Induction of Telithromycin Resistance by Erythromycin in Isolates of Macrolide-Resistant Staphylococcus spp.

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Staphylococcal isolates were examined for possible macrolide-inducible resistance to telithromycin. All macrolide-resistant isolates demonstrated telithromycin D-shaped zones. This result did not discriminate between resistance due to an efflux mechanism (msrA) or a ribosomal target modification (ermA or ermC). Inducible telithromycin resistance in staphylococci does not appear to be analogous to inducible clindamycin resistance.

Telithromycin is the first commercially available ketolide. Ketolides are a recently developed class of antimicrobial agents that belong to the macrolide-lincosamide-streptogramin B (MLS\textsubscript{B}) family. Ketolides possess significant structural differences from macrolides, including a second site of interaction with the ribosome at domain II on the 23S rRNA of the 50S ribosomal subunit (4). This is in addition to the interaction at domain V, which is where 14- and 15-membered-ring macrolides act (2). These and other modifications improve the stability of ketolides in acidic environments, prevent the induction of MLS\textsubscript{B} resistance, and maintain activity against organisms that develop inducible resistance to MLS\textsubscript{B} antimicrobials (2). Mechanisms that confer resistance to MLS\textsubscript{B} antimicrobials include target site modification and active antimicrobial efflux (1). Target site modification is encoded by constitutive or inducible \textit{erm} genes (16) that may require exposure to subinhibitory concentrations of erythromycin for optimal expression (18). The active antimicrobial efflux pumps that have been described for \textit{Staphylococcus aureus} are encoded by the \textit{msrA}, \textit{msrB}, and \textit{NorA} genes (11, 16).

We previously reported a practical disk approximation method which identified 97% of \textit{S. aureus} strains and 100% of coagulase-negative staphylococcus (CoNS) strains with inducible MLS\textsubscript{B} resistance during routine disk diffusion susceptibility testing (6). A similar method involves placing erythromycin and clindamycin disks in close proximity on standard sheep blood agar plates used for verification of inoculum purity when broth-based susceptibility tests are performed (10). These tests are intended to detect strains with inducible MLS\textsubscript{B} resistance in order to avoid potential clinical failures with clindamycin therapy (5, 7, 15, 17). The goal of the present study was to determine if inducible telithromycin resistance, like inducible clindamycin resistance, might occur in macrolide-resistant staphylococci.

A group of 100 \textit{S. aureus} clinical isolates and 100 CoNS clinical isolates, some of which have been previously described (6), were selected for study. All isolates were macrolide resistant by standard Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) disk diffusion testing (14). An additional 10 \textit{S. aureus} isolates that were susceptible to erythromycin were included. Standard CLSI disk diffusion testing (14) was performed on all isolates by use of Mueller-Hinton agar (Becton-Dickinson Microbiology Systems, Cockeysville, MD) with standard 15-\textmu g erythromycin disks, 2-\mu g clindamycin disks, and 15-\mu g telithromycin disks (Becton-Dickinson). Two sets of three disks were placed on the same agar plate (Fig. 1). Each set consisted of a centrally placed erythromycin disk with either clindamycin or telithromycin disks placed at 20 mm and 26 mm on opposite sides of the erythromycin disk. Zone diameters were carefully measured and evaluated for the formation of a D-shaped zone (D zone) following incubation for 16 to 18 h at 35°C.

A second method to assess possible inducible telithromycin resistance was performed on a subset of isolates by determining telithromycin MICs. This was completed by standard broth dilution testing (13) with and without the addition of a sub-
inhibitory concentration of erythromycin (0.5 µg/ml). MIC testing was then repeated with the addition of one of three known efflux pump inhibitors: reserpine (10, 25, 50, and 100 µg/ml), 2,4-dinitrophenol (20 µg/ml), or carbonyl cyanide m-chlorophenylhydrazone (CCCP; 0.5 µg/ml) (all obtained from Sigma Chemical Company, St. Louis, MO) (8, 9, 12). Both reserpine and CCCP were initially dissolved in dimethyl sulfoxide (American Type Culture Collection, Manassas, VA) prior to serial dilutions in sterile Mueller-Hinton broth (Becton-Dickinson). The reversal of macrolide-induced telithromycin MIC elevation in the presence of an efflux pump inhibitor would infer that resistance was due to active antimicrobial efflux.

Preparation of whole-cell DNA, PCR for the ermA, ermC, and msrA genes and detection of amplified DNA was completed as previously described (6). Control strains for disk diffusion tests and molecular analysis included S. aureus ATCC 25923 (macrolide and clindamycin susceptible; negative for ermA, ermC, and msrA), S. aureus RN1551 (containing ermA), S. aureus RN4220 (with plasmid pE194 containing ermC), and S. aureus RN4220 (with plasmid pAT10 containing msrA) (6).

