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Effects of Sub-MICs of Erythromycin and Other Macrolide Antibiotics on Serum Sensitivity of *Pseudomonas aeruginosa*

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We examined the effects of sub-MICs of erythromycin (EM) and other macrolide antibiotics on the serum sensitivity of *Pseudomonas aeruginosa*. *P. aeruginosa* S-6 grown for 36 and 48 h on agar with 10 μg of EM per ml (1/10th the MIC) showed significantly increased sensitivity to human serum bactericidal activity compared with those of bacteria grown on agar without EM ($P < 0.05$). No changes in serum sensitivity were observed in bacteria grown for less than 24 h. This increased sensitivity was apparent even at a concentration of 1.5 μg of EM per ml (1/67th the MIC) in bacteria grown for 48 h ($P < 0.01$). Among the other macrolide antibiotics tested, clarithromycin also enhanced sensitivity to serum, but there were no changes in the sensitivities of bacteria grown on agar with kitasamycin, josamycin, rokitamycin, or oleandomycin even at a concentration of 12 μg/ml (1/16th, 1/16th, 1/8th, and 1/33rd the MICs, respectively). *P. aeruginosa* S-6 grown on agar with subinhibitory concentrations of EM showed decreased cell surface hydrophobicity in a dose-dependent manner, whereas oleandomycin and rokitamycin, even at a concentration of 12 μg/ml, induced a slight decrease in hydrophobicity which was approximately equivalent to that of 1.5 μg of EM per ml. Among six other strains of the nonmucoid phenotype, three strains became more sensitive to serum by exposure to 10 μg of EM per ml for 48 h. In contrast, no evident correlation between EM treatment and a change in serum sensitivity was observed in six strains of the mucoid phenotype, as judged by the results of experiments with both 2 and 0.4% serum. These results show that EM at subinhibitory concentrations enhances the serum sensitivity of some *P. aeruginosa* strains. Since induced serum sensitivity was accompanied by a decrease in bacterial cell surface hydrophobicity, EM may render *P. aeruginosa* more serum sensitive by changing the cell surface structure(s) of this organism.

Pseudomonas aeruginosa is one of the most frequently encountered bacterial pathogens in patients with chronic pulmonary infections, including cystic fibrosis (20, 31) and diffuse panbronchiolitis (12). Once *P. aeruginosa* colonizes the lungs of these patients, the organism is rarely eradicated, despite the use of various antibiotics, and ultimately, patients die of respiratory failure or consequences of respiratory infection (8). It is most important to inhibit *P. aeruginosa* infection or to eradicate this organism from such patients.

Recently, it has been reported that erythromycin (EM) improves the clinical symptoms of and prognosis for patients with chronic pulmonary infections, including those caused by *P. aeruginosa* (14). Since EM at therapeutic doses is neither bactericidal nor bacteriostatic to *P. aeruginosa*, it has been speculated that EM may affect the virulence factors of this organism (14), host defense mechanisms (6, 7, 13), or both. Kita et al. (14) have shown that EM suppresses the production of proteases and leukocidin by *P. aeruginosa* without affecting cell growth. Some investigators have reported that EM enhances such polymorphonuclear neutrophil functions as chemotaxis, ingestion, and bactericidal activity (6, 7). More recently, Hirakata et al. (10, 11) reported the efficacy of EM against *P. aeruginosa* bacteremia in experimental mouse models (11) and the potential for use of this antibiotic as an immunomodulator or bacterial virulence-suppressing agent (10). Because EM interferes with bacterial protein synthesis, it is possible that a subinhibitory concentration of this antibiotic may alter superficial components of bacteria, inhibit extracellular products, and consequently, reduce the virulence of *P. aeruginosa*. However, the exact mechanisms of the efficacy of EM against *P. aeruginosa* infection are still unclear.

Serum bactericidal activity has been regarded as one of the most important host defense mechanisms against bacterial infections (27). In the past several years, various classes of antibiotics at sub-MICs have been reported to enhance bacterial sensitivity to serum (1–3, 21, 28, 29). However, to our knowledge there have been no reports concerning the effects of macrolide antibiotics on the serum sensitivity of *P. aeruginosa*.

The aim of the present study was to investigate the effects of subinhibitory concentrations of EM and other macrolide antibiotics on the serum sensitivity of *P. aeruginosa*. In the present investigation, the serum sensitivity of bacteria grown on agar with or without antibiotic was compared on the basis of the percentage of viable bacteria present after incubation with serum. The change in the cell surface hydrophobicity of *P. aeruginosa* S-6 after treatment with macrolide antibiotics was also tested.

MATERIALS AND METHODS

Bacterial strain. *P. aeruginosa* S-6 was used to examine the effects of growth time, concentrations of EM, heat inactivation of serum, various macrolides, and other classes of antibiotics on serum sensitivity and was also used for experiments of hydrophobicity. This strain was a clinical isolate from the sputum of a patient with chronic pulmonary

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disease from Nagasaki University Hospital, Nagasaki, Japan. It showed a nonmucoid phenotype. To investigate whether EM is able to sensitize other strains of *P. aeruginosa* to serum bactericidal activity, an additional 12 strains of clinical isolates (6 strains of the nonmucoid phenotype and 6 strains of the mucoid phenotype) were used for serum sensitivity assays.

Antibiotics. The following 14-membered macrolide antibiotics were kindly provided by the indicated manufacturers: EM, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan; clarithromycin (CAM), Taisho Pharmaceutical Co., Ltd., Tokyo, Japan; and oleandomycin (OM), Pfizer Laboratories, Groton, Conn. The following 16-membered macrolide antibiotics were provided by the indicated manufacturers: kitasamycin (LM), Asahi Chemical Industry Co., Ltd., Tokyo, Japan; josamycin (JM), Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan; and rokitamycin (RKM), Asahi Chemical Industry Co., Ltd. Four antibiotics representing nonmacrolide classes—ceftazidime (CAZ; Tanabe Pharmaceutical Co., Ltd., Osaka, Japan), ofloxacin (OFLX; Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), tobramycin (TOB; Shionogi Pharmaceutical Co., Ltd., Osaka, Japan), and clindamycin (CLDM; Japan UpJohn Co., Ltd., Tokyo, Japan)—were used.

Antimicrobial susceptibility testing. The MICs of the antibiotics for *P. aeruginosa* S-6 were determined by the agar dilution method by using serial twofold dilutions of each individual antibiotic in Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.). Approximately 10^5 log-phase organisms were inoculated onto the agar containing antibiotics, and the MICs were determined after 18 h of incubation at 35°C and were defined as the lowest concentrations of antibiotics that inhibited the visible growth of bacteria (19).

Serum. Blood was obtained by venipuncture from one of three healthy volunteers for each experiment and was allowed to clot at 4°C for 2 h. After centrifugation at 1,000 × *g* for 15 min at 4°C, serum was diluted to suitable concentrations in physiological saline and was used immediately for serum sensitivity assays. Specific antibody against *P. aeruginosa* S-6 was not detected in the serum by the bacterial slide agglutination test. To inactivate complement, serum was heated at 56°C for 30 min. Among the sera from the three donors, no substantial differences in the results of serum sensitivity were observed.

Serum sensitivity assay. Basically, after growth on Mueller-Hinton agar in the presence or absence of various antibiotics, the bacteria were harvested and suspended in physiological saline. After washing by centrifugation, each bacterial suspension was adjusted to a concentration equivalent to that of a 0.5 McFarland standard. Twenty-microliter portions of the bacterial suspensions were mixed with 2 ml of freshly prepared human serum solution. The bacterium-serum mixtures were incubated with gentle shaking at 35°C. Samples (20 μl) were removed after each incubation time for plate count analysis. The remaining viable bacterial counts were determined by using serially diluted samples spread on Mueller-Hinton agar and then overnight incubation at 35°C. Serum sensitivity was expressed as the percentage of viable bacteria before and after incubation with human serum. The *P. aeruginosa* strains used in the present study were substantially resistant to saline alone, and their viabilities after incubation in saline without serum were more than or were at least equal to the viabilities of nontreated bacteria incubated with serum.

Determination of bacterial surface hydrophobicity. Bacterial cell surface hydrophobicity was determined by examining the adherence of the bacteria to liquid hydrocarbon as described previously (24). Bacteria grown at 35°C for 48 h on agar with EM (1.5, 3, and 12 μg/ml), OM (12 μg/ml), RKM (12 μg/ml), or no antibiotic were suspended in physiological saline. After washing by centrifugation, the concentration of each bacterial suspension was adjusted to an optical density at 660 nm of 0.400. Two milliliters of *n*-hexadecane was added to 2 ml of each duplicate sample, and the two phases were mixed by vortexing for 2 min. The two phases were allowed to separate by letting the solution stand at room temperature for 20 min. The lower aqueous phase was collected, and the optical density at 660 nm was measured. The results were expressed as the percent decrease in the absorbance of the lower aqueous phase compared with the absorbance of the initial cell suspension.

