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In Vitro Development of Resistance to Erythromycin, Other Macrolide Antibiotics, and Lincomycin in *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae was made highly resistant to erythromycin in vitro by serial subculture in broth media containing erythromycin. The resistance developed to erythromycin was 200 μg/ml with the Mac strain, a prototype of *M. pneumoniae*, and 10 μg/ml with the Fukumura strain, an isolate. The erythromycin resistance was accompanied by cross resistance to other macrolide antibiotics (leucomycin, josamycin, spiramycin, and oleandomycin) and to lincomycin, but there was no resistance to vernamycin B. Resistance to the antibiotics developed in vitro or in vivo was stable after the microorganisms were repeatedly transferred in antibiotic-free media.

Only a few studies have been reported on the development of antibiotic resistance in *Mycoplasma pneumoniae*. We first reported a case of *M. pneumoniae* pneumonia in which *M. pneumoniae* acquired high resistance to erythromycin, other macrolide antibiotics, and lincomycin during the administration of erythromycin (15). Masuda (14) reported decreased susceptibility to streptomycin in *M. pneumoniae* after four subcultures in media containing streptomycin.

We have succeeded in making *M. pneumoniae* highly resistant to erythromycin with cross resistance to other macrolide antibiotics and lincomycin by serial subcultures in broth media containing erythromycin. The resistance developed was stable after the microorganisms were repeatedly subcultured in antibiotic-free media.

MATERIALS AND METHODS

Media. The broth medium for propagation of the microorganisms consisted of seven parts 2.1% pleuropneumonia-like organism (PPLO) broth (Difco), two parts unheated horse serum, one part 25% yeast extract, 1% glucose, 500 U of penicillin G per ml, 500 μg of thallium acetate per ml, and 0.002% phenol red according to the formula of Chanock et al. (4). In broth media used for antibiotic susceptibility tests, penicillin was omitted and the pH was adjusted to 7.8 before addition of the antibiotics. Agar media were prepared by replacing PPLO broth with 3.4% PPLO agar (Difco) in the above formula for propagation.

Strains of M. pneumoniae. The strains used were the Mac strain, a prototype of *M. pneumoniae*, the Fukumura strain, which was isolated by us from a child with pneumonia, and Hosokawa strains no. 1 to 5, which were isolated from a child with pneumonia before and during erythromycin therapy. The Mac and Fukumura strains were susceptible to erythromycin. Of the Hosokawa strains, no. 1 to 3 were susceptible to erythromycin, other macrolide antibiotics, and lincomycin, and strains no. 4 and 5 were resistant to all of the antibiotics, as reported elsewhere (15).

Antibiotics. The following antibiotics were used: erythromycin gluceptate (Ilotycin, Eli Lilly & Co.), leucomycin tartrate (Toyo Jozo, josamycin tartrate (Yamanouchi), spiramycin (Kyowa Hakko), oleandomycin phosphate (Sankyo), lincomycin hydrochloride (Lincomycin, Upjohn Co.), vernamycin A, and vernamycin B (E. R. Squibb & Sons).

Development of resistance. Broth media were prepared to contain erythromycin in fourfold concentrations of 0.0015, 0.006, 0.025, 0.1, 0.4, and 1.6 μg/ml. For the Mac strain, media were further prepared to contain erythromycin concentrations of 6.25, 25, 100, and 400 μg/ml. The medium containing each concentration of erythromycin was distributed in 2 ml into each of two tubes. The tubes were inoculated with the microorganisms, incubated at 37 C, and observed for a month. To test for the development of resistance, 0.1 ml of the fluid of the broth culture of *M. pneumoniae* was inoculated into each tube of two series of broth media containing fourfold increments in erythromycin concentration. Then, a pool of cultures grown in the highest concentration of erythromycin that permitted the color to change to yellow similar to the control (as read when the control medium without erythromycin changed in color to yellow and indicative of rich growth) was serially subcultured in two series of media containing fourfold concentrations of erythromycin. Besides this procedure, the pooled fluids of cultures grown in relatively higher concentra-

tions of erythromycin during subculture were tested for their resistance to erythromycin, as described below, directly or after being subcultured in antibiotic-free broth media.

Susceptibility test. Broth media containing twofold concentrations of erythromycin, leucomycin, josamycin, oleandomycin, spiramycin, lincomycin, vernamycin A, and vernamycin B were prepared and distributed in 1-ml portions into two tubes. A 0.1-ml portion of the broth culture of *M. pneumoniae* was inoculated into each tube of two series of broth media containing the twofold concentrations of the antibiotics, and the tubes were incubated at 37 C and observed for a month.

Growth tests and definitions. The color of the media was read when the control medium without antibiotic first changed to a yellow color, indicating a rich growth. The minimal growth-inhibitory concentration is defined as the lowest concentration of an antibiotic that completely prevented any color change, indicating a complete inhibition of growth. The maximal growth concentration is defined as the highest concentration of an antibiotic that permitted a growth comparable with the control as regards the color of the medium.

The color change of the media proceeded further and finally stopped at a definite concentration of an antibiotic during a month of incubation. The final minimal growth-inhibitory concentration was the lowest concentration that completely inhibited growth during a month of incubation; the final maximum growth concentration was the highest concentration of an antibiotic that permitted the growth during a month of incubation.

RESULTS

Development of erythromycin resistance. Two strains of *M. pneumoniae*, Mac and Fukumura, were tested for the development of erythromycin resistance by serial subculture in broth media containing fourfold increments in concentrations of erythromycin.

Mac strain. Twenty-one subcultures were made (Fig. 1). The maximal growth concentration of erythromycin was 0.0015 μg/ml in the 1st through the 3rd subcultures and 0.006 μg/ml in the 4th through 21st subcultures, showing only a fourfold increase in resistance. A high level of resistance could not be obtained by this method. However, later in the 18th through 21st subcultures, the microorganisms could be grown in 100-μg or higher concentrations of erythromycin per ml during a month of incubation, thus showing the development of higher levels of resistance to erythromycin.

Five broth cultures, A through E in Fig. 1, grown in relatively higher concentrations of erythromycin during a month of incubation, were tested for their susceptibility to erythromycin with broth media containing varying concentrations of erythromycin (Table 1). Culture B showed no decrease in susceptibility, but cultures C and E showed low-level resistance as compared with the parent susceptible culture. Culture A showed a maximal resistance to 100 μg of erythromycin per ml when tested directly with media containing fourfold concentrations of erythromycin and a maximal resistance to 200 μg/ml when tested with media containing twofold concentrations of erythromycin after one subculture in antibiotic-free medium. Culture D was similar to culture A, and its corresponding values were 100 and 200 μg/ml, respectively. Thus, variants with maximum resistance to 200 μg of erythromycin per ml could be obtained in the Mac strain.

Fukumura strain. Eighteen subcultures were made by the method used in the Mac strain (Fig. 2). The results were somewhat different from that of the Mac strain. The maximal growth concentration increased from 0.0015 to 0.006 μg of erythromycin per ml after only 12 subcultures, and the final maximal growth concentration increased from 0.006 to 0.4 μg/ml in the 4th subculture but remained at 0.1 μg/ml in most of the further subcultures. Thus, the Fukumura strain did not develop higher levels of resistance to erythromycin by this method, as compared with the Mac strain.

