phenotypic Group I yielded amplicons consistent with \( \text{bla}_{\text{CTX-M-15}} \). The gene was confirmed as \( \text{bla}_{\text{CTX-M-15}} \) in representative isolates by sequencing the entire open-reading frame using the following external primers: CTX-M-15-SF, 5'-CACACGTGGAATTTA GGGACT-3' and CTX-M-15-SR, 5'-GCCGTCTAAGGCGAT AAACA-3'. These results were consistent with the findings in a previous study reporting the predominance of \( \text{bla}_{\text{CTX-M-15}} \) in a small number of isolates from Southern India. Some of the Group I isolates were positive for \( \text{bla}_{\text{TEM}} \) or \( \text{bla}_{\text{SHV}} \). The sequences of representative amplicons were consistent with \( \text{bla}_{\text{TEM-1}} \) and either \( \text{bla}_{\text{SHV-1}} \) or \( \text{bla}_{\text{SHV-25b}} \), respectively, all of which are non-ESBL genes. All five isolates in Group II were positive for \( \text{bla}_{\text{TEM}} \), an ESBL gene, and negative for \( \text{bla}_{\text{TEM}} \) or \( \text{bla}_{\text{CTX-M-15}} \).

The prevalence of ESBL producers in this study was strikingly high, particularly given the fact that more than half of the isolates were obtained from outpatients. One of the reasons contributing to the high prevalence of ESBL may be the crowded hospital conditions precluding implementation of optimal hygiene practices. The epidemic in the community is then likely fuelled by unrestricted use of antimicrobials that may be purchased without prescription. A recent study reported that ESBL-producing Enterobacteriaceae, including \( \text{K. pneumoniae} \), were responsible for community-onset infections in India. Dissemination of ESBL-producing \( \text{K. pneumoniae} \) in the community has a serious implication in the empirical management of complicated urinary tract infections caused by this organism, given their tendency for co-resistance to non-\( \beta \)-lactam classes of antimicrobials, as was observed in the present study. CTX-M-15 is known to be an ESBL that has peculiar association with community-onset \( \text{Escherichia coli} \) infections. It may therefore be speculated that CTX-M-15-producing \( \text{E. coli} \) is already established in the community in this area and that the responsible gene is being exchanged between \( \text{E. coli} \) and \( \text{K. pneumoniae} \) by means of conjugal transfer, both in the healthcare and in the community environments.

### Table 1. Phenotypic and genotypic characteristics of the \( \text{K. pneumoniae} \) clinical isolates

<table>
<thead>
<tr>
<th>Phenotypic</th>
<th>No. of isolates ((n = 270))</th>
<th>CAZ</th>
<th>CTX</th>
<th>FEP</th>
<th>FOX</th>
<th>ETP</th>
<th>IPM</th>
<th>CIP</th>
<th>CHL</th>
<th>GEN</th>
<th>AMK</th>
<th>ESBLs</th>
<th>Pulsotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>subgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>a</td>
<td>155</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S/I</td>
<td>R/S</td>
<td>S/I</td>
</tr>
<tr>
<td>b</td>
<td>71</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S/I</td>
<td>R/S</td>
<td>S/I</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>c</td>
<td>18</td>
<td>I</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S/I</td>
<td>R/S</td>
<td>S/I</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>d</td>
<td>7</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S/R</td>
<td>S</td>
<td>S</td>
<td>I/R</td>
<td>I/R</td>
<td>R/S</td>
<td>S</td>
<td>S</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>II</td>
<td>—</td>
<td>5</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I/S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>III</td>
<td>—</td>
<td>10</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I/R</td>
<td>S/S</td>
<td>S</td>
<td>none</td>
</tr>
</tbody>
</table>

CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; ETP, ertapenem; IPM, imipenem; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; AMK, amikacin.

Susceptibility results were interpreted according to the CLSI criteria: R, resistant; I, intermediate; S, susceptible.

### Transparency declarations

None to declare.

### References


### Journal of Antimicrobial Chemotherapy

doi:10.1093/jac/dkn105

Advance Access publication 15 March 2008

### Daptomycin resistance in *Enterococcus faecalis*

**prosthetic valve endocarditis**

Alicia I. Hidron1, Audrey N. Schuetz2, Frederick S. Nolte3, Carolyn V. Gould4, and Melissa K. Osborn1

1Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA, USA; 2Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA;

---

**Research letters**

---

**Funding**

D. L. P. is supported in part by NIH grant R01AI070896. Y. D. is supported by NIH grant T32AI007333.
Sir,

Daptomycin resistance is unusual. We report a case of clinical and bacteriological failure in a patient with *Enterococcus faecalis* endocarditis treated with daptomycin and review available data on daptomycin resistance.

