistance to tobramycin (MIC > 500 mg/L) emerged after seven passages in tobramycin-containing broth and to kanamycin (MIC > 250 mg/L) after eight passages in kanamycin-containing broth. Resistance to gentamicin (MIC > 500 mg/L) was observed only after 14 passages in gentamicin-containing broth. Development of streptomycin resistance was associated with increased resistance to tobramycin but not to the other aminoglycosides tested. In contrast, variants resistant to neomycin, kanamycin, tobramycin or gentamicin were cross-resistant to these aminoglycosides but remained susceptible to streptomycin (table IV). In all

but one case, resistance persisted for at least 17 passages in aminoglycoside-free broth.

Discussion

There are relatively few reports of the antimicrobial susceptibilities of *M. fermentans*. In the most comprehensive study,[14] the susceptibilities of 24 *M. fermentans* strains from various sources to 10 antimicrobial agents were compared. The results of the current study are broadly consistent with earlier results, despite differences in the MIC methods used in the different laboratories, and also provide new data on previously unreported compounds.

Most striking was the greater anti-*M. fermentans* activity of azithromycin than that of the related macrolides, erythromycin and clarithromycin. Since mycoplasmacidal concentrations of azithromycin are likely to be achieveable in human blood and tissues, this antibiotic should be considered for the treatment of systemic *M. fermentans* infections in man, particularly as it persists in serum, is concentrated within tissues and penetrates mammalian cells,[28] which are all sites of *M. fermentans* infection.[11,13,29] The importance of adequate blood and tissue levels of antibiotics was also stressed by Hayes *et al.*[14] who concluded that drugs which satisfy those criteria and which are sufficiently active against *M. fermentans* for human use include ciprofloxacin, levofloxacin (not tested in the current study), clindamycin, lincomycin, doxycycline and tetracycline.

The fluoroquinolones, together with the veterinary antibiotic tiamulin and the topical antibiotic mupirocin, appear to be mycoplasmacidal at concentrations close to the MIC. Tiamulin is not licensed for human use and mupirocin is unstable *in vivo*.[30] However, these drugs and azithromycin might offer alternative means of eradicating *M. fermentans* from tissue culture cells. Ciprofloxacin has already been found to be effective in this respect.[31]

Mycoplasma infections may be very difficult to eradicate from immunocompromised patients.[32,33] The persistance of *M. pneumoniae* in a patient with hypogammaglobulinaemia despite prolonged treatment with tetracycline, erythromycin and doxycycline has been reported.[34] It has been suggested[32,34] that in hypogammaglobulinaemic patients infected with *M. pneumoniae*, antibiotic treatment should be supplemented with plasma obtained from immunocompetent patients known to have high circulating antibody. Elimination of *Ureaplasma urealyticum* has been achieved in an aggammaglobulinaemic patient with simultaneous antibiotic and hyperimmune goat antiserum (P. M. Furr, personal communication). Such cures could be due either to synergy between certain antibiotics and specific antibodies in the presence of serum complement or to reductions in the mycoplasma load sufficient for cellular immune mechanisms to cope with the infection. Since synergy was not found in the current study, but mycoplasma numbers were markedly reduced by the antiserum and guinea-pig complement, the second of these hypotheses seems more likely.

Marked resistance of some *M. fermentans* strains to certain aminoglycosides has been reported previously.[14,35] In those studies, *M. fermentans* incognitus and other *M. fermentans* strains derived from urine sediments from AIDS patients were compared with isolates from an outbreak of acute respiratory infection in Canada, an isolate from a patient with leukaemia,[2] various cell culture isolates and the type strain PG-18. Significant differences in the broth MICs of aminoglycosides for *M. fermentans* strains from different sources were not apparent. However, the present results show clearly that early *M. fermentans* isolates obtained in cell-free media were susceptible to all of the aminoglycosides tested, whereas strains isolated in cell cultures were highly resistant to streptomycin, an aminoglycoside commonly incorporated in tissue culture media to suppress bacterial contamination. The experiments also showed that *M. fermentans* strains obtained more recently without the use of cell cultures were also resistant to streptomycin, suggesting that resistance to streptomycin had been acquired through use of the drug. These strains and the earlier isolates were all susceptible to the deoxystreptamine group of aminoglycosides (neomycin, kanamycin, gentamicin, tobramycin). However, *M. fermentans* incognitus and all strains derived from cell cultures showed cross-resistance to all aminoglycosides tested. In the case of the cell culture strains, this may have been due to exposure to streptomycin and other aminoglycosides during attempts to remove bacterial or mycoplasmal contaminants from tissue culture cell stocks, as kanamycin, gentamicin and neomycin are often used for this purpose.[10] The demonstration of aminoglycoside cross-resistance in *M. fermentans* incognitus throws doubt on the origin of this strain since it was detected originally during transfection studies with NIH/3T3 cells.

To ascertain the ease with which *M. fermentans* develops resistance to individual aminoglycosides and the cross-resistance patterns which arise following development of resistance to particular aminoglycosides, habituation experiments were performed *in vitro*. These tests showed that high resistance to the streptidine-containing aminoglycoside, streptomycin, developed within five passages in streptomycin-containing broth, and that such variants were not cross-resistant to deoxystreptamine-containing aminoglycosides, other than tobramycin. Conversely, deoxystreptamine-containing aminoglycosides induced cross-resistance to other members of that group, but not to streptomycin. These induced resistances were generally stable, indicating that once resistance had developed it was permanent. The mechanisms by which aminoglycoside resistance developed were not studied.

These results indicate that aminoglycoside resistance in *M. fermentans* is associated with previous exposure to these antibiotics, but that exposure to streptomycin alone does not trigger multiple resistance. The use of streptomycin and other aminoglycosides in human medicine and the frequent inclusion of some of these drugs in cell cultures to combat bacterial and mycoplasmal contamination may account for the high single or multiple aminoglycoside resistances in recent *M. fermentans* isolates. The rapid development of multiple aminoglycoside resistance demonstrated *in vitro* indicates that such resistance could develop in patients treated with any aminoglycoside. Naturally susceptible *M. fermentans* strains could also develop multiple aminoglycoside resistance during the isolation process if tissue cultures containing streptomycin or other aminoglycosides of the neomycin group were used, although such strains could be tissue culture contaminants. Since the antibiotic treatment history of the AIDS patient from which *M. fermentans* incognitus was isolated is unknown (S.-C. Lo, personal communication), the multiple aminoglycoside resistances seen in *M. fermentans* incognitus could be due to any of these possibilities.

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