Statistics. Student's *t* test was used to compare means, and a level of 5% was considered significant.

TABLE 1. Effects of growth time on serum sensitivity of *P. aeruginosa* S-6

Growth time (h)[a]	% Viable bacteria after incubation with 10% human serum for[b]:			
	30 min		90 min	
	Nontreated	EM treated	Nontreated	EM treated
12	107 ± 21	153 ± 32	108 ± 5	145 ± 38
24	152 ± 18	147 ± 22	105 ± 18	108 ± 30
36	155 ± 19	17 ± 3[c]	86 ± 7	3.6 ± 1.4[c]
48	124 ± 14	6.0 ± 0.4[c]	34 ± 7	2.6 ± 0.4[d]

[a] *P. aeruginosa* S-6 was grown on agar with or without 10 μg of EM per ml for various times.

[b] The percentage of viable bacteria after incubation with 10% human serum for 30 and 90 min is expressed as mean ± standard deviation (n = 3).

[c] $P < 0.01$ compared with the percentage of viable bacteria in the corresponding nontreated group.

[d] $P < 0.05$ compared with the percentage of viable bacteria in the corresponding nontreated group.

RESULTS

MICs of macrolides and other classes of antibiotics for *P. aeruginosa* S-6. The MICs of macrolide antibiotics for *P. aeruginosa* S-6 were 200, 200, 100, 100, 100, and 400 μg/ml for LM, JM, RKM, EM, CAM, and OM, respectively. The MICs of the other classes of antibiotics were 3.13, 0.78, 0.78, and >400 μg/ml for CAZ, OFLX, TOB, and CLDM, respectively.

Effects of growth time on serum sensitivity of *P. aeruginosa* S-6. In *P. aeruginosa* S-6 grown for 12 and 24 h on agar with 10 μg of EM per ml, there were no significant changes in the serum sensitivity between EM-treated and nontreated bacteria, although the viabilities of the bacteria grown for 12 h with EM increased after incubation with serum. In contrast, bacteria grown with EM for 36 and 48 h became significantly more sensitive than nontreated bacteria to serum bactericidal effects (Table 1). For bacteria grown for 48 h without EM, survival was 34% ± 7% after incubation for 90 min with 10% serum, whereas for bacteria grown for 48 h with EM, survival was only 2.6% ± 0.4% ($P < 0.05$).

Effects of concentrations of EM on serum sensitivity of *P. aeruginosa* S-6. As shown in Table 2, EM increased the serum sensitivity of *P. aeruginosa* S-6 in a concentration-dependent manner. Even at a concentration of 1.5 μg/ml, bacteria grown with EM were significantly more sensitive

TABLE 2. Effects of EM concentrations on serum sensitivity of *P. aeruginosa* S-6

EM concn (μg/ml)[a]	% Viable bacteria after incubation with 10% human serum for[b]:	
	30 min	90 min
No antibiotic	80 ± 25	56 ± 5
1.5	57 ± 2	26 ± 3[c]
3	30 ± 7[d]	10 ± 4[c]
6	11 ± 6[d]	7 ± 2[c]
12	4 ± 1[d]	2.3 ± 0.4[c]

[a] *P. aeruginosa* S-6 was grown for 48 h on agar with various concentrations of EM.

[b] The percentage of viable bacteria after incubation with 10% human serum for 30 and 90 min is expressed as mean ± standard deviation (n = 3).

[c] $P < 0.01$ compared with the percentage of viable bacteria in the corresponding nontreated group.

[d] $P < 0.05$ compared with the percentage of viable bacteria in the corresponding nontreated group.

than nontreated bacteria to serum after incubation for 90 min with 10% serum ($P < 0.01$).

Effects of heat inactivation of serum on serum sensitivity enhanced by EM. When 10% heat-inactivated serum was used, bacterial killing was not observed in bacteria grown with and without 12 μg of EM per ml (data not shown). This result suggests that the increased serum sensitivity of *P. aeruginosa* S-6 following exposure to EM was due to enhanced sensitivity to complement-mediated killing.