A 53-year-old male was hospitalized for native mitral valve endocarditis due to methicillin-susceptible *Staphylococcus aureus*. Treatments with nafcillin and then vancomycin were discontinued due to a severe rash with both agents. Daptomycin was initiated followed by mitral valve replacement. The patient had a complicated, 2 month hospital course during which he developed a stage IV decubitus ulcer colonized with vancomycin-resistant *E. faecalis*. He completed 8 weeks of daptomycin at 6 mg/kg/day.

One month after discharge, the patient was diagnosed with prosthetic mitral valve endocarditis caused by vancomycin-resistant *E. faecalis*. A transesophageal echocardiogram showed multiple vegetations on the posterior leaflet of the mitral valve. Owing to penicillin allergy, treatment with daptomycin was initiated.

Blood cultures on day 1 grew three different strains of *E. faecalis*, including a vancomycin-resistant daptomycin-susceptible *E. faecalis* (MIC >512 and 4 mg/L, respectively), a vancomycin-susceptible daptomycin-non-susceptible *E. faecalis* (MIC 2 and >8 mg/L, respectively), and a vancomycin-resistant daptomycin-non-susceptible *E. faecalis* (MIC >512 and >8 mg/L, respectively). Susceptibility testing was performed using MicroScan broth microdilution (Dade Behring Inc., West Sacramento, CA, USA). Daptomycin MICs were performed using Etest strips (AB Biodisk North America Inc., Piscataway, NJ, USA) on Mueller–Hinton plates and were confirmed at the CDC. Daptomycin susceptibility methods were supplemented with additional calcium, as recommended by the CLSI.

Blood cultures on hospital days 2 and 4 again grew vancomycin-resistant, daptomycin-non-susceptible *E. faecalis*. Penicillin desensitization was attempted unsuccessfully. Linezolid was started on hospital day 7 and blood cultures cleared on day 9. Because he had heparin-induced thrombocytopenia, repeat valve replacement presented a high risk of stroke while on cardiac bypass. The patient therefore elected medical management. He was discharged on hospital day 16 on oral linezolid therapy for palliation. Ten days after discharge, bacteremia returned. He elected hospice care and expired shortly thereafter.

Antibacterial agents with activity against emerging multidrug-resistant Gram-positive pathogens are limited. Daptomycin is a novel cyclic lipopeptide that binds the cell membrane in a calcium-dependent fashion causing depolarization and release of intracellular ions with arrest in macromolecular synthesis and cell death. It is bactericidal against both susceptible and resistant Gram-positive organisms and has a low toxicity profile. However, it is not approved for use in vancomycin-resistant *Enterococcus* infections. Little is known about the potential emergence of daptomycin resistance among these species and there are no defined MIC breakpoints for a resistant category for *Enterococcus* according to the USA-FDA and CLSI.

Case reports of clinical failures with daptomycin in *S. aureus* infections emerged shortly after approval. However, resistance among *Enterococcus* species has been reported in only seven published cases (Table 1). Currently, there are no studies on mechanisms of daptomycin resistance in enterococcal species. For *S. aureus*, changes in membrane structure and function correlate with in vivo development of daptomycin non-susceptibility, and mutations in proteins involved in cell permeability have been implicated in daptomycin MIC increases. Daptomycin, a large molecule, may get trapped in the cell wall, unable to bind its target in the cell membrane. Supporting this hypothesis, elevated daptomycin MICs have been found to correlate with decreased susceptibility to vancomycin in >90% of the *S. aureus* isolates, and the level of resistance for both drugs has been found to correlate positively with cell wall thickness in glycopeptide-intermediate *S. aureus* isolates.

