Pneumococcal Resistance to Macrolides, Lincosamides, Ketolides, and Streptogramin B Agents: Molecular Mechanisms and Resistance Phenotypes

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The macrolides, lincosamides, ketolides, and streptogramin B agents (the MLKS\textsubscript{B} antimicrobial agents) have related chemical structures and share similar molecular targets on the 50S ribosomal subunit of \textit{Streptococcus pneumoniae}. Mutations in rRNA or ribosomal proteins generate a variety of resistance phenotypes. The M phenotype of \textit{S. pneumoniae}, which predominates in North America, affords low-level resistance to macrolides only (excluding macrolides with 16-member rings) by means of an efflux pump encoded by the \textit{mefA} gene. The MLS\textsubscript{B} phenotype, which predominates in Europe, affords high-level resistance to macrolides, lincosamides, and streptogramin B agents and arises, in most cases, from dimethylation of adenine 2058 in the 23S rRNA of the 50S ribosomal subunit. Other, less common, phenotypes arise from other 23S rRNA modifications (ML and K phenotypes) or from amino acid substitution (MS\textsubscript{B} phenotype) or insertion (MKS\textsubscript{B} phenotype) into the 50S subunit ribosomal protein L4. In all cases, the decrease in susceptibility to ketolides (for example, telithromycin) is less than the decrease in susceptibility for other MLKS\textsubscript{B} agents.

\textit{Streptococcus pneumoniae} is widely acknowledged as an important bacterial cause of respiratory tract infections and as the predominant pathogen causing community-acquired pneumonia [1, 2]. As physicians continue to seek effective therapy for these infections, it is disconcerting to note the continuing worldwide emergence of strains of \textit{S. pneumoniae} resistant to a number of well-known antimicrobial agents, including the macrolide erythromycin [3].

Macrolides, lincosamides, ketolides, and streptogramins, known as the MLKS\textsubscript{B} antimicrobial agents, have similar antimicrobial activities and similar mechanisms of resistance. Several different MLKS\textsubscript{B} resistance phenotypes of \textit{S. pneumoniae} occur, some of which confer resistance only to macrolides. With knowledge of the mechanism of resistance and its spectrum of resistance, it is possible to determine which MLKS\textsubscript{B} agents retain activity against pneumococci. Without this information, there is a danger of considering resistance to one of these agents as resistance to all such agents and thereby overestimating the resistance problem in \textit{S. pneumoniae} and underestimating the clinical usefulness of some antibacterial agents.

Here I focus on the mechanisms underlying the resistance of \textit{S. pneumoniae} to the MLKS\textsubscript{B} agents, excluding other \textit{Streptococcus} and \textit{Staphylococcus} species, which may exhibit resistance phenotypes different from those of \textit{S. pneumoniae}. The following is reviewed: current knowledge of ribosomal structure and peptide assembly, which is important in understanding the mechanisms of pneumococcal resistance; the molecular structures of the macrolides and related drugs and their mechanisms of action, the phenotypic resistance patterns encountered, and the molecular mechanisms of resistance responsible for these pathogens; and reasons that, under certain conditions, ketolides remain active, whereas the other classes of agent do not.
RIBOSOME STRUCTURE AND PEPTIDE ASSEMBLY

The bacterial 70S ribosome is made up of a large (50S) and a small (30S) subunit, each of which comprises a mixture of ribosomal RNA (rRNA) and ribosomal proteins (figure 1). The 50S subunit contains 23S and 5S rRNA and 31 structural proteins, designated L1 to L31 [4]. The small subunit contains 16S rRNA associated with 21 ribosomal proteins (S1–S21). The ribosomal proteins account for about one-third of the total mass of the ribosome.

The MLKS_{B} agents share similar molecular targets on the bacterial 50S ribosomal subunit. They work in 2 ways: by interfering with the peptidyl transferase activity of 23S rRNA in the 50S subunit and by disrupting assembly of the 50S subunit.

The process of protein synthesis involves both ribosomal subunits. As messenger RNA (mRNA) passes through a groove at the interface of the 30S and 50S subunits, with the 5′ end of the mRNA leading the way, a peptide is formed that eventually exits from a tunnel that passes through the center of the 50S subunit. All of the drugs under discussion bind in the vicinity of the 50S subunit tunnel, blocking elongation and exit of the peptide from the 50S ribosomal subunit.

The second mechanism of action of these drugs involves interference with 50S rRNA subunit assembly. The normal pathway of assembly involves 23S rRNA transcription and the creation of 32S and 43S intermediate forms. When macrolides, for example, are present, they bind to their active site during assembly and induce a defective intermediate, which cannot fold correctly and is degraded by ribonuclease to yield rRNA fragments and ribosomal proteins [5].

