INDUCIBLE RESISTANCE TO A 16-MEMBERED MACROLIDE, MYCINAMICIN, IN *STAPHYLOCOCCUS AUREUS* RESISTANT TO 14-MEMBERED MACROLIDES AND STREPTOGRAMIN B ANTIBIOTICS

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Staphylococcus aureus 8325(pEP2104), a transductant derived from *S. aureus* PM2104 isolated clinically in Hungary (L. Janosi, and E. Ban, *Acta Microbiol. Acad. Sci. Hung.*, **29**: 187-200, 1982), exhibited an inducible resistance to the 14-membered macrolides [erythromycin (EM) and oleandomycin (OL)] and streptogramin B (MKM-B) antibiotics, but not to the 16-membered macrolides and lincosamides. This resistance was referred to as PMS-resistance phenotype (L. János, Y. Nakajima, and H. Hashimoto, *Microbiol. Immunol.*, **34**: 723-735, 1990). In addition to EM, OL, and MKM-B, however, the strain was recently and first observed to have inducible resistance to mycinamicin, a 16-membered ring macrolide. Thereby, we propose that the reference stated just above as PMS-resistance has to be extended to such 16-membered macrolides as mycinamicin. An optimum concentration of erythromycin or oleandomycin for induction of PMS-resistance was $1.35 \mu\text{g/ml}$ in the strain 8325(pEP2104). The concentration was about 30 times as great as that ($0.05 \mu\text{g/ml}$) required for induction of well-known co-resistance to macrolide-lincosamide-streptogramin B antibiotics in *S. aureus* ISP447.

KEYWORDS — *Staphylococcus aureus*; macrolide antibiotics; inducible resistance; mycinamicin

INTRODUCTION

Staphylococcus aureus PM2104 isolated clinically in Hungary revealed a novel type of resistance to macrolide antibiotics: it carried a plasmid pEP 2104 with encoded resistances to 14-membered ring macrolides, i.e., erythro-

mycin (EM) and oleandomycin (OL), and streptogramin type B (MKM-B) antibiotics.^{2, 3} The plasmid was transducible into *S. aureus* NCTC8325 strain using a bacteriophage 80.⁴ The transductant 8325(pEP2104) has been recognized to express inducible resistances to some drugs, such as 14-membered ring macrolides (MIC 100 μ g/ml) and MKM-B (MIC 6.25), but susceptibility to 16-membered ring macrolides (MIC 0.78-3.13, except that the MIC of mycinamicin (MCM) is 0.39-0.78, if the drug-resistance being uninduced) and lincosamides (MIC 0.1-0.39). A similar resistance phenotype which is resistant to 14-membered macrolides and type B streptogramins except 16-membered macrolides and lincosamides has been observed in clinical isolates of coagulase-negative staphylococci from USA⁵ and the UK^{6, 9}: this phenotype has been designated as an MS-resistance.

During the course of study on inducibility of resistance to several 16-membered macrolides by EM using the transductant 8325(pEP2104), this strain was discovered to show inducible resistance to MCM, a 16-membered macrolide, in addition to showing inducible resistance to 14-membered macrolides and MKM-B.

The present paper concerns inducible resistance to MCM in the strain 8325 (pEP2104).

MATERIALS AND METHOD

Bacteria — *Staphylococcus aureus* 8325(pEP2104), a strain which is a transductant of NCTC8325, a typically susceptible standard strain, with pEP2104 from a wild type *S. aureus* strain PM2104,^{2, 3, 4} was used. The plasmid pEP2104 has been described as a plasmid which encodes resistance to 14-membered ring macrolides and streptogramin type B antibiotic (PMS), Hg²⁺ and Cd²⁺ ion resistance and penicillinase production. *S. aureus* strain ISP447, formerly U9,¹⁰ shows inducible MLS-type resistance.

Induction of Resistance — (1) The inducibility of EM was assayed by the agar disc diffusion test. Soft agar (8ml) including bacteria (10⁶ cells) was poured on to previously hardened base agar (40ml) containing peptone, 5; yeast extract, 5; K₂HPO₄, 1; glucose, 2; agar, 20g per liter. The soft agar had the same composition as the base agar except for 5g in substitution for 20g agar. Discs impregnated with the antibiotics indicated in Table I were laid on each plate seeded previously with a tested strain, so as to be arranged in three rows. However, all discs in the second row included EM [10 μ g/disc (A, B, and C)] or OL [10 μ g/disc (D)]. The plate was incubated at 37°C for 18 to 20 h.

(2) The Induction of resistance was assayed by alternative and turbidimetric methods. An overnight culture of the strain grown in trypticase soy broth was diluted 1:9 (ca. 10^7 cells/ml) with fresh culture medium H (pH 7.6), i.e., 50 mM potassium *N*-2-hydroxyethyl-piperazine-*N'*-2-ethane-sulfonate (K-HEPES) together with tryptone, 5; beef extract, 3; yeast extract, 2g per liter. After 3-h of incubation at 37°C, each 1-ml culture was further diluted 1:9 with the medium H containing EM or OL (0.017, 0.05, 0.15, 0.45, 1.35, or 4.05 μ g/ml), and a single 1-ml culture with antibiotic-free medium H (as a control). After the cultures were incubated at 37°C for 3 h while being shaken, they were diluted 1:3 with the medium H containing the challenging drugs indicated in the legends of Fig. 3 and 4. Bacterial growth was turbidimetrically determined either after 5-h incubation in the MCM-medium H (Fig. 3) or at 1-h intervals (Fig. 4).