Five cultures, F through L in Fig. 2, grown in higher concentrations of erythromycin during a month of incubation, were inoculated into a series of broth media containing varying concentrations of erythromycin and were tested for their susceptibility to erythromycin (Table 2). With the cultures, F, G, and K, the maximal growth concentration of erythromycin was 0.1 μg/ml, thus showing the development of a low level of resistance to erythromycin as compared

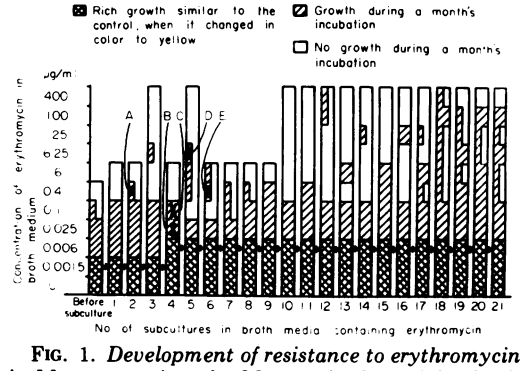

FIG. 1. *Development of resistance to erythromycin in M. pneumoniae, the Mac strain, by serial subcultures in broth media containing varying concentrations of erythromycin. Cultures A through E were tested for susceptibility to erythromycin (Table 1), and cultures A and D were tested for susceptibility to erythromycin and other antibiotics (Table 3).*

TABLE 1. *Erythromycin susceptibility of M. pneumoniae, Mac strain, grown in broth medium containing higher concentrations of erythromycin*[a]

Culture origin	Maximal growth concn[b] (μg/ml)	Final maximal growth concn[b] (μg/ml)
Parent susceptible culture before exposure to erythromycin	0.0015 (5)[c]	0.1 (17)
Culture A[d]	100 (7)	800 (7)
Subculture[e]	200 (7)	≧800 (7)
Culture B	0.0015 (6)	0.006 (6)
Culture C	0.025 (15)	0.1 (20)
Culture D	100 (5)	400 (7)
Subculture	200 (5)	≧800 (7)
Culture E	0.1 (12)	0.1 (12)

[a] Concentrations of erythromycin were fourfold increments (Fig. 1) in the test made directly, and twofold increments in the test made after a subculture.

[b] See text.

[c] Time of reading in days of incubation.

[d] See Fig. 1.

[e] Subculture was made once in the antibiotic-free broth medium.

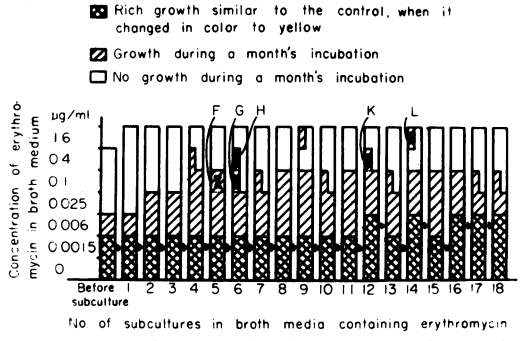

FIG. 2. *Development of resistance to erythromycin in M. pneumoniae, Fukumura strain, by serial subcultures in broth media containing varying concentrations of erythromycin. Cultures F through L were tested for susceptibility to erythromycin (Table 2), and cultures H and L were tested for susceptibility to erythromycin and other antibiotics (Table 3).*

with the parent susceptible culture. The maximal growth concentration was 25 μg/ml with culture H and 6.25 μg/ml with culture L, when tested directly with media containing fourfold concentrations of erythromycin. When both H and L were tested with media containing twofold concentrations of erythromycin after two subcultures in erythromycin-free broth medium, the maximal growth concentration for both was 10 μg/ml. Thus, with the Fukumura strain as well, variants with resistance to 10 or 25 μg of erythromycin per ml could be obtained.

Cross resistance to other macrolide antibiotics and lincomycin. The erythromycin-resistant variants obtained in vitro were tested for cross resistance to other macrolide antibiotics and lincomycin.