Effects of other macrolide antibiotics on serum sensitivity of *P. aeruginosa* S-6. To examine whether the induced serum sensitivity is EM specific, the serum sensitivity of *P. aeruginosa* S-6 grown on agar with other macrolide antibiotics was examined (Fig. 1 and 2). Of the other macrolide antibiotics tested, CAM increased the serum sensitivity of *P. aeruginosa* S-6 in a concentration-dependent manner, whereas there were no changes in the serum sensitivities of bacteria treated with LM, JM, RKM, or OM, even at a concentration of these antibiotics of 12 μg/ml (1/16th, 1/16th, 1/8th, and 1/33rd the MICs, respectively).

Effects of other classes of antibiotics on serum sensitivity of *P. aeruginosa* S-6. As shown in Table 3, there were no changes in the serum sensitivity of *P. aeruginosa* S-6 grown for 24 and 48 h on agar with 0.1 μg of TOB per ml (1/8th the MIC), 0.1 μg of OFLX per ml (1/8th the MIC), 0.3 μg of CAZ per ml (1/10th the MIC), or 10 μg of CLDM per ml (<1/40th the MIC).

Change of cell surface hydrophobicity of *P. aeruginosa* S-6 after treatment with macrolide antibiotics. Bacteria grown with EM showed decreased cell surface hydrophobicities in a concentration-dependent manner. In particular, by treatment with 3 and 12 μg of EM per ml, bacterial hydrophobicity decreased to less than 50% of that for nontreated bacteria (36.6 and 29.7%, respectively). On the other hand, OM and RKM, even at a concentration of 12 μg/ml, induced only a slight decrease in cell surface hydrophobicity (63.7 and 51.3% of those for nontreated bacteria, respectively); this decrease in hydrophobicity was approximately equivalent to that obtained with 1.5 μg of EM per ml (Table 4).

Effects of EM on serum sensitivity of other strains of *P. aeruginosa*. The viabilities of six untreated strains of the nonmucoid phenotype were between 29.5 and 68.4% after incubation with 10% serum. Among the strains of the nonmucoid phenotype, three strains became more serum sensitive by exposure to EM; the viabilities of these three strains grown with EM decreased to less than 50% of that of nontreated bacteria after incubation with 10% serum (Fig. 3A). In contrast, the results obtained with bacteria of the mucoid phenotype were more variable. All six mucoid strains were highly serum sensitive, and incubation with 2% serum decreased the viabilities of nontreated bacteria to less than 0.1% (Fig. 3B). By exposure to EM, the viabilities of some mucoid strains decreased in experiments with 0.4% serum but, conversely, increased in experiments with 2% serum. The results of the serum sensitivity assay with 2 and 0.4% serum indicated no evident correlation between EM

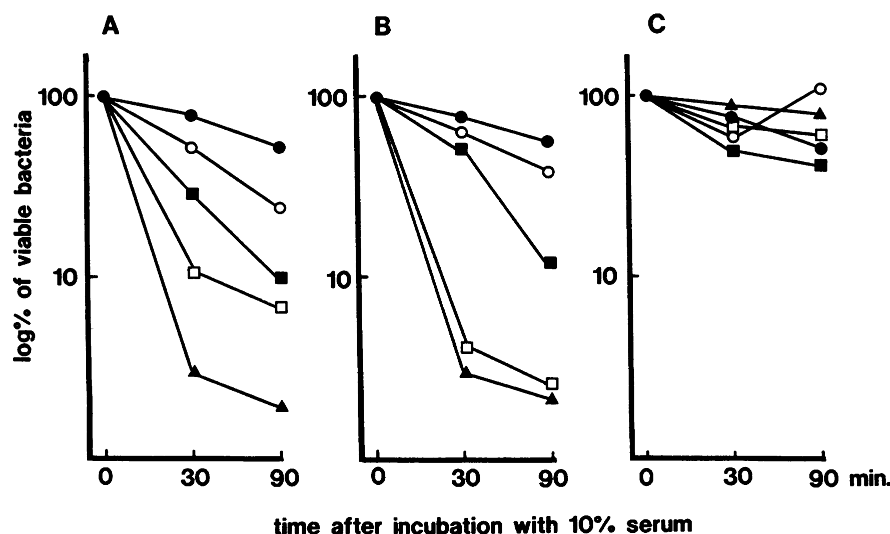

FIG. 1. Effects of 14-membered macrolide antibiotics on serum sensitivity of *P. aeruginosa* S-6. *P. aeruginosa* S-6 was incubated for 48 h on agar with EM (A), CAM (B), or OM (C). Symbols: ●, no antibiotic (control); ○, 1.5 μg/ml; ■, 3 μg/ml; □, 6 μg/ml; ▲, 12 μg/ml. Each bacterial suspension was incubated with 10% human serum for 30 and 90 min. The results are expressed as the mean percentages of viable bacteria (n = 2).