Initial disk diffusion testing demonstrated that 74 S. aureus isolates and 45 CoNS isolates were susceptible to telithromycin (zone diameter of ≥22 mm) (Table 1), with all but two S. aureus isolates and three CoNS isolates susceptible to clindamycin (zone diameter of ≥21 mm). Unexpectedly, disk approximation testing revealed that all macrolide-resistant, telithromycin-susceptible staphylococcal isolates produced telithromycin D zones (Table 1 and Fig. 1). None of the erythromycin-susceptible S. aureus isolates demonstrated a flattening of the zones of inhibition. In contrast, inducible clindamycin resistance predicted the presence of an inducible erm gene, except for three CoNS isolates with msrA genes (Table 2). The telithromycin MIC of five selected S. aureus isolates with only the msrA genotype was 0.06 µg/ml, which increased to 0.5 µg/ml in the presence of a subinhibitory concentration of erythromycin (Table 3). Likewise, the telithromycin MIC was also induced by erythromycin for S. aureus isolates that contained only ermA or ermC. The negative-control strain, S. aureus ATCC 25923, did not demonstrate an elevated telithromycin MIC in the presence of erythromycin. The addition of reserpine, 2,4-dinitrophenol, or CCCP did not significantly decrease the induced telithromycin MICs.

All macrolide-resistant staphylococcal isolates in this study, irrespective of genotype, unexpectedly demonstrated positive telithromycin D-zone induction tests. Our previous study demonstrated that a positive macrolide induction test with clindamycin was a marker for those isolates that contained only a ribosomal-modification ermA or ermC gene and not the msrA efflux mechanism gene (6). A positive macrolide induction test with telithromycin did not discriminate between these resistance mechanisms. We initially postulated that inducible telithromycin resistance in these isolates was due to an alternate efflux pump that we had not identified. However, the addition of known inhibitors of staphylococcal efflux pumps did not reverse the erythromycin-induced telithromycin MICs, implying that either the pump was not affected by these inhibitors or there is an alternate mechanism of this inducible resistance.

### Table 1. Staphylococcal resistance phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Total no. of isolates tested</th>
<th>No. of isolates with positive induction test result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ery/Cli disks were separated by:</td>
<td>Ery/Tel disks were separated by:</td>
</tr>
<tr>
<td></td>
<td>20 mm</td>
<td>26 mm</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clr Tels</td>
<td>72</td>
<td>26</td>
</tr>
<tr>
<td>Clr Telr</td>
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<td>0</td>
</tr>
<tr>
<td>Clr Teli</td>
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<td>NA</td>
</tr>
<tr>
<td>Ery Tels</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>CoNS</td>
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<td></td>
</tr>
<tr>
<td>Clr Tels</td>
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<td>27</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td>Clr Teli</td>
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### Table 2. Staphylococcal genotype results

<table>
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<th>Phenotype</th>
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<th>No. of isolates with genotype:</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>S. aureus</td>
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<td>46</td>
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<tr>
<td>Inducible Clr</td>
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<td>Constitutive Clr</td>
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<td>Tel</td>
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<tr>
<td>CoNS</td>
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<tr>
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<td>Tel</td>
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<tr>
<td>Constitutive Tel</td>
<td></td>
<td>55</td>
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### Table 3. MICs for select S. aureus isolates

<table>
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<tr>
<th>Isolate</th>
<th>Genotype identified</th>
<th>Tel MIC (µg/ml) with:</th>
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<tr>
<td></td>
<td>Ery</td>
<td>Ery and reserpine</td>
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<td>UH 23</td>
<td>msaA</td>
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<tr>
<td>UH 24</td>
<td>msaA</td>
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<tr>
<td>UH 25</td>
<td>msaA</td>
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<tr>
<td>UH 26</td>
<td>msaA</td>
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<td>UH 27</td>
<td>msaA</td>
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<td>UH 31</td>
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<tr>
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<tr>
<td>UH 40</td>
<td>ermC</td>
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</tr>
<tr>
<td>UH 33</td>
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<tr>
<td>UH 39</td>
<td>ermC</td>
<td>4.0</td>
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</tbody>
</table>

* The original telithromycin MIC for all isolates was 0.06 µg/ml. Tel, telithromycin; Ery, erythromycin; ND, not done.
  
  a Erythromycin concentration, 0.5 µg/ml.
  b Reserpine concentration, 25 µg/ml.
  c Dinitrophenol, 20 µg/ml.
  d CCCP concentration, 0.5 µg/ml.
The mechanism of macrolide-induced telithromycin resistance may or may not be target site modification in those strains that contained *ermA* or *ermC*. Further work is needed to explain the exact mechanism of inducible telithromycin resistance observed in this study.

Regardless of the mechanism for this observation, we do not recommend routinely testing clinical isolates for inducible telithromycin resistance. It is unclear what, if any, clinical significance this observation provides, for two reasons. First, our results did not demonstrate a discriminating cause for the positive telithromycin D test, as is the case with the clindamyacin D test (6). Second, to our knowledge, there have been no reports of clinical failure of telithromycin therapy for patients who have infections caused by telithromycin-susceptible, erythromycin-resistant isolates. It is concerning that the erythromycin induction of telithromycin resistance did elevate the telithromycin MIC above the resistance breakpoint (3) for one of the *ermC*-containing strains. Any potential clinical relevance of this phenomenon may become apparent with the expanded use of telithromycin. For now, clinical laboratories should not test for inducible telithromycin resistance unless further investigation reveals its cause and demonstrates that it is a relevant finding.

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REFERENCES