In vitro activity of retapamulin against Staphylococcus aureus isolates resistant to fusidic acid and mupirocin

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Objectives: We determined the in vitro activity of retapamulin, a novel pleuromutilin antibiotic, against 664 Staphylococcus aureus isolates from the UK, including many resistant to fusidic acid and/or highly resistant to mupirocin.

Methods: MICs were determined on Mueller–Hinton agar in accordance with the CLSI guidelines. Susceptibility was categorized using CLSI criteria, where available; otherwise the European Committee for Antimicrobial Susceptibility Testing (EUCAST)/BSAC criteria were used (for mupirocin and fusidic acid). Mutations in the rplC gene, which encodes ribosomal protein L3, were sought by PCR and DNA sequencing.

Results: The S. aureus included 488 (73%) methicillin-resistant isolates (oxacillin MICs >2 mg/L), 336 isolates (51%) resistant to fusidic acid (MICs >1 mg/L) and 254 (38%) with high-level mupirocin resistance (MICs >256 mg/L); 103 (16%) isolates were resistant both to fusidic acid and to high levels of mupirocin. Retapamulin inhibited 663 (99.9%) isolates at ≤0.25 mg/L. A single methicillin-resistant S. aureus isolate, also with high-level mupirocin resistance, required a retapamulin MIC of 2 mg/L, but its reduced susceptibility to retapamulin was not associated with any mutation in ribosomal protein L3.

Conclusions: Retapamulin demonstrated excellent activity in vitro against S. aureus isolates, irrespective of their level of resistance to other antibacterials. These results support the EUCAST epidemiological cut-off value for retapamulin of ≤0.5 mg/L against S. aureus.

Keywords: S. aureus, MRSA, mechanisms

We determined the activity of retapamulin in vitro against current S. aureus isolates from the UK. We included many isolates resistant to fusidic acid and/or highly resistant to mupirocin, and a large number of MRSA isolates.

Materials and methods

Bacterial isolates
Six hundred and sixty-four isolates of S. aureus were recovered from storage either at −70°C or at room temperature; these had been submitted to the HPA Centre for Infections for reference investigation from laboratories throughout the UK. Four hundred and eighty-eight (73%) isolates were MRSA (oxacillin MICs >2 mg/L).

Susceptibility testing
MICs were determined following the CLSI guidelines on Mueller–Hinton agar. The agents tested were retapamulin, fusidic acid,
Mupirocin, oxacillin, erythromycin, clindamycin and gentamicin. Susceptibility was categorized using CLSI criteria, where available; the European Committee for Antimicrobial Susceptibility Testing (EUCAST)/BSAC criteria were used for mupirocin and fusidic acid. 'Wild-type' susceptibility to retapamulin and mupirocin was defined using epidemiological cut-off values of ≤0.5 mg/L, as recommended by EUCAST (http://www.srga.org/eucastwt/MICTAB/index.html).

Investigation of mechanisms of reduced pleuromutilin susceptibility

Mutations in rplC, which encodes ribosomal protein L3, were sought in isolates with reduced retapamulin susceptibility. Primers used to amplify and sequence an 822 bp fragment, including the entire rplC gene, were: 5'-AAC CTG ATT TAG TTC CGT CTA and 5'-GTT GAC GCT TTA ATG GGC TTA. Selected PCR products, from isolates with reduced susceptibility and from fully susceptible isolates (used as controls), were sequenced with dye terminator chemistry using a CEQ8000 Genetic Analyser (Beckman–Coulter, High Wycombe, UK). The sequences were compared with GenBank depositions using BLAST algorithms.

Results and discussion

The susceptibilities of the 664 isolates to oxacillin, erythromycin, clindamycin and gentamicin are summarized in Table 1, while Table 2 shows the MIC distributions of those agents prescribed for topical use versus impetigo. The majority of the isolates were MRSA (488, 73%, which reflects the bias in S. aureus submissions to the national reference laboratories). The isolates studied included 254 (38%) with high-level mupirocin resistance (MICs >256 mg/L) and 49 with low-level resistance (MICs 8–256 mg/L), which may result in slower eradication by mupirocin than for isolates with full susceptibility. A total of 324 (49%) isolates were above the epidemiological cut-off value for wild-type mupirocin susceptibility (MICs ≤ 0.5 mg/L) set by EUCAST (http://www.srga.org/eucastwt/MICTAB/index.html). Over half of the isolates (336, 51%) were resistant to fusidic acid (MICs >1 mg/L), and 103 (16%) were resistant both to fusidic acid and to high levels of mupirocin.

