81 conferred a ciprofloxacin-resistance level of 4 mg/L where glycine had been substituted by cysteine with no mutation concurrent at codon Ser-83. This mutation has already been described in *Escherichia coli*. The GyrA mutations do not explain why isolates with the same mutation have different MICs of ciprofloxacin. This variation in MICs could be explained by a mutation in the *parC* gene. In *A. baumannii*, topoisomerase IV is a target of quinolones and mutation at residues Ser-80 and Glu-84 of *ParC* contribute to decreased fluoroquinolone susceptibility. In the present study, only a change at these particular codons can be determined by the digestion of PCR products with *Hin* fI site at the codons for amino acids Asp-82–Ser-80 was intact, and suggesting that mutation either at codon Ser-82 or Ser-83 had occurred. *Hin* fI restriction of PCR products of *parC* produced two DNA fragments of 144 bp and 53 bp, respectively, in all isolates, indicating that the *Hin* fI restriction site at the codons for amino acids Asp-79–Ser-80 was intact, and suggesting the absence of mutation at codon Ser-80.

In conclusion, we showed that mutations in the amino acids corresponding to Gly-81 and Gly-78 in GyrA and ParC, respectively, contribute to decreased ciprofloxacin susceptibility in *A. baumannii*. Furthermore, it is also possible that other mutations at other locations within *gyrA*, *parC*, or in other genes may also contribute to the modulation of the MIC level since these mutations did not entirely explain resistance.

### Acknowledgements

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### References


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### Susceptibility of capsular *Staphylococcus aureus* strains to some antibiotics, triclosan and cationic biocides

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Keywords: *S. aureus*, capsule polysaccharides, antimicrobial susceptibility

*Corresponding author. Tel: +44-29-20875768; Fax: +44-29-20-874305; E-mail: day@cardiff.ac.uk*

Sir,

Approximately 90% of *Staphylococcus aureus* isolates produce one of 11 serotypes of capsular polysaccharides. Serotypes CP5 and CP8 account for ~25% and 50%, respectively, of isolates recovered from humans, offering support for their
The importance and relevance of these capsule types is confirmed by the development of a conjugate vaccine, StaphVAX that includes type 5 and 8 capsule polysaccharides. CP5 and CP8 serotypes are considered as microcapsules because they are much smaller than those produced by the mucoid serotypes 1 and 2. The microcapsules of CP5 and CP8 are extracellular, uronic acid-containing polysaccharides that are too small to be visualized by negative stains such as India ink. These two capsules are very similar and differ only in the position of O-acetyl groups and the linkages between the amino sugars. The function of CP5 and CP8 in S. aureus virulence has been investigated in great depth, especially with regard to their role in impeding phagocytosis. However, few authors have reported upon the potential for a capsule polysaccharide to present a permeability barrier to antimicrobial agents. Gram-positive bacteria possess a cell wall that is usually permeable and does not limit the incursion of antimicrobials. However, resistance through reduced penetration has been shown to occur, for example vancomycin-intermediate resistant S. aureus (VISA) strains produce a distinctly thickened cell wall. Furthermore, in 1984 Kolawole discussed the effect of a mucoid capsule upon disinfectant and antiseptic susceptibility in S. aureus. He concluded that the thick capsule, as associated with serotypes CP1 and CP2, does provide a permeability barrier to common biocides but fell short of testing the more common and clinically important serotypes. We wish to report how CP5 and CP8 microcapsules affect the susceptibility of S. aureus to several antibiotics and three widely used biocides: triclosan, chlorhexidine gluconate and cetylpyridinium chloride.

A range of 14 antibiotic discs were purchased from Oxoid (Basingstoke, UK) and used to analyse S. aureus Reynolds and two isogenic capsule mutants. Antibiotic susceptibility was established as per the BSAC standardized disc susceptibility testing methodology (Table 1). The MICs of nine of these clinically relevant antibiotics were elucidated by Etest strips (AB Biodisk, Sweden) according to the manufacturer’s recommendations. MICs for the three biocides were calculated using Iso-Sensitest agar (Oxoid, Basingstoke, UK), multipoint inoculator (Denley; Mast Diagnostics, Bootle, UK) and incubation in air at 37°C for 18–20 h, in accordance with BSAC guidelines. Triclosan (Irgasan DP300) was a gift from Ciba Speciality Chemicals; chlorhexidine gluconate and cetylpyridinium chloride were purchased from ICN Biomedicals Inc. (Ohio, USA). Of the three S. aureus Reynolds strains, one was wild-type, expressing a serotype CP5 capsule, one a mutant expressing CP8 and the third a second mutant, lacking a capsule (CP–). The acapsular Reynolds strain was constructed by replacing the serotype-specific capsule genes cap5HIJK on the bacterial chromosome with an erm(B) gene, conferring erythromycin resistance. S. aureus NCTC 6571 (Oxford) was included alongside the capsule strains as a control. The MICs were defined as the lowest concentration

<table>
<thead>
<tr>
<th>Antibiotic susceptibility</th>
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<tbody>
<tr>
<td>Strain</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>CP5</td>
</tr>
<tr>
<td>CP8</td>
</tr>
<tr>
<td>CP–</td>
</tr>
<tr>
<td>6571</td>
</tr>
</tbody>
</table>

R, resistant; S, susceptible; PEN, penicillin; OXA, oxacillin; VAN, vancomycin; MUP, mupirocin; CHL, chloramphenicol; TET, tetracycline; ERY, erythromycin; GEN, gentamicin; FD, fusidic acid; AMP, ampicillin; CEC, cefaclor; CRO, ceftriaxone; RIF, rifampicin; TEC, teicoplanin.

