Introduction of \textit{erm}(C) into a linezolid- and methicillin-resistant \textit{Staphylococcus aureus} does not restore linezolid susceptibility

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Sir,

Oxazolidinones are a new class of compounds with activity against resistant Gram-positive organisms such as methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and vancomycin-resistant \textit{Enterococcus} spp. (VRE). However, resistance to linezolid, the only oxazolidinone available for clinical use, has emerged in enterococci\textsuperscript{1} as well as MRSA.\textsuperscript{2,3} In both reports of linezolid-resistant MRSA published to date, resistance was due to a mutation in the 23S rRNA at position 2576 in the peptidyl transferase centre of domain V.\textsuperscript{2,3} We found that strain selection by passage in vitro sometimes lost resistance to erythromycin. Our study of serial isolates that were indistinguishable by pulsed-field gel electrophoresis (PFGE) obtained from a patient with prolonged MRSA bacteraemia showed that the loss of erythromycin resistance conferred by constitutive ribosomal methyltransferase activity occurred coincidentally with an increase in linezolid MIC from 0.5 to 2 mg/L (G. Sakoulas, H. S. Gold, R. C. Moellering, Jr & G. M. Eliopoulos, unpublished observation). We hypothesized that mutations conferring linezolid resistance might be incompatible with expression of macrolide resistance due to methylation at A2058. Therefore, we undertook a study to determine whether introduction of a methyltransferase gene into a linezolid-resistant MRSA might restore susceptibility to linezolid.

The absence of ribosomal methyltransferase \textit{erm} genes conferring macrolide–lincosamide–streptogramin B (MLS\textsubscript{B}) resistance within \textit{S. aureus} A7819, one of the linezolid-resistant MRSA isolates (MIC > 32 mg/L) that we recently characterized,\textsuperscript{3} was confirmed using PCR with degenerate \textit{erm} primers according to published methods.\textsuperscript{5} \textit{S. aureus} A7819 was made electrocompetent using ice-cold 10% glycerol. Plasmid pE194 containing inducible \textit{erm}(C) was purified from \textit{S. aureus} RN2442 (Wizard Plus Miniprep; Promega, Madison, WI, USA) and introduced via electroporation into \textit{S. aureus} A7819 (100 Ω, 25 μF, 2.3 kV, time constant 2 s). Transformed cells were selected on brain–heart infusion agar plates containing erythromycin 20 mg/L. Authenticity of the clones derived from \textit{S. aureus} A7819 was verified by PFGE. Inducible MLS\textsubscript{B} resistance was confirmed by measuring zone sizes around erythromycin and clindamycin discs on Mueller–Hinton agar plates and on plates supplemented with erythromycin 0.1 mg/L. The presence of \textit{erm}(C) in the transformants was confirmed by PCR as above.\textsuperscript{5}

Using NCCLS methods\textsuperscript{6} we determined MICs of linezolid on agar. We also determined MICs of linezolid in agar with erythromycin 0.1 mg/L to study the effect of methyltransferase induction on MIC of linezolid. Growth curves were performed in antibiotic-free Mueller–Hinton (II) broth (MHB), and MHB containing linezolid 10 mg/L or erythromycin 0.1 mg/L, or both. Twenty millilitre cultures were maintained in 250 mL Erlenmeyer flasks incubated at 35°C without agitation.

For six erythromycin-resistant transformants [\textit{erm}(C)] of \textit{S. aureus} A7819, growth in the presence or absence of an inducing concentration of erythromycin did not restore susceptibility to linezolid (MICs remained >32 mg/L). Growth curves showed that the \textit{erm}(C) transformants did not demonstrate impaired growth in the presence of linezolid 10 mg/L and an inducing concentration of erythromycin (Figure 1).

Our central observation is that linezolid resistance mediated by the G2576U 23S rRNA mutation in \textit{S. aureus} is not incompatible with macrolide resistance due to methylation. We felt that exploring this possibility was important because incompatibility of these resistance mechanisms might have provided clinicians the option of concomitant administration of a macrolide with linezolid to prevent the development of oxazolidinonone resistance. Furthermore, if resistance to oxazolidinones required loss of methyltransferase-mediated
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Despite these negative findings, we believe that additional study of the relationship between macrolide resistance and linezolid resistance among clinical isolates of staphylococci is warranted. The possible interaction of macrolide resistance mechanisms with ribosomal mutations conferring oxazolidinone resistance will be a subject of interesting future investigation.

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References