Retapamulin inhibited 663 (99.9%) isolates at ≤0.25 mg/L, with a unimodal MIC distribution, centred at 0.06 mg/L. These data are consistent with the epidemiological cut-off value for wild-type susceptibility of ≤0.5 mg/L set by EUCAST. A single MRSA isolate in our study, also with high-level mupirocin resistance, required a retapamulin MIC of 2 mg/L. As reduced susceptibility and resistance to pleuromutilins has been associated with mutations in ribosomal protein L3, the 663 bp rplC gene of this isolate was sequenced alongside that of a fully retapamulin-susceptible MRSA isolate (MIC 0.06 mg/L). The sequence obtained from the fully susceptible isolate was identical to rplC of MRSA252 (GenBank BX571856); that of the isolate with reduced susceptibility varied at three positions known to show polymorphism between S. aureus strains (C198T, C492T and T600C). Importantly, each of these changes in the DNA sequence was silent, with no amino acid substitutions in the predicted L3 protein. Reduced susceptibility to pleuromutilins in staphylococci may also involve production of the Cfr methyltransferase, which also confers cross-resistance to phenics, lincosamides, oxazolidinones and streptogramin A compounds, or non-target-specific efflux mediated via VgaAv, which also affects streptogramin A compounds (Summary of Product Characteristics, http://www.emea.europa.eu/humandocs/PDFs/EPAR/altargo/H-757-PI-en.pdf). The presence of cfr was not indicated in our isolate, which remained susceptible to linezolid (MIC 2 mg/L), quinupristin/dalfopristin (MIC 1 mg/L) and clindamycin (MIC 0.5 mg/L); the possible contribution of efflux requires further study.

Our collection of S. aureus isolates was selected to include large numbers of fusidic acid- and mupirocin-resistant strains since these agents would frequently be considered for the topical treatment of impetigo, the licensed indication for retapamulin in the USA and one of its indications in Europe.

### Table 1. Summary of susceptibilities of 664 S. aureus isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MICs (mg/L)</th>
<th>MIC50</th>
<th>MIC90</th>
<th>% resistance (MIC breakpoint, mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>0.06 to &gt;128</td>
<td>128</td>
<td>128</td>
<td>73 (&gt;2)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤0.125 to &gt;128</td>
<td>128</td>
<td>128</td>
<td>72 (&gt;4)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤0.03 to &gt;128</td>
<td>0.125</td>
<td>128</td>
<td>36 (&gt;4)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.015–64</td>
<td>0.5</td>
<td>64</td>
<td>36 (&gt;8)</td>
</tr>
</tbody>
</table>

### Table 2. MIC distributions of retapamulin, mupirocin and fusidic acid for 664 S. aureus isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retapamulin</td>
<td>≤0.008</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>7</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>3</td>
</tr>
</tbody>
</table>

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Retapamulin showed excellent activity against these resistant isolates in vitro, including against those resistant both to fusidic acid and to high levels of mupirocin. Recently, O’Neill et al.\textsuperscript{10,11} described an epidemic European fusidic acid-resistant impetigo clone (EEFIC) of \textit{S. aureus}, which has a \textit{fusB} determinant and typical fusidic acid MICs of 4 mg/L. We did not determine whether representatives of this clone were included in our study collection. However, retapamulin retained activity in vitro against many \textit{S. aureus} isolates with high- and low-level forms of fusidic acid resistance (see the trimodal distribution in Table 2). It is likely that those with low-level resistance (MICs 2–16 mg/L) had \textit{fusB}, whereas high-level resistance (MICs 32 to >256 mg/L) is more likely to be caused by mutations in elongation factor G (EF-G; \textit{fusA} genotype).\textsuperscript{11} By inference, it is likely that retapamulin would also retain activity against the EEFIC strain, although this requires formal evaluation.

In summary, retapamulin demonstrated excellent activity in vitro against this collection of \textit{S. aureus} isolates, which was greatly biased towards MRSA and to isolates with resistance to mupirocin or fusidic acid. Reduced susceptibility (MIC 2 mg/L) was seen in only a single isolate and was not associated with any mutations in ribosomal protein L3; the mechanism(s) underlying the reduced susceptibility of this isolate warrants further investigation. These results support the EUCAST epidemiological cut-off value for retapamulin of \textless 0.5 mg/L against \textit{S. aureus}.

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References