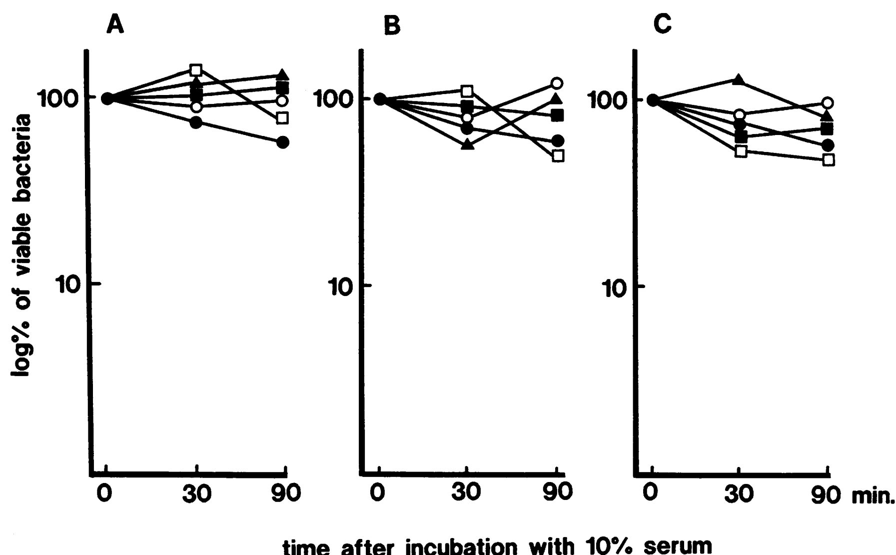

FIG. 2. Effects of 16-membered macrolide antibiotics on serum sensitivity of *P. aeruginosa* S-6. *P. aeruginosa* S-6 was incubated for 48 h on agar with LM (A), JM (B), or RKM (C). Symbols: ●, no antibiotic (control); ○, 1.5 μg/ml; ■, 3 μg/ml; □, 6 μg/ml; ▲, 12 μg/ml. Each bacterial suspension was incubated with 10% human serum for 30 and 90 min. The results are expressed as the mean percentages of viable bacteria (n = 2).

treatment and a change in serum sensitivity in these mucoid strains (Fig. 3B and C).

DISCUSSION

At the beginning of the present experiments, we noticed that longer exposure of *P. aeruginosa* S-6 to EM induced more apparent differences in the colony morphologies between EM-treated and nontreated bacteria; the rough characteristic of the colony morphology of nontreated bacteria changed to less rough by a longer exposure to EM. Upon microscopic examination of Gram-stained smears, no apparent alteration except for a weaker fuchsin color in EM-treated bacteria was observed. Since changes in colony morphology are usually caused by alterations in the cell surface structures of bacteria, which frequently lead to changes in susceptibility to serum, we examined the serum sensitivity of *P. aeruginosa* S-6 at various growth times with EM.

This study showed that EM at sub-MICs was able to render *P. aeruginosa* S-6 significantly more susceptible to serum. This observation was apparent only for bacteria grown with EM for more than 36 h. However, the enhanced serum sensitivity was observed not only in EM-treated bacteria but also, to a lesser extent, in bacteria grown for 48 h in the absence of EM, as shown in Table 1. This observation suggested that longer growth on agar, by itself, may make bacteria more fragile to the serum bactericidal effect, and EM may augment this fragility of bacteria.

