Activity of mecillinam against Escherichia coli resistant to third-generation cephalosporins

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Objectives: To assess the activity of mecillinam against two groups of Escherichia coli: (i) a selection of international isolates with mechanisms of resistance caused by the presence of defined β-lactamases; and (ii) isolates resistant to third-generation cephalosporins referred from across Wales.

Methods: Antibiotic susceptibility testing with mecillinam, meropenem, amoxicillin, co-amoxiclav, cefotaxime, piperacillin/tazobactam, ciprofloxacin, nitrofurantoin, trimethoprim and gentamicin was performed using the BSAC agar dilution method against 30 international strains of E. coli with known β-lactamase presence. Antibiotic susceptibility testing with mecillinam using the same method was performed against 325 regional isolates of E. coli resistant to third-generation cephalosporins.

Results: The susceptibility results showed that the only antibiotics to which the 30 international isolates were consistently susceptible were mecillinam (100%) and meropenem (100%), irrespective of the presence of β-lactamases. Of the local isolates, 93.5% (304/325) were susceptible to mecillinam, having MICs ≤ 8 mg/L.

Conclusions: Our results show that mecillinam has excellent in vitro activity against a range of E. coli exhibiting β-lactamase activity, some with the production of multiple β-lactamases. It is time to further evaluate the clinical utility of mecillinam in the treatment of infections caused by such organisms.

Keywords: ESBLs, susceptibilities, resistance

Introduction

Escherichia coli is the most common cause of acute urinary tract infections. It is also the most common genuine isolate from bacteraemias in Wales, accounting for a rate of 44 per 100000 bed days in 2007.1 Since the late 1990s there has been a widespread and increasing emergence of antibiotic-resistant E. coli. The changing UK and worldwide prevalence of E. coli producing extended-spectrum β-lactamases (ESBLs), especially the CTX-M enzyme type, has been well documented,2–4 with CTX-M-15 showing a particular propensity for spread.1–4 Other enzymes such as plasmid-borne AmpC-type and OXA β-lactamases are being increasingly recognized, and in some parts of the world E. coli strains with multiple resistance mechanisms are becoming more common.6 Multiple antibiotic resistances are often found in these organisms, with consistent susceptibility only shown to carbapenems and nitrofurantoin. However, many isolates submitted to reference laboratories are from community sources, especially urinary tract infections,1,2 and the lack of orally available antibiotics can be problematic.

Mecillinam, is a β-lactam antibiotic that is active against many Enterobacteriaceae but not against Gram-positive organisms. Unlike most β-lactams, which bind to Gram-negative PBP-1A, -1B or -3, mecillinam binds to Gram-negative PBP2, an enzyme that is critical for the establishment and maintenance of bacillary cell shape.5 Pivmecillinam is the orally administered pro-drug that is hydrolysed to give the active agent mecillinam. Clinical use of pivmecillinam is largely confined to Scandinavian countries where experience and studies confirm the efficacy and safety of this agent in the treatment of acute cystitis.6–8

Given the increasing resistance problems in E. coli and the subsequent limited therapeutic options available, the aim of the work presented here was to evaluate mecillinam susceptibility in a range of E. coli exhibiting enzymatic mechanