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

Yoshinori NAKAJIMA,* László JÁNOSI,** Kikutarou ENDOU,* Mayumi MATSUOKA,* and Hajime HASHIMOTO***

Division of Microbiology, Hokkaido Institute of Pharmaceutical Sciences,* 7-1 Katsuraoka-cho, Otaru, Hokkaido 047-02, National Institute of Hygiene,** Budapest, Hungary, and Department of Microbiology, Gunma University School of Medicine,*** Maebashi, Gunma 371, Japan

_Staphylococcus aureus_ 8325(pEP2104), a transductant derived from _S. aureus_ PM2104 isolated clinically in Hungary (L. Jánosi, 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. Jánosi, 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 μg/ml in the strain 8325(pEP2104). The concentration was about 30 times as great as that (0.05 μg/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.\textsuperscript{2, 3}\textsuperscript{2} The plasmid was transducible into S. aureus NCTC8325 strain using a bacteriophage 80.\textsuperscript{4} The transductant 8325(pEP2104) has been recognized to express inducible resistances to some drugs, such as 14-membered ring macrolides (MIC 100 \(\mu\) g/ml) and MKM-B (MIC 6.25), but susceptibility to 18-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 USA\textsuperscript{2} and the UK\textsuperscript{6, 9}: this phenotype has been designated as an MS-resistance.

During the course of study on inducibility of resistance to several 18-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).

\textbf{MATERIALS AND METHOD}

\textbf{Bacteria} — \textit{Staphylococcus aureus} 8325(pEP2104), a strain which is a transductant of NCTC8325, a typically susceptible standard strain, with pEP 2104 from a wild type S. aureus strain PM2104,\textsuperscript{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), Hg\(^{2+}\) and Cd\(^{2+}\) ion resistance and penicillinase production. S. aureus strain ISP447, formerly U8,\textsuperscript{10} shows inducible MLS-type resistance.

\textbf{Induction of Resistance} — (1) The inducibility of EM was assayed by the agar disc diffusion test. Soft agar (8\textsuperscript{cml}) including bacteria (10\textsuperscript{6} cells) was poured on to previously hardened base agar (40\textsuperscript{cml}) containing peptone, 5; yeast extract, 5; \(K_2HPO_4\), 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 \(\mu\) g/disc (A, B, and C)] or OL [10 \(\mu\) g/disc (D)]. The plate was incubated at 37\textdegree C for 18 to 20 h.
The Induction of resistance was assayed by alternative and turbidimetric methods. An overnight culture of the strain grown in tryptcase soy broth was diluted 1:9 (ca. $10^7$ cells/ml) with fresh culture medium H (pH7.6), 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 μg/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 (μg) per Disc Laid on the Plates Shown in Fig. 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>OL</th>
<th>TS</th>
<th>MDM</th>
<th>MOM</th>
<th>RSM</th>
<th>LCM</th>
<th>SPM</th>
<th>JM</th>
<th>RKM</th>
<th>MCM</th>
<th>MKM-B</th>
<th>CLDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCTC8325</td>
<td>25</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>25</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>8325(pEP2104)</td>
<td>25</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>25</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

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

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

![Chemical structures of Erythromycin A, Rosaminic, and Mycinamicin](image)

Fig. 1. The Chemical Structure of Erythromycin A, Rosaminic and Mycinamicin.

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<sup>9</sup>, N<sup>9</sup>-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. This observation confirmed the report of Lai and Weisblum. 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, the optimal concentrations for the induction were 1.35 μg EM or OL/ml in strain NCTC8325, and 0.05 μg 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. The most favorable concentrations for the induction of EM-, OL-, and MCM-resistance in the strain 8325(pEP2104) were 1.35 μg of either EM or OL per ml (Fig. 3). However, data on the induction of resistance to EM and OL, as challenging drugs, (20 μg/ml) were not shown in this text.

A marked difference was found between the optimal EM-concentration (1.35 μg/ml) required for induction of PMS-resistance and that (0.05 μg/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(pEP 2104) 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. have demonstrated that an active (energy-dependent) efflux of EM from cells of S. aureus RN4220 containing mrsA 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 marA gene responsible for MS-resistance. A study on the mechanism of PMS-resistance is in progress.

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REFERENCES


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