On the other hand, neither LM, JM, or RKM (16-membered) nor OM (14-membered) increased the serum sensitivity of *P. aeruginosa* S-6. EM and CAM are both 14-membered macrolide antibiotics, and they share similar structures except for a difference of one side chain residue of the macrolide aglycone ring. Like EM and CAM, OM is also a 14-membered macrolide antibiotic. Although 1/67th the MIC of EM (1.5 μg/ml) induced a significant increase in the serum sensitivity of strain S-6, as shown in Table 2, no changes in serum sensitivity following exposure to a greater MIC of OM (1/33rd the MIC; 12 μg/ml) were observed (Fig. 1). These results suggest that the activities of EM and CAM for enhancing the serum sensitivity may be related to the fine structures of the antibiotics, such as the substitutions on the lactone ring and/or the sugar compositions of these antibiotics, in addition to their simple classification as being 14 or 16 membered. It is also possible that the effects of EM and CAM on the serum sensitivity of *P. aeruginosa* S-6 may be

TABLE 3. Effects of other classes of antibiotics on serum sensitivity of *P. aeruginosa* S-6

Growth time (h)[a]	% Viable bacteria after incubation with 10% human serum for 60 min[b]					
	No antibiotic	EM	TOB	OFLX	CAZ	CLDM
24	102	37	76	74	64	98
48	64	8	75	67	58	61

[a] *P. aeruginosa* S-6 was grown for 24 and 48 h on agar with EM (10 μg/ml), TOB (0.1 μg/ml), OFLX (0.1 μg/ml), CAZ (0.3 μg/ml), CLDM (10 μg/ml), or no antibiotic (control).
[b] The results are the means of two experiments.

TABLE 4. Change in cell surface hydrophobicity of *P. aeruginosa* S-6 after treatment with macrolide antibiotics

Antibiotic[a]	Concn (μg/ml)	% Hydrophobicity[b] (% of that with no antibiotic)
No antibiotic		43.5 (100.0)
EM	1.5	24.3 (55.9)
	3	15.9 (36.6)
	12	12.9 (29.7)
OM	12	27.7 (63.7)
RKM	12	22.3 (51.3)

[a] *P. aeruginosa* S-6 was grown for 48 h on agar with various concentrations of macrolide antibiotics.
[b] The results are expressed as the means of two experiments.

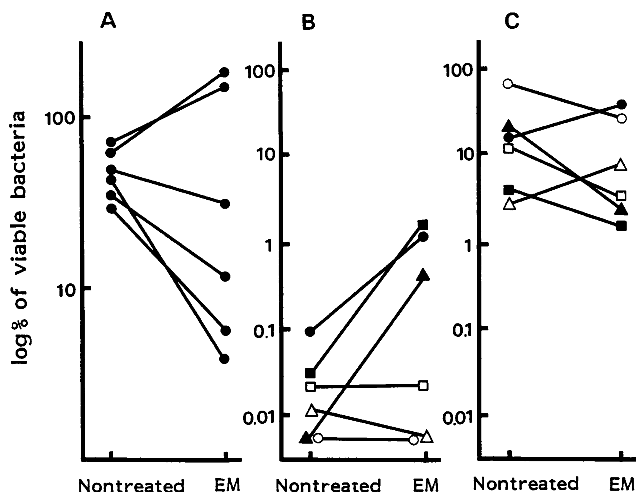

FIG. 3. Effects of EM on serum sensitivity of other strains of *P. aeruginosa*. Nonmucoid (six strains) and mucoid (six strains) phenotype strains of *P. aeruginosa* were grown for 48 h with or without 10 μg of EM per ml. Nonmucoid strains were incubated for 90 min with 10% serum (A), and mucoid strains were incubated for 90 min with 2% serum (B) and 0.4% serum (C). The results are expressed as the mean percent viability of nontreated and EM-treated bacteria (n = 2). In panels B and C, corresponding strains are represented by same symbols.

controlled by other mechanisms besides the direct bactericidal activities of these antibiotics.

For the other classes of antibiotics tested (TOB, OFLX, CAZ, and CLDM), no effects on the serum sensitivity of *P. aeruginosa* S-6 grown for 24 and 48 h were observed with these antibiotics. Like EM, TOB and CLDM are both protein synthesis inhibitors of bacteria. It is possible that enhancement of the serum sensitivity of *P. aeruginosa* S-6 after growth on agar with antibiotics is specific for some macrolide antibiotics such as EM and CAM.