### Table 1. Daptomycin non-susceptible *Enterococcus* species isolates: case reports

<table>
<thead>
<tr>
<th>Patient age (years)</th>
<th>Underlying diagnosis</th>
<th>Daptomycin treatment duration</th>
<th>Indication for daptomycin use</th>
<th>Daptomycin MIC (mg/L)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>ESRD, cirrhosis</td>
<td>+/− 2 weeks</td>
<td>VR-<em>E. faecalis</em> bacteraemia</td>
<td>16</td>
<td>Munoz-Price et al.3</td>
</tr>
<tr>
<td>37</td>
<td>AML</td>
<td>17 days</td>
<td>VR-<em>E. faecium</em> bacteraemia</td>
<td>&gt;32</td>
<td>Long et al.5</td>
</tr>
<tr>
<td>62</td>
<td>myeloblastosis</td>
<td>28 days</td>
<td>VR-<em>E. durans</em> bacteraemia</td>
<td>32</td>
<td>Green et al.6</td>
</tr>
<tr>
<td>55</td>
<td>DM, ESRD</td>
<td>18 days</td>
<td><em>E. faecalis</em> endocarditis with PCN, vancomycin allergy</td>
<td>32</td>
<td>Kanafani et al.8</td>
</tr>
<tr>
<td>22</td>
<td>HL/AML</td>
<td>17 days</td>
<td>VR-<em>E. faecium</em> bacteraemia</td>
<td>&gt;32</td>
<td>Lewis et al.7</td>
</tr>
<tr>
<td>84</td>
<td>CKD, CHF</td>
<td>no prior exposure</td>
<td>VR-<em>E. faecium</em> endocarditis</td>
<td>6</td>
<td>Lesho et al.9</td>
</tr>
<tr>
<td>NR</td>
<td>Crohn’s disease</td>
<td>no prior exposure</td>
<td>VR-<em>E. faecium</em> bacteraemia</td>
<td>16</td>
<td>Fraher et al.10</td>
</tr>
</tbody>
</table>

ESRD, end-stage renal disease; AML, acute myeloid leukaemia; DM, diabetes mellitus; HL, Hodgkin’s lymphoma; CKD, chronic kidney disease; CHF, congestive heart failure; VR, vancomycin-resistant; PCN, penicillin; ref, reference; NR, not reported.
More recently, emergence of daptomycin heteroresistance was demonstrated for a vancomycin-heteroresistant *E. faecium* after exposure to vancomycin, suggesting the same underlying resistance mechanism. During therapy with daptomycin for *S. aureus* bacteraemia and endocarditis, 16% of the patients failed with a persistent or relapsing infection with increasing isolate MICs. Most of these failures were in deep-seated infections for which a necessary surgical intervention was not performed. In the case of *Enterococcus*, either a chronic indwelling line or persistent focus of infection was reported for all but two of the seven reported cases of daptomycin clinical treatment failures, and non-susceptibility developed on treatment after an average of 19 days.

The mechanisms of non-susceptibility to daptomycin are thus diverse and not completely understood. Increasing MICs in the setting of pre-exposure to either vancomycin or daptomycin and/or evidence of a persistent focus of infection seem to be a frequent occurrence for clinical failures among both *S. aureus* and *Enterococcus* species. Despite lack of experimental data, it is plausible that the mechanisms that explain enterococcal non-susceptibility parallel those for *S. aureus*. The use of daptomycin in patients with prior prolonged vancomycin or daptomycin therapy, or with a focus of infection that cannot be removed, should be undertaken with caution, as clinical failure can occur.

**Funding**

No specific funding was received for this study.

**Transparency declarations**

None to declare.

**References**


**Journal of Antimicrobial Chemotherapy**

doi:10.1093/jac/dkn117

Advance Access publication 3 April 2008

**Severe hypokalaemia caused by flucloxacillin**

Ewout J. Hoorn and Robert Zietse

Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

Keywords: aldosterone, collecting duct, kaliuresis, solute diuresis, spironolactone, spondylodiscitis

*Corresponding author. Tel: +31-10-4635335; Fax: +31-10-4633008; E-mail: ejhoorn@gmail.com

Sir,

Hypokalaemia has been reported during treatment with penicillin G and broad-spectrum penicillins. We report, to our knowledge for the first time, a case of severe hypokalaemia during treatment with yet another penicillin, namely sodium flucloxacillin (floxacillin). Our aim was to illustrate that hypokalaemia is a class-effect of penicillins, to reiterate the importance of penicillin-induced hypokalaemia and to further investigate its mechanism and potential treatment.

A 67-year-old woman (height 165 cm and weight 45 kg) was treated with flucloxacillin (2 g six times daily) for spondylodiscitis during two admissions. Spondylodiscitis had developed after an elective colectomy for diverticulosis, which was