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Effects of Subminimal Inhibitory Concentrations of Erythromycin, Clindamycin, and Pristinamycin on the Penicillinase Production of *Staphylococcus aureus*

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The influence of subminimal inhibitory concentrations of erythromycin, clindamycin, and pristinamycin on the penicillinase production of *Staphylococcus aureus* was tested in 12 strains. Of the 36 experiments performed, 16 (44%) showed a lack of influence, 10 (28%) displayed an increase, and 10 revealed a decrease in penicillinase activity. The maximal effect produced was generally induced by concentrations ranging from ¼ to 1/32 the minimal inhibitory concentration, irrespective of the susceptibility of the strain to the drug. In spite of the fact that the drugs are closely related, they sometimes produced opposite effects on the same strain.

The problem of the effects of subminimal inhibitory concentrations (subMIC's) of antibacterial drugs has been investigated in the last few years and was recently discussed by Lorian (9) and Rolinson (15). Most of the available data concern the influence of various agents on bacterial growth (2, 6, 17) and the morphological changes induced by the subMIC's of β-lactam antibiotics (4, 8, 10, 12). Only a few papers have dealt with the effects of the subMIC on bacterial enzymatic systems. Inducible enzymes were shown to be affected by the subMIC's of nitrofurans (7) and chloramphenicol (18). Gemmell has reported the influence of the subMIC's of antibiotics, especially clindamycin, on the biosynthesis of some staphylococcal toxins and enzymes during experimental pyogenic infection (5). We have shown that the subMIC of chloramphenicol can inhibit β-lactamase production in gram-negative bacilli (13) and in *Staphylococcus aureus* (14) and that this inhibition sometimes leads to a synergistic bactericidal effect in vitro (13, 14) and in vivo (16). More recently, Allen and Epp have shown that erythromycin can inhibit penicillinase induction in strains of *S. aureus* (1). To the best of our knowledge, a comparative study on the effect of closely related drugs on penicillinase production has not been reported in the literature. The purpose of the present paper was, therefore, to investigate the influence of subMIC's of erythromycin, clindamycin, and pristinamycin on the penicillinase production of strains of *S. aureus*.

MATERIALS AND METHODS

Twelve strains of penicillin G-resistant *S. aureus* were used. These were isolated in the diagnostic laboratory of the Department of Clinical Microbiology, Hadassah University Hospital, Mount Scopus, Jerusalem, from sputum, wound exudates, urine, and blood. Each was tested for β-lactamase production by the iodometric method, as described by Workman and Farrar (21). The minimal inhibitory concentrations (MICs) of erythromycin, clindamycin, and pristinamycin for these strains were determined by using a microdilution technique. Serial twofold dilutions of the antibiotic were made in tryptic soy broth (TSB; Difco Laboratories) in sterile Microtiter U-plates (Cook Engineering Co.). A suitable dilution of a 4-h culture of the bacteria in TSB at 37°C was prepared in TSB and added to the wells of the microtiter plates to give a final inoculum of 10^5 colony-forming units (CFU)/ml. The plates were incubated at 37°C, and the results were recorded at 24 h. Erythromycin-inducible resistance was tested by the disk method, as described by Weisblum and Demohn (20).

The influence of the subMIC's of erythromycin, clindamycin, and pristinamycin on penicillinase production was tested by incubating the test organism, with shaking, at 37°C in TSB containing 0.5 μg of methicillin per ml as an inducer of β-lactamase production and various concentrations of erythromycin, clindamycin, or pristinamycin, ranging from 1:2 to 1:1,024 of the respective MICs. Controls were performed in TSB containing 0.5 μg of methicillin per ml. After overnight incubation, the number of CFU per milliliter was determined in each sample, and the culture was centrifuged at 11,000 × *g* for 10 min. The supernatant fluid was then tested for penicillinase activity by using the timed iodometric method as described by Citri (3). Each experiment was duplicated or triplicated.

The results were interpreted by the following criteria: (i) only concentrations giving a number of CFU per milliliter equal to that of the control were considered; (ii) the penicillinase activity in these subMIC's was compared with that of the control, and if the difference was less than 50% of the activity of the control, the result was classified as a lack of influence

of the subMIC's of the antibiotics; when, in the presence of a subMIC, the penicillinase activity was 150% or more than that of the control, the results were classified as an increase in penicillinase activity in the presence of this subMIC; when the penicillinase activity was 50% or less than that of the control, the results were classified as a decrease in penicillinase activity by the particular subMIC of the drug.

The influence of erythromycin, clindamycin, and pristinamycin on preformed penicillinase was tested on a crude preparation of the enzyme obtained from each strain grown in TSB containing 0.5 μg of methicillin per ml as an inducer. After overnight incubation, with shaking, at 37°C, the cultures were centrifuged at 11,000 × *g* for 10 min. The antibiotic was then added to a portion of the supernatant fluid at a final concentration of 500 μg/ml, and another portion was used as control. After 30 min at 37°C, the penicillinase activity was measured in both samples.