Increased serum sensitivity was accompanied by a greater than 50% decrease in bacterial surface hydrophobicity in strain S-6 grown with 3 and 12 μg of EM per ml (Table 4). The bacterial structures that affect cell surface hydrophobicity include outer membrane proteins, lipoproteins, phospholipids, lipopolysaccharides (LPSs), and fimbriae (pili) (17, 18). Among these components, the contribution of fimbriae in *P. aeruginosa* to cell surface hydrophobicity has been investigated extensively (23, 30). The fimbriae of *P. aeruginosa* are known to be proteinaceous polar structures with highly hydrophobic domains (23, 30) and are classified as *N*-methylphenylalanine fimbriae (23). Speert et al. (26) reported that *P. aeruginosa* grown on agar is piliated, hydrophobic, and susceptible to nonopsonic phagocytosis, whereas bacteria grown to the stationary phase in broth with shaking were nonpiliated, less hydrophobic, and resistant to phagocytosis. Recently, it was reported (9) that sub-MICs of azithromycin, a new 15-membered ring azalide synthesized from EM, inhibit the expression of pili in *Neisseria gonorrhoeae*, which is known to possess the same *N*-methylphenylalanine pili as *P. aeruginosa* (23). However, there were no reports concerning the relation between piliation and serum sensitivity in *P. aeruginosa*.

It is known that LPS is an important component responsible for the serum sensitivity of *P. aeruginosa* (25). Concerning the effects of antibiotics on bacterial LPS, monobactam has been reported to inhibit O side chain formation in *Escherichia coli* (22). Kusser and Ishiguro (15) have also reported that aminoglycoside antibiotics, which are bacterial protein inhibitors like macrolide antibiotics, influence the LPS synthesis of *E. coli*. We speculate that EM may enhance the serum sensitivity of *P. aeruginosa* S-6 by changing the cell surface structure(s). Further experiments are necessary to investigate the effects of EM on the molecular changes of bacterial components, such as expression of fimbriae, the structure and amount of LPS, or other factors responsible for enhanced serum sensitivity.

Colonizing strains of *P. aeruginosa* in patients with cystic fibrosis have been known to be, for the most part, mucoid strains (5). In the present study, no apparent correlation between EM treatment and a change in serum sensitivity was observed in six strains of the mucoid phenotype. All of these mucoid strains were highly serum sensitive, and incubation with 2% serum decreased the viabilities of nontreated bacteria to less than 0.1%. It is possible that these mucoid strains are so delicate to serum bactericidal activity that viability may be easily influenced by factors other than the presence or absence of EM, which may lead to variable results, as shown in Fig. 3B and C. In contrast, three of six strains of the nonmucoid phenotype became more serum sensitive by exposure to EM. It is likely that administration of EM prior to the isolation of *P. aeruginosa* may provide a chance to select for bacterial populations that are resistant to such effects of EM. Further studies are required to investigate the exact frequency of this phenomenon in clinical isolates of *P. aeruginosa* by considering the history of EM administration in each patient.

Since there is no standard method for estimating the serum sensitivity of bacteria, it has been reported that differences in in vitro conditions may lead to remarkable differences in the outcomes of comparable investigations (4). DeMatteo et al. (4) reported that the survival of *P. aeruginosa* in serum was highly dependent on in vitro conditions, and the factors that appeared to contribute most to variations in survival were the medium used for inoculum preparation, the concentration of serum in the reaction mixture, and the growth phase of the inoculum. Usually, bacteria grown in broth medium are used to assay serum sensitivity. In the present study, we used bacteria grown on agar with or without antibiotics for the preparation of inocula. In the respiratory tract or alveolar space of patients with persistent *P. aeruginosa* infections, bacteria may exist with secreted mucus, with host cell debris, or on the surface of respiratory epithelial cells. It is speculated that some of those bacteria may grow not only in an environment like liquid broth medium but also in an environment like an agar plate. The effects of EM on bacteria growing on agar may serve as a model for the interactions between antibiotics and the bacteria growing on surfaces in an in vivo environment.

The concentrations of EM used in the present study are still far greater than those that can be achieved clinically, although 1.5 μg of EM per ml was reported to be an achievable concentration in the plasma of patients with chronic bronchitis (16). In addition, the milieu in the lung of the patient with chronic *P. aeruginosa* infection may be very different from that in serum. The clinical importance of the effects of EM and other macrolide antibiotics on the serum sensitivity of *P. aeruginosa*, or even whether this phenomenon occurs in vivo, remains to be determined.

In conclusion, we showed that sub-MICs of EM can increase the serum sensitivity of some *P. aeruginosa* strains grown on agar with this antibiotic.

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