Mac strain. The two broth cultures, A and D in Fig. 1, after a single transfer in erythromycin-free medium, and the parent susceptible culture before exposure to erythromycin were all tested for susceptibility to the antibiotics (Table 3). With erythromycin, the maximal growth concentration increased from 0.004 to 200 μg/ml in both cultures, showing the development of a high-level resistance to erythromycin. With leucomycin, josamycin, spiramycin, and oleandomycin, maximal growth concentration increased from 0.008 to 1.0, 0.008 to 3.2, 0.05 to 6.4, and 0.064 to ≧1,600 μg/ml, respectively. With lincomycin, the maximal growth concentration increased from 1.6 to 100 μg/ml in culture A and to 50 μg/ml in culture D. The microorganisms in the two cultures had acquired higher levels of resistance to all the antibiotics tested.

Fukumura strain. The two broth cultures, H and L in Fig. 2, after a single transfer in erythromycin-free medium, and the parent

TABLE 2. *Erythromycin susceptibility of M. pneumoniae, Fukumura strain, grown in broth medium containing higher concentrations of erythromycin*[a]

Culture origin	Maximal growth concn[b] (μg/ml)	Final maximal growth concn[b] (μg/ml)
Parent susceptible culture before exposure to erythromycin	0.0015 (14)[c]	0.006 (20)
Culture F[d]	0.1 (7)	0.4 (13)
Culture G	0.1 (7)	1.6 (30)
Culture H	25 (12)	100 (18)
Subculture[e]	10 (12)	200 (18)
Culture K	0.1 (4)	1.6 (14)
Culture L	6.25 (5)	400 (23)
Subculture	10 (5)	200 (23)

[a] Concentrations of erythromycin were fourfold increments (Fig. 2) in the test made directly, and twofold increments in the test made after two cultures.

[b] See text.

[c] Time of reading in days of incubation.

[d] See Fig. 2.

[e] Subculture was made twice in the antibiotic-free broth medium.

TABLE 3. *Cross resistance to M. pneumoniae, which acquired resistance to erythromycin, to other macrolide antibiotics and to lincomycin*[a]

Strain	Culture origin	Growth-permitting concn[b]	Concn of antibiotics (μg/ml)					
			Erythromycin	Leucomycin	Josamycin	Spiramycin	Oleandomycin	Lincomycin
Mac	Parent susceptible culture[c]	MGC	0.004 (3)[d]	0.008 (3)	0.008 (3)	0.05 (3)	0.064 (3)	1.6 (3)
		FMGC	0.064 (20)	0.4 (22)	0.8 (22)	0.8 (16)	0.8 (16)	10(6)
	Culture A[e]	MGC	200 (3)	1.0 (3)	3.2 (5)	6.4 (5)	≧1,600 (3)	100 (5)
		FMGC	≧800 (5)	20 (31)	10 (13)	40 (12)	≧1,600 (3)	400 (12)
	Culture D	MGC	200 (4)	1.0 (4)	3.2 (4)	6.4 (4)	≧1,600 (4)	50 (4)
		FMGC	≧800 (5)	20 (12)	20 (16)	80 (14)	≧1,600 (4)	400 (14)
Fukumura	Parent susceptible culture[c]	MGC	0.002 (6)	0.008 (6)	0.008 (6)	0.05 (6)	0.064 (6)	0.8 (6)
		FMGC	0.032 (26)	0.2 (32)	0.1 (28)	0.4 (18)	0.8 (28)	3.2 (9)
	Culture H[f] (culture M)[c]	MGC	10 (6)	100 (6)	50 (6)	400 (6)	100 (6)	10 (6)
		FMGC	200 (12)	400 (12)	200 (8)	≧800 (6)	800 (14)	80 (30)
	Culture L	MGC	10 (5)	50 (5)	10 (5)	400 (5)	100 (5)	10 (5)
		FMGC	400 (30)	400 (10)	400 (26)	≧800 (5)	≧1,600 (16)	80 (16)

[a] Cultures A, D, H, and L were tested after one or two subcultures in the antibiotic-free broth medium.
[b] Abbreviations: MGC, maximal growth concentration; FMGC, final maximal growth concentration. See text.
[c] Susceptibility to vernamycin A and vernamycin B is shown in Table 5.
[d] Time of reading in days of incubation.
[e] See Fig. 1.
[f] See Fig. 2.

