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Molecular Mechanism of Macrolide-Lincosamide-Streptogramin B (MLS) Resistance

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MLS antibiotics 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 by rRNA methylase, encoded by *erm* gene family, resulting in a decreased ribosomal affinity for MLS antibiotics. MLS resistance of this type is divided into two subtypes. One is inherent constitutive resistance and the other is inducible type, where the bacteria become resistant only with prior or simultaneous exposure to one or another MLS antibiotics. To study regulation of *ermK*, showing inducible resistance, we introduced mismatches into terminators. Because of transcriptional pausing in the proposed terminators, in wild type *ermK*, only truncated transcription products were detected. In the case of terminator region mutants, full-length transcripts were synthesized regardless of induction. These results firmly proved that transcriptional attenuation rather than translational attenuation primarily regulate the expression of *ermK*. We also tested the possible minor contribution of translational attenuation control by constructing a triple mutant (terminator1 + terminator2 + methylase SD region). A higher level of beta-gal synthesis was seen in the triple mutant. Therefore, unlike previously described attenuators, it can be concluded that both transcriptional and translational attenuation contribute to the regulation of ermK, although transcriptional attenuation mainly contributes to its regulation.

The inducers of MLS resistance are generally 14 membered-ring macrolides and lincosamides. On the contrary, 16 membered-ring macrolides have been known for their inability to induce MLS resistance, and the molecular mechanisms for the descrimination between inducers and non-inducers are not clear. We clinically isolated *Enterococcus faecalis* 373, in which 16 membered-ring macrolides induced MLS resistance more strongly than 14 membered-ring macrolides did. Further research on this strain will help us to understand the molecular basis for the induction mechanism of MLS resistance.

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Molecular Mechanism of β-lactam Resistance of *Streptococcus pneumoniae*

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Antimicrobial resistance of *S. pneumoniae* has become a global problem in recent decades, while Korea is the hottest spot in the world with regard to penicillin- and multidrug-resistance (MDR) which ranged from 70-77 % and 30-40 %, respectively. Resistance to β-lactam antibiotics in pneumococci is due to alterations of penicillin-binding proteins (PBPs) with decreased affinity for the antibiotics. High-level resistance to cephalosporins is associated with alterations of PBP 1A and 2X, whereas high-level penicillin resistance additionally requires alterations of PBP 2B. Sequencing of *pbp 2b* gene of Korean MDR strains showed relatively uniform alterations in nucleotides and amino acids, while hypervariable region which contained 90 % of changes in nucleotides and amino acids was noted between 389 and 993 bp in most strains. Unique alterations in amino acids were detected in Korean MDR strains. Sequencing of pbp 2b gene in strains with increasing MIC to penicillin showed that more extensive changes in nucleotides and amino acids were noted with increase in penicillin MIC. Characteristic alterations in amino acids were detected according to the specific level of penicillin MIC. Sequencing of *pbp 2x* gene of Korean MDR strains showed uniform pattern of alterations in nucleotides and amino acids as in pbp 2b gene. Sequence variations of *pbp 2x* gene were relatively scattered. Further studies to clarify the structure and function of other PBPs in developing antimicrobial resistance of pneumococci are warranted.