Table I. Antibiotics Used and Their Amount (μ g) per Disc Laid on the Plates Shown in Fig. 2

Strain	Antibiotic (μ g/disc)											
	OL	TS	MDM	MOM	RSM	LCM	SPM	JM	RKM	MCM	MKM-B	CLDM
NCTC8325	25	10	10	10	10	5	25	10	10	10	25	5
8325(pEP2104)	25	10	10	10	10	5	25	10	10	10	25	5
ISP447	25	25	25	25	25	5	50	25	25	25	50	5

Abbreviations: OL, oleandomycin; TS, tylosin; MDM, midecamycin; MOM, miokamycin; RSM, rosamicin; LCM, lincomycin; SPM, spiramycin; JM, josamycin; RKM, rokitamycin; MCM, mycinamicin; MKM-B, streptogramin B, CLDM, clindamycin.

Antibiotics — Chemical structures of EM, MCM, and rosamicin (RSM), nearly similar to MCM are shown in Fig. 1.

Fig. 1. The Chemical Structure of Erythromycin A, Rosamicin⁷⁾ and Mycinamicin I¹¹⁾

RESULTS AND DISCUSSION

Resistance to MCM and MKM-B in the PMS-resistant strain was induced by EM or OL, since the strain formed a D-shape-inhibited zone around the discs of MCM and MKM-B (Fig. 2). On the contrary, induction (by EM) of resistance to

all macrolide, lincosamide and MKM-B (MLS) antibiotics was observed in an inducible MLS-resistant strain ISP447. The amount of N^6 , N^6 -dimethyladenine in 23S ribosomal RNA from EM-induced cells of the strain ISP447 was twice as much as that from (uninduced) cells of the same strain.¹⁾ This observation confirmed the report of Lai and Weisblum.⁶⁾ A susceptible strain NCTC8325 produced no D-shape zones (Fig. 2).

When the mode of induction of resistance by EM or OL was turbidimetrically assayed by using a slightly modified liquid medium,⁵⁾ the optimal concentrations for the induction were $1.35 \mu\text{g}$ EM or OL/ml in strain NCTC8325, and $0.05 \mu\text{g}$ EM/ml in strain ISP447 (Fig. 3).

Figures 2 and 4 show that strain 8325(pEP2104) is inducibly resistant to MCM (the 16-membered macrolide but not to RSM), EM, OL, and MKM-B. The inducible resistance to the latter three antibiotics has already been described in a previous paper.^{2, 4)} The most favorable concentrations for the induction of EM-, OL-, and MCM-resistance in the strain 8325(pEP2104) were $1.35 \mu\text{g}$ of either EM or OL per ml (Fig. 3). However, data on the induction of resistance to EM and OL, as challenging drugs, ($20 \mu\text{g}/\text{ml}$) were not shown in this text.

A marked difference was found between the optimal EM-concentration ($1.35 \mu\text{g}/\text{ml}$) required for induction of PMS-resistance and that ($0.05 \mu\text{g}/\text{ml}$) required for induction of MLS-resistance (Fig. 3).

Fig. 2. Effect of Erythromycin or Oleandomycin on the Sensitivity of *Staphylococcus aureus* NCTC8325, 8325(pEP2104), and ISP447

Resistance to the other 16-membered macrolides, josamycin, rokitamycin, tylosin, and RSM, on the contrary, was not inducible by EM (Fig. 2 and 4), or OL (Fig. 2).

MCM, though a 16-membered macrolide antibiotic, appears to belong to EM or OL, since they have a common feature, two monosaccharides.

Fig. 3. Effect of Concentration of Either Erythromycin or Oleandomycin on Ability to Induce Mycinamicin-Resistance in *Staphylococcus aureus* 8325(pEP2104) and ISP447

Symbols: ● and ○, strain 8325(pEP2104) exposed to the indicated concentrations of EM and OL, respectively, was challenged by MCM (3 μg/ml); △, strain ISP447 exposed to those of EM was challenged by the same concentration as the MCM.

Fig. 4. Effect of Erythromycin on Induction of Resistance to Macrolide and Streptogramin B Antibiotics in *Staphylococcus aureus* 8325(pEP2104)

The concentrations of challenge antibiotics: erythromycin (A), oleandomycin (B), streptogramin B (C), mycinamicin I (D), josamycin (E), rokitamycin (F), tylosin (G), and rosamicin (H) were 20, 20, 15, 3, 10, 10, 5, and 5 μg/ml, respectively. Some cells were grown in the presence of a challenging drug after being exposed (●) or not (○) to an inducer EM (1.35 μg/ml), and other cells were grown without exposures to the inducer or a challenging drug (△).

* The results obtained in the tests of F, G, H were also the same profile as the result of E.

Recently, Ross et al.⁶³ have demonstrated that an active (energy-dependent) efflux of EM from cells of *S. aureus* RN4220 containing *msrA* gene (located on a plasmid pUL5050 which originated from one coagulase-negative strain of *S.*

epidermidis) takes place. It is still uncertain whether a gene encoding for PMS-resistance is related to the *msrA* gene responsible for MS-resistance.⁸⁾ A study on the mechanism of PMS-resistance is in progress.

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