TABLE 1. *Susceptibility of S. aureus to erythromycin, clindamycin, and pristinamycin (12 strains)*

Strain no.	MIC (μg/ml)		
	Erythromycin	Clindamycin	Pristinamycin
1	>500.0	>500.0	0.50
2	1.0	0.25	0.25
3	0.25	0.25	0.25
4	1.0	0.25	0.50
5	0.50	0.25	0.25
6	>500.0	>500.0	1.0
7	1.0	0.25	0.50
8	>500.0	0.25	0.50
9	>500.0	>500.0	0.50
10	64.0	0.25	0.25
11	500.0	>500.0	4.0
12	>500.0	>500.0	0.25

RESULTS

The susceptibility of the strains to the drugs is shown in Table 1. Two strains were resistant to erythromycin alone (no. 8 and 10), five were resistant to both erythromycin and clindamycin (no. 1, 6, 9, 11, and 12), and all but one were susceptible to pristinamycin (strain no. 11 was relatively resistant to this drug). None of the erythromycin-susceptible strains was found to be inducibly resistant to this antibiotic.

The maximal effects produced by a particular subMIC of the drugs are presented in Table 2. The results obtained in duplicated or triplicated experiments were identical. We observed fluctuations of only about 10% in the penicillinase activity of a particular strain in the presence of the same concentration of drug. In the 36 experiments performed, 16 (44%) showed a lack of influence, 10 (28%) showed an increase, and 10 showed a decrease in penicillinase activity. The subMIC's producing the maximal effect varied from <1:2 to 1:256 MIC for erythromycin, from 1:8 to 1:64 MIC for clindamycin, and from 1:4 to 1:32 MIC for pristinamycin. Other concentrations produced weaker effects.

The maximal increase observed ranged from +100 to +145% with erythromycin, from +104 to +280% with clindamycin, and from +125 to +235% with pristinamycin. The maximal decrease varied from −58 to −100% with erythromycin, from −56 to −100% with clindamycin, and from −69 to −100% with pristinamycin.

The strains reacted differently to the subMIC's of the three drugs; for example, in strain 4, we found an increase with the three drugs; in strain 8 there was a decrease with the

TABLE 2. *Maximal effects produced by the subMIC's of erythromycin, clindamycin, and pristinamycin on the penicillinase production of S. aureus (10 strains)*[a]

Strain no.	Control PA[b]	Erythromycin			Clindamycin			Pristinamycin		
		Concn[c]	PA[b]	Effect[d]	Concn[c]	PA[b]	Effect[d]	Concn[c]	PA[b]	Effect[d]
1	40	<1/4	0	−100			—[e]			—
3	20			—	1/16	45	+125	1/32	45	+125
4	20	1/128	49	+145	1/8	76	+280	1/8	67	+235
5	225	1/16	95	−58	1/32	100	−56			—
6	90			—	<1/4	184	+104			—
7	11	1/256	22	+100			—	1/32	30	+173
8	90	<1/2	18	−80	1/64	20	−78	1/16	28	−69
10	90			—	1/8	33	−63			—
11	50	1/16	106	+112			—	1/16	0	−100
12	32	<1/8	0	−100	<1/32	0	−100	1/4	89	+178

[a] In strains 2 and 9, no effects were observed in duplicated experiments.
[b] PA, Penicillinase activity in units per milliliter (average of triplicated experiments).
[c] Expressed as a fraction of the MIC.
[d] Expressed as the percentage of increase (+), or decease (−) of the penicillinase activity by comparison with the control.
[e] —, No effect observed at any subMIC ranging from 1/2 to 1/1,024 the MIC.

three drugs; in strain 11 the subMIC of erythromycin produced an increase, the subMIC of pristinamycin produced a decrease, and the subMIC of clindamycin did not influence penicillinase activity. Increase and decrease in penicillinase activity was observed in both susceptible and resistant strains in the presence of subMIC's of the respective antibiotics.

The 500-μg/ml concentration of erythromycin, clindamycin, and pristinamycin failed to produce any significant change in the activity of preformed penicillinase.

DISCUSSION

To the best of our knowledge, this is the first report on the comparative effects of the subMIC's of closely related drugs on the production of staphylococcal penicillinase. Although the drugs used in this study are members of chemically distinct classes of antibiotics, they are known to bind to the 50S ribosomal subunit, interfering with the process of translocation (19).

The method used in the present work for the determination of the penicillinase activity is known to be accurate within ±10% (3). Our results indicate that subMIC's of erythromycin, clindamycin, and pristinamycin can affect the penicillinase production of *S. aureus*. The maximal effect produced was generally induced by a subMIC ranging from 1:4 to 1:32 of the MIC, irrespective of the absolute value of the MIC, i.e., of the susceptibility of the strain to the drugs. This supports the initial concept of "minimal antibiotic concentration," as defined by Lorian and Popoola (11).

In 28% of our experiments, we found that the penicillinase production was depressed by some subMIC's of the drugs. This is in accordance with the data on the inhibition of the β-lactamase production by other antibacterial drugs that inhibit protein synthesis (1, 7, 13, 14, 18). On the other hand, we found the same percentage of experiments in which subMIC's of the drugs increased the penicillinase production. Such a stimulation has not been reported for staphylococcal penicillinase.

Another point has to be emphasized. In spite of the fact that the drugs tested in this study act on the 50S ribosomal subunit, they sometimes induced opposite effects on the same strain. All these findings indicate the need for further investigations to clarify the mechanism by which erythromycin, clindamycin, and pristinamycin interfere with the process of penicillinase production in *S. aureus*.

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