broth culture, before exposure to erythromycin, were tested for susceptibility to the antibiotics (Table 3). With erythromycin, maximal growth concentration increased from 0.002 to 10 μg/ml in both of the cultures, showing the development of a high level of resistance to erhthromycin. With leucomycin, the maximal growth concentration increased from 0.008 to 100 μg/ml in culture H and to 50 μg/ml in culture L; with josamycin, it increased from 0.008 to 50 μg/ml in culture H and to 10 μg/ml in culture L; with spiramycin, it increased from 0.05 to 400 μg/ml; with oleandomycin, it increased from 0.064 to 100 μg/ml; and with lincomycin, it increased from 0.8 to 10 μg/ml in both of the cultures. The microorganisms in the two cultures had acquired higher levels of resistance to all the antibiotics tested.

Stability of the resistance. The stability of the resistance acquired to erythromycin, other macrolide antibiotics, and lincomycin in vitro and in vivo was tested after repeated subcultures of the microorganisms in broth and agar media without the antibiotics (Table 4).

Mac strain. The level of resistance was compared among the broth culture once transferred in antibiotic-free medium from culture D in Fig. 1, the culture after it was subcultured five times on antibiotic-free agar medium, and the culture after it was subcultured nine times in antibiotic-free broth medium. The maximal growth concentration was the same among the three cultures for erythromycin (200 μg/ml) and was nearly the same among them for each of the other antibiotics tested. The resistance acquired to the antibiotics in vitro is thus stable after repeated subcultures of the resistant microorganisms in antibiotic-free medium.

Hosokawa no. 4 strain. This strain had acquired higher levels of resistance to erythromycin, the other macrolide antibiotics, and lincomycin in vivo (15). The level of resistance was compared among the 3rd subculture in antibiotic-free broth medium from the first isolate from the patient, the broth culture after five subcultures on antibiotic-free agar medium, and the culture after 15 subcultures in antibiotic-free broth medium. The maximal growth concentration was the same among the three cultures for erythromycin (200 μg/ml) and was nearly the same among all of them for each of the other antibiotics tested. These facts suggested that the resistance acquired in vivo is also stable after repeated subcultures of the resistant microorganisms in antibiotic-free medium.

Susceptibility to vernamycin A and vernamycin B. The susceptibility to vernamycin A

and vernamycin B was tested in the parent erythromycin-susceptible cultures and in cultures that had acquired erythromycin resistance in vitro (Table 5). All of the erythromycin-susceptible cultures or strains were susceptible to the other macrolide antibiotics and lincomycin, and all the erythromycin-resistant cultures or strains were resistant to them. The in vitro and in vivo development of resistance to macrolide antibiotics and lincomycin did not accompany cross resistance to vernamycin A and vernamycin B.

DISCUSSION

The Mac strain of *M. pneumoniae* was reported to be streptomycin susceptible by Jao and Finland (8), whereas the Mac strain used here was streptomycin resistant, as reported by others (1, 5, 10, 14). This suggests that the streptomycin resistance might have developed in the Mac strain by the exposure to streptomycin in some laboratory. We first reported in 1970 the isolation of strains of *M. pneumoniae* highly resistant to erythromycin from a girl with pneumonia given erythromycin therapy (15). From these facts, we assumed that *M. pneumoniae* might develop resistance to erythromycin by serial subcultures in broth media containing erythromycin.

Two strains of *M. pneumoniae* were repeatedly subcultured in broth media containing varying concentrations of erythromycin. For the two strains, the level of resistance to erythromycin increased only fourfold. Higher levels of resistance could not be obtained by this method of subculturing.

