Resistance to Macrolide-Lincosamide-Streptogramin B Antibiotics is Induced by 16 Membered-ring Macrolide Antibiotics in *Enterococcus faecalis* 373

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Macrolide, lincosamide, and streptogramin B (MLS) antibiotics are chemically distinct but have a similar mode of action. They act by binding to the 50S ribosomal subunit of ribosome and consequently inhibit protein synthesis. The most common mechanism of resistance to these antibiotics is the specific methylation of 23S rRNA resulting in a decreased ribosomal affinity for MLS antibiotics (Lai *et al.*, 1971). The specific rRNA methylase is encoded by *erm* gene family, which is widespread among the bacteria including *Staphylococcus* (Lai *et al.*, 1971), *Enterococcus* (Couvalin *et al.*, 1972), *Streptococcus* (Horinouchi *et al.*, 1983), *Bacteroides* (Salaki *et al.*, 1976), *Corynbacterium* (Coyle *et al.*, 1979), *Bacillus* (Kim *et al.*, 1993, Kwak *et al.*, 1991), *Clostridium* (Wilkins *et al.*, 1973), and *Streptomyces* (Fujisawa *et al.*, 1981).

MLS resistance of this type is divided into two subtypes. One is constitutively expressed one where the bacteria is resistant to all of the MLS antibiotics without prior exposure to them. The other is inducible type, where the bacteria become resistant to MLS antibiotics only with prior or simultaneous exposure to one or another MLS antibiotics (Weisblum *et al.*, 1969). In case of *ermC*, inducible resistance is regulated by translational atteunuation mechanism in which inducing antibiotics interact with susceptible ribosomes and inhibit translation of leader peptide during induction (Horinouchi *et al.*, 1980). This results in the conformational change of leader sequence and consequent translation of rRNA methyltransferase. The inducers of MLS resistance are generally 14 membered-ring macrolides (erythromycin and oleandomycin), and lincosamides (celesticetin and clindamycin). Erythromycin (EM) and oleandomycin (OM) can induce the expression of most *erm* gene family such as *ermC* (Horinouchi *et al.*, 1980), *ermA* (Murphy *et al.*, 1985), *ermAM* (Horinouchi *et al.*, 1983) and *ermK* (Kwak *et al.*, 1991). On the contrary, 16 membered-ring macrolides such as tylosin and josamycin, have been known for their inability to induce MLS resistance except in the cases of *Streptomyces* (Fujisawa *et al.*, 1981, Kamimiya *et al.*, 1997) and selected mutant strain (Tanaka *et al.*, 1974).

In the screening of MLS resistance strains among the clinically isolated gram-positive bacteria in Korea, we selected 34 EM resistance strains of different genera from 84 isolates, and characterized the resistance phenotypes of 34 EM resistance strains by agar disk diffusion test. Among them, eight strains of *Staphylococcus aureus* and one *Enterococcus faecalis* showed inducible phenotype. In this communication, we report the resistance phenotype of EM resistant *Enterococcus faecalis* 373 in detail, because it showed interesting inducible phenotype.

Enterococcus faecalis 373 was grown in Brain Heart Infusion broth (Difco) for agar disk diffusion test to characterize the inducible resistance phenotype. The disks used for determining the resistance phenotype pattern contained kinds of MLS antibiotics. Interestingly, EM did not induce resistance to 16 membered-ring macrolides such as josamycin, kitasamycin, and tylosin. On the other hand, 16 membered-ring macrolides such as josamycin, kitasamycin, and tylosin strongly induced resistance to EM and to themselves. Strong

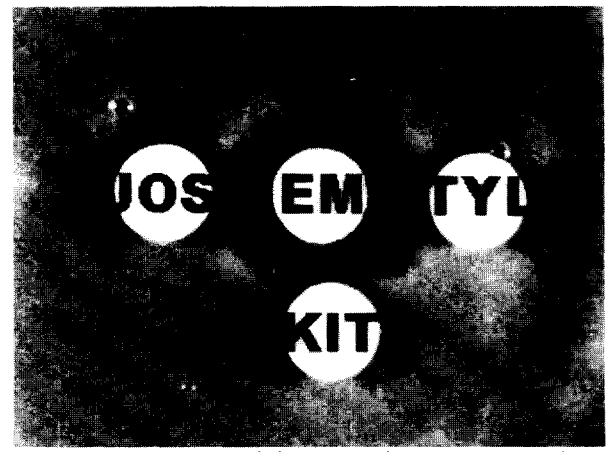

Fig. 1. Disk agar susceptibility test of *Enterococcus faecalis* 373. EM, erythromycin (80 μg); JOS, josamycin (40 μg); KIT, kitasamycin (40 μg); TYL, tylosin (40 μg).

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Table I. MICs of various MLS antibiotics for *Enterococcus faecalis* 373.

	Antibiotic	MIC (μg/ml)
14 Membered-ring macrolide	Clarithromycin	16
	Erythromycin	16
	Oleandomycin	2048
16 Membered-ring macrolide	Josamycin	1024
	Kitasamycin	2048
	Rokitamycin	1024
	Tylosin	>2048
Lincosamide	Clindamycin	256
	Lincomycin	1024
Streptogramin B	Mikamycin	32

inducibility of resistance to MLS antibiotics by 16 membered-ring macrolides in bacteria excepting actinomycetes has not yet been reported. The ability of 16 membered-ring macrolides to induce resistance to EM is revealed by the distorted EM inhibition zone (Fig. 1).

MICs were determined by the broth dilution technique with Brain Heart Infusion broth according to the procedures published by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). MICs of various 16 membered-ring macrolides for *E. faecalis* 373 were at least 1024 μg/ml (Table I) and MIC of EM was 16 μg/ml. In contrast to MIC of EM, MIC of OM which is one of 14 membered-ring macrolide was 2048 μg/ml. High MICs of various 16 membered-ring macrolides and OM show the possibility that these antibiotics can strongly induce resistance to themselves but EM can not strongly induce resistance to itself.

The kinds of inducer antibiotics for MLS resistance and their strength in *E. faecalis* 373 are different from those in other bacteria excepting actinomycetes. Of particular interest in this study is the fact that EM seems to lose its MLS resistance inducibility while 16 membered-ring macrolides seem to obtain their MLS resistance inducibility in this strain. Although the molecular mechanism of MLS resistance induction is well-elucidated (Weisblum, 1995), little is known about the molecular basis for the distinction of inducing antibiotics and non-inducing antibiotics, except that some amino acid residues in leader peptide is crucial to inducible resistance and that site-specific mutation of the crucial amino acid residues markedly changes the phenotype of inducible resistance (Mayford *et al.*, 1990). According to current knowledge, the specificity of induction of MLS resistance by various MLS antibiotics does not seem to be related to the class of erm determinant but depends on the sequence of the regulatory region upstream from the structural gene for the methylase. Further research of MLS resistance of *E. faecalis* 373 will help us to understand the genetic environment that distinguishes inducing antibiotics from non-inducing MLS antibiotics.

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