Table 2. MICs for S. aureus Reynolds expressing either capsule serotype CP5, CP8 or CP– (acapsular)

<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th>PEN</th>
<th>OXA</th>
<th>VAN</th>
<th>MUP</th>
<th>CHL</th>
<th>TET</th>
<th>ERY</th>
<th>GEN</th>
<th>FD</th>
<th>Tric</th>
<th>CHX</th>
<th>CPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP5</td>
<td>+</td>
<td>16</td>
<td>0.75</td>
<td>1.0</td>
<td>0.125</td>
<td>6</td>
<td>0.28</td>
<td>0.38</td>
<td>0.25</td>
<td>0.064</td>
<td>0.13</td>
<td>2.0</td>
</tr>
<tr>
<td>CP8</td>
<td>+</td>
<td>16</td>
<td>0.75</td>
<td>1.0</td>
<td>0.125</td>
<td>6</td>
<td>0.38</td>
<td>0.38</td>
<td>0.25</td>
<td>0.064</td>
<td>0.13</td>
<td>2.0</td>
</tr>
<tr>
<td>CP–</td>
<td>+</td>
<td>16</td>
<td>0.75</td>
<td>1.5</td>
<td>0.125</td>
<td>4</td>
<td>0.5</td>
<td>&gt;256</td>
<td>0.19</td>
<td>0.064</td>
<td>0.13</td>
<td>2.0</td>
</tr>
<tr>
<td>6571</td>
<td>–</td>
<td>0.023</td>
<td>0.125</td>
<td>1.0</td>
<td>0.19</td>
<td>4</td>
<td>0.064</td>
<td>0.19</td>
<td>0.19</td>
<td>0.094</td>
<td>0.13</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Calculated by Etest (AB Biodisk, Sweden).
*bCalculated in accordance with BSAC guidelines.

PEN, penicillin; OXA, oxacillin; VAN, vancomycin; MUP, mupirocin; CHL, chloramphenicol; TET, tetracycline; ERY, erythromycin; GEN, gentamicin; FD, fusidic acid; Tric, triclosan; CHX, chlorhexidine gluconate; CPC, cetylpyridinium chloride.
of antimicrobial with which there was no visible growth of the organism and are shown in Table 2, along with the Etest data. In addition, all strains were investigated for β-lactamase production by nitrocefin stick (Oxoid, Basingstoke, UK; Table 2).

The antibiogram for NCTC 6571, as deduced by antibiotic disc susceptibility testing, was as expected. No difference in antibiotic susceptibility was observed between the capsule strains other than for erythromycin, resistance to which was found in CP—. This was as expected due to the construction of the CP— strain by disruption of the serotype-specific capsule genes with an erm(B) gene. The nitrocefin test implies that penicillin and ampicillin resistance is conferred by β-lactamase production in S. aureus Reynolds. MICs for NCTC 6571 were within plus or minus one two-fold dilution of the expected MIC. The capsule strains all demonstrated MICs for each antimicrobial within plus or minus one two-fold dilution of each other. The only exception to this was—once again—erythromycin, confirming the results of the susceptibility disc test. From this we deduced that there is no significant difference between the antibiotic and biocide susceptibilities of the strains investigated herein. Consequently, we conclude that the capsule polysaccharide serotypes CP5 and CP8 do not present a permeability barrier to the antibiotics used in this investigation, or to triclosan, chlorhexidine gluconate or cetylpyridinium chloride.

These results indicate that whereas S. aureus capsule polysaccharides are implicated in virulence, they are not involved in conferring reduced antibiotic or biocide susceptibility. Clinical implications are pertinent through the choice of clinically relevant antibiotics and biocides regularly used in the hospital setting.

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Correspondence

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BSAC Respiratory Resistance Surveillance Programme (2002–2003): comparative susceptibility of Streptococcus pneumoniae, cultured from patients in Great Britain and Ireland with community-acquired lower respiratory tract infection, to gemifloxacin

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Keywords: S. pneumoniae, RTIs, antibacterials, resistance

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Sir, Streptococcus pneumoniae is an important pathogen of the respiratory tract where it may be responsible for a wide range of infections including community-acquired pneumonia, acute exacerbations of chronic bronchitis and obstructive airways disease, sinusitis and otitis media.1

Therapeutic choice for infections which are likely to include S. pneumoniae among the causative bacteria is increasingly complicated by resistance to a range of otherwise suitable antimicrobials with the prevalence of resistance reaching alarming proportions in the Far East region, the USA and some countries within Europe.2 Global, national and local surveillance programmes are essential aids in defining rates and the evolution of antimicrobial resistance.3

The BSAC Respiratory Resistance Surveillance Programme began in 1999 and exists to provide long-term, large-scale antimicrobial susceptibility surveillance of the major pathogens causing community-acquired lower respiratory tract infections in Great Britain and Ireland, including S. pneumoniae.4

Gemifloxacin is a novel, fluorinated, naphthyridone antibacterial characterized by excellent activity against Gram-negative species typical of most fluoroquinolones, whilst possessing enhanced potency against Gram-positive bacterial pathogens, most notably S. pneumoniae, which exceeds that of other currently licensed members of this class of antimicrobials.4

During the 2002–2003 ‘cold season’, as part of the BSAC Resistance Surveillance Programme, the susceptibility of 772 isolates of S. pneumoniae to various antimicrobials, including gemifloxacin, was determined using BSAC procedures and interpretation of results.5 The results are presented in Table 1.

Non-susceptibility to penicillin (intermediate, MIC 0.12–1 mg/L, 8.3% + high-level resistance, MIC ≥2 mg/L, 0.3%) was found in a total of 8.6% of the isolates of S. pneumoniae. Erythromycin resistance was found in 10% of the isolates of S. pneumoniae with 4.7% resistant to clindamycin suggesting...