In later subcultures, however, the two strains were able to be grown in higher concentrations of erythromycin for longer periods than in the control cultures during a month of incubation. In some of these cultures, the microorganisms had acquired high levels of resistance to eryth-

TABLE 4. *Stability of resistance of M. pneumoniae to erythromycin, other macrolide antibiotics, and lincomycin*

Culture	Conditions	Growth-permitting concn[a]	Concn of antibiotics (μg/ml)					
			Erythromycin	Leucomycin	Josamycin	Spiramycin	Oleandomycin	Lincomycin
Mac strain	Before following subcultures in antibiotic-free medium[b]	MGC	200 (4)[c]	1.0 (4)	3.2 (4)	6.4 (4)	≧1,600 (4)	50 (4)
		FMGC	≧800 (6)	20 (12)	20 (16)	80 (14)	≧1,600 (4)	400 (14)
	After five subcultures on antibiotic-free agar medium and then a subculture in antibiotic-free broth medium	MGC	200 (5)	1.0 (5)	3.2 (5)	10 (5)	≧1,600 (5)	100 (5)
		FMGC	≧800 (6)	10 (12)	10 (14)	40 (10)	≧1,600 (5)	400 (10)
	After nine subcultures in antibiotic-free broth medium (culture N[d])	MGC	200 (5)	1.0 (5)	3.2 (5)	6.4 (5)	≧1,600 (14)	50 (5)
		FMGC	≧800 (12)	10 (10)	10 (14)	40 (10)	≧1,600 (28)	200 (7)
Hosokawa no. 4 strain	Before following subcultures in antibiotic-free broth medium[e] (culture O[d])	MGC	200 (17)	1.0 (14)	3.2 (6)	6.4 (13)	800 (14)	50 (14)
		FMGC	400 (33)	10 (17)	10 (6)	20 (18)	≧1,600 (28)	100 (19)
	After 5 subcultures on antibiotic-free agar medium and then a subculture in antibiotic-free broth medium	MGC	200 (6)	1.0 (6)	3.2 (6)	6.4 (6)	≧1,600 (6)	50 (6)
		FMGC	≧800 (10)	20 (14)	40 (32)	40 (16)	≧1,600 (6)	200 (14)
	After 15 subcultures in antibiotic-free broth medium	MGC	200 (5)	1.0 (5)	3.2 (5)	6.4 (5)	≧1,600 (5)	50 (5)
		FMGC	≧800 (10)	40 (24)	20 (14)	80 (24)	≧1,600 (5)	400 (14)

[a] Abbreviations as in Table 3.

[b] Culture D, which was grown in medium containing 6.25 μg of erythromycin per ml in the 5th subculture (Table 1), was tested after being subcultured once in antibiotic-free broth medium.

[c] Time of reading in days of incubation.

[d] Susceptibility to vernamycin A and vernamycin B is shown in Table 5.

[e] Strain no. 4 was isolated in our laboratory and reported elsewhere (15). The test was made of the 3rd subculture in antibiotic-free broth medium from the 1st isolate.

TABLE 5. *Comparison of susceptibility to vernamycin A and B between erythromycin-susceptible and -resistant strains*

Strain	Culture	Susceptibility to erythromycin	Vernamycin A		Vernamycin B	
			MGIC[a]	FMIC	MGIC	FMIC
Mac	Parent strain	Susceptible	0.8 (4)[b]	80 (14)	20 (4)	200 (10)
	Culture N[c]	Resistant	0.4 (4)	80 (14)	20 (4)	200 (14)
Fukumura	Parent strain	Susceptible	0.8 (8)	80 (26)	10 (8)	100 (33)
	Culture M[d]	Resistant	3.2 (7)	80 (16)	1.6 (7)	40 (33)
Hosokawa	No. 1	Susceptible	0.8 (8)	80 (26)	6.4 (8)	200 (33)
	No. 2	Susceptible	0.8 (12)	40 (17)	6.4 (12)	80 (16)
	No. 3	Susceptible	0.8 (9)	40 (26)	6.4 (9)	80 (16)
	No. 4[e]	Resistant	0.8 (14)	80 (26)	10 (14)	200 (33)
	No. 5	Resistant	0.8 (9)	80 (26)	10 (9)	200 (33)