MOLECULAR STRUCTURE OF MLKS_{B} AGENTS

The macrolide class of antibiotic agents includes compounds with 14-membered (erythromycin, clarithromycin, and others), 15-membered (azithromycin and others), and 16-membered (rokitamycin and others) ring structures (figure 2). The lincosamides, such as clindamycin and lincomycin, have a completely different structure, yet have the same cellular target as the macrolides. Streptogramin antibiotics, such as dalfopristin-quinupristin, contain 2 active components, type A and type B, which synergistically inhibit peptide elongation [6]. The B component is the only component against which pneumococcal macrolide resistance may develop. Examples of streptogramin B agents include quinupristin and pristinamycin IA.

The ketolides are modified macrolides. For example, the ketolide telithromycin is generated from erythromycin by removing a cladinose sugar and replacing it with a keto group (figure 2). In addition, the C-11/C-12 region of the erythromycin molecule is bridged by a carbamate. Finally, to ensure activity, all of the ketolides require a large organic side group. In the case of telithromycin, the side group is an aryl-alkyl extension of the carbamate; for cethromycin, the large organic side group is attached at the C-6 position [7].

PHENOTYPIC RESISTANCE PATTERNS

There are 4 phenotypic resistance patterns among S. pneumoniae, designated M, MLS_{B}, MS_{B}, and ML, according to whether resistance to macrolides (M) and/or lincosamides (L) and/or streptogramin B agents (S_{B}) is observed. Of these, the M and MLS_{B} phenotypes account for the vast majority of cases of drug-resistant infection. However, the distribution of these different phenotypes varies considerably by geographic region. In France and Spain, for example, the vast majority of resistant strains are of the MLS_{B} phenotype (accounting for ∼95% of resistant isolates), and a low proportion are of the M phenotype (4%) [8–10]. By contrast, in North America, the M phenotype predominates, accounting for ∼50%–75% of resistant isolates, and the MLS phenotype accounts for ∼25%–50% of such cases [8, 11–13].

The M phenotype arises from the presence of an efflux pump

Figure 1. Assembly of the 70S ribosome of prokaryotes. Reproduced from [4] with permission.
Figure 2. Chemical structures of the macrolides, lincosamides, ketolides, and streptogramin B agents (the MLKS₉ antimicrobials)
Table 1. Cellular mechanisms responsible for MLKS<sub>B</sub> resistance phenotypes (conferring resistance to macrolides, lincosamides, ketolides, and streptogramin B agents) in *Streptococcus pneumoniae.*

<table>
<thead>
<tr>
<th>Mechanism (gene)</th>
<th>Resistance phenotype</th>
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<tbody>
<tr>
<td>Efflux pump (<em>mefA</em>)</td>
<td>M   MLS&lt;sub&gt;B&lt;/sub&gt;  MS&lt;sub&gt;B&lt;/sub&gt; ML MKS&lt;sub&gt;B&lt;/sub&gt; K</td>
</tr>
<tr>
<td>Ribosome modification</td>
<td></td>
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<tr>
<td>Methylation of adenine 2058 in 23S rRNA (<em>ermB</em>)</td>
<td>– + – – – –</td>
</tr>
<tr>
<td>23S rRNA mutations</td>
<td>– + – + – +</td>
</tr>
<tr>
<td>Ribosomal protein L4 mutations</td>
<td>– – +&lt;sup&gt;a&lt;/sup&gt; – 4&lt;sup&gt;b&lt;/sup&gt; –</td>
</tr>
</tbody>
</table>

<sup>a</sup> 3-Amino acid substitution.  
<sup>b</sup> 18-Amino acid insertion.

encoded by the *mefA* (macrolide efflux) gene, which removes drug from the bacterial protoplasm (table 1). The MLS<sub>B</sub> phenotypes are attributable to ribosome-structure modifications that prevent the drugs from binding to the 50S rRNA subunit (table 1). The predominant modification involves dimethylation of adenine 2058 in the 23S rRNA, encoded by the *ermB* (erythromycin ribosomal methylase) gene. Other ribosomal modifications include modifications of the ribosomal proteins and rRNA mutations.

In general, therefore, the M phenotype is related to drug efflux and the MLS<sub>B</sub> phenotype to ribosomal modifications. However, studies have highlighted a disagreement of ∼10% between phenotypic and molecular classifications [8]. In most cases, this can be accounted for by strains with the MLS<sub>B</sub> phenotype, in which the efflux pump is present but not detectable by means of standard phenotypic methods.

**MOLECULAR MECHANISMS OF RESISTANCE**

Presence of the efflux pump is a common cause of macrolide resistance among streptococci [14]. The efflux pump protein MefA is specific for macrolides, excluding those with a 16-membered ring. The M phenotype results in relatively low-level resistance, generating strains with an MIC ∼16-fold greater than that of wild type strains (table 2) [15–17]. Expression of *mefA* also yields a slight decrease in the susceptibility of *S. pneumoniae* to the ketolide telithromycin, but the MIC<sub>90</sub> remains very low.

With regard to 23S rRNA modifications, 3 different molecular mechanisms have been reported. Dimethylation of adenine 2058 is encoded by the *ermB* gene and accounts for 98% of ribosomal modifications. The remaining cases are attributable to mutations of the 23S rRNA or to modification of the L4 protein with or without 23S rRNA mutation. All of these modifications affect the critical 23S rRNA region of the peptide synthesis tunnel of the large 50S subunit, yielding a strain with the MLS<sub>B</sub> phenotype.