[a] Abbreviations: MGIC, minimal growth-inhibitory concentration; FMIC, final minimal growth-inhibitory concentration.
[b] Time of reading in days of incubation.
[c] See Table 4.
[d] See Table 3.
[e] Culture O in Table 4.

romycin. The resistance to erythromycin developed in vitro in *M. pneumoniae* was accompanied by cross resistance to other macrolide antibiotics and lincomycin, similar to the resistance developed in vitro (15). Cross resistance to vernamycin B developed neither in vitro nor in vivo. The resistance of *M. pneumoniae* developed to these antibiotics in vitro or in vivo was stable after the microorganisms were repeatedly subcultured in antibiotic-free media.

Erythromycin resistance of microorganisms has been extensively studied in *Staphylococcus aureus*. Strains of *S. aureus* isolated from patients can be classified into two types: one of which has the dissociated resistance reported by Garrod (6), i.e., resistance to erythromycin only, and the other which shows cross resistance to erythromycin as well as other macrolides (2, 3, 6, 11, 12), lincomycin (2, 12), and streptogramin B-type antibiotics (3). Jones et al. (9) reported that *S. aureus* was rendered resistant to erythromycin, carbomycin, oleandomycin, spiramycin, and streptogramin by repeated subcultures in the presence of increasing concentrations of these antibiotics, accompanied by cross resistance to all of these agents except streptogramin. *S. aureus* showing dissociated erythromycin resistance was found to be resistant to other macrolides (2, 6) and lincomycin (2, 7) in the presence of erythromycin. These facts are accounted for by the induction of resistance by erythromycin. Weaver and Pattee (16) reported that subinhibitory concentrations of erythromycin can induce resistance to itself in *S. aureus* that shows dissociated erythromycin resistance, and that this induced resistance is completely lost after a single subculture of induced cells in the absence of erythromycin.

Weisblum and Demohn (17) defined the classes of antibiotics whose action is antagonized by erythromycin and reported that, in strains of *S. aureus* showing dissociated erythromycin resistance, erythromycin induced resistance exclusively toward inhibitors of 50*S* ribosomal subunit function, and of these, it was restricted to three groups: other macrolides, lincosaminide, and streptogramin group B-type antibiotics of the six known classes of inhibitors that act on this subunit. Weisblum et al. (18) selected constitutive erythromycin-resistant strains of *S. aureus* on media containing antibiotics that belong to any of one of the three classes of antibiotics and found two patterns of constitutive resistance: one is generalized constitutive resistance, which involves resistance to all members to each of the three cited classes of 50*S* subunit inhibitors; the other is partial constitutive resistance, which involves different degrees of resistance to various members of the three classes. They reported that 50*S* ribosomal subunits isolated from induced or constitutively resistant cells showed decreased ability to bind erythromycin and lincomycin. Lai and Weisblum (13) subsequently found that a base, tentatively identified as [^{6}N]dimethyladenine, was present in 23*S* ribosomal ribonucleic

acid obtained from induced- and constitutive-resistant cells but not in 23*S* ribonucleic acid from susceptible, uninduced cells.

Erythromycin developed cross resistance to erythromycin, other macrolides, and lincomycin in *M. pneumoniae* as it does in *S. aureus*, but it did not develop cross resistance to vernamycin B. The mode of action of macrolide antibiotics and lincomycin and the mechanism of resistance to these antibiotics may be similar in *M. pneumoniae* and *S. aureus*. Why the erythromycin-resistant strains do not show cross resistance to vernamycin B remains a problem.

We obtained variant strains of *M. pneumoniae* with high levels of stable resistance to erythromycin, other macrolide antibiotics, and lincomycin in vitro. We have found that the use of the resistant strain of *M. pneumoniae* enables the fermentation inhibition test for the measurement of serum antibody to *M. pneumoniae* to be practicable in sera drawn from patients receiving macrolide antibiotics and lincomycin (15a).

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