The 3-dimensional structure of the 50S subunit clearly identifies the centrally located peptide synthesis tunnel. Near the exit site of the nascent peptide in the tunnel are located nucleotides primarily derived from 2 domains of the 23S RNA, domains II and V [18]. Mutation of any of these nucleotides (752, 2057, 2058, 2059, 2609, and 2611) can generate the MLS<sub>B</sub> phenotype, by altering the binding of the antimicrobials to the nucleotide and thereby reducing the ability of the drugs to physically interfere with peptide synthesis. It is interesting that mutation of nucleotide 2609 is associated with ketolide-specific resistance [18, 19]. The effect of *ermB*-mediated dimethylation of adenine 2058 in domain V is to reduce by 100,000-fold the binding affinity of the macrolide for the 50S subunit, so that it is no longer able to block the egress of the peptide chain from the subunit.

The 23S rRNA mutations all involve single-base changes in critical regions of domain II or V. Each mutation alters the binding affinity of a macrolide for a critical site. Pneumococci have 4 copies of the ribosome per cell [19]. For the mutations to have a phenotypic effect, identical mutations must occur in at least 2 of these ribosomes. As the number of ribosomes carrying the mutation increases, so does the level of resistance associated with it.

In the case of the L4 ribosomal protein, which in part determines the conformation of the 50S subunit, 2 modifications have been reported thus far: a 3-amino acid change in the critical region and an 18-amino acid insertion [19]. Both mutations result in a narrowing of the peptide synthesis tunnel and a displacement of the macrolide-binding site away from the tunnel, which may even prevent the macrolide from binding [20]. Some of these phenotypes may be associated with poor growth of the host bacteria and reversion to wild type. This is particularly true of the MKS<sub>B</sub> phenotype that results from the 18-amino acid insertion.

Modifications of the L22 ribosomal protein have been reported in laboratory mutants of macrolide-resistant pneumococci and in 1 clone of macrolide-resistant pneumococci isolated from Japanese patients [21, 22]. In both cases, the L22
mutations were point mutations and were all found in combination with 23S modifications, making it difficult to attribute resistance solely to the L22 modifications. Close examination of laboratory-created mutants did find strains with solely L22 mutations [22]. These laboratory-derived mutations conferred MICs of the MLKSB agents that were 2-fold to 64-fold greater than those of the wild type but did not result in significant levels of resistance to any of the agents; the mutations conferred no change, up to a 4-fold increase in clindamycin MICs. When L22 mutations were cloned into a wild type background, the same general changes were observed, with the greatest increase in MIC noted for pristinamycin, but even still outside the resistant range [22]. It is predicted that L22 mutations alter the geometry of the tunnel to prevent macrolide binding [20], but thus far no L22 mutation has been reported to confer high-level macrolide resistance. These mutations are unlikely to be undetected by routine antimicrobial susceptibility testing.

The most common ribosomal modification, ermB-mediated methylation, results in an MLSB phenotype (table 2) [15–17]. Other less common mutations include rRNA mutations, resulting in MLSa, ML, or K phenotypes, and ribosomal protein mutations, resulting in MSa and MKSB phenotypes. The MKSB phenotype is associated with the 18-amino acid insertion in the L4 protein, causing poor growth and reversion to wild type; the clinical significance of this phenotype is unclear.

The impact of ermB expression on the resistance profile of *S. pneumoniae* is quite different from that of mefA. The erythromycin MIC is considerably higher for the ermB-expressing pneumococci than for the wild type (table 2). Expression of ermB causes large increases in resistance to all macrolides, lincosamides, and streptogramin B agents. A smaller effect on the telithromycin MIC is also observed.

**WHY DO KETOLIDES “BREAK THE RULES?”**

It is clear from the preceding discussion that the changes in susceptibility of *S. pneumoniae* to MLKSB agents resulting from methylation, results in an MLSB phenotype (table 2) [15–17]. Other less common mutations include rRNA mutations, resulting in MLSa, ML, or K phenotypes, and ribosomal protein mutations, resulting in MSa and MKSB phenotypes. The MKSB phenotype is associated with the 18-amino acid insertion in the L4 protein, causing poor growth and reversion to wild type; the clinical significance of this phenotype is unclear.

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<table>
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<tr>
<th>Agent (class)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; in μg/mL</th>
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<tbody>
<tr>
<td>Erythromycin (14-member macrolide)</td>
<td>0.25</td>
</tr>
<tr>
<td>Roxitumycin (16-member macrolide)</td>
<td>0.25</td>
</tr>
<tr>
<td>Clindamycin (lincosamide)</td>
<td>0.12</td>
</tr>
<tr>
<td>Streptogramin B agents</td>
<td>4</td>
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<tr>
<td>Telithromycin (ketolide)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**NOTE.** Adapted from [15–17].

Erythromycin (14-member macrolide) 0.25 4 >64
Telithromycin (ketolide) 0.008 0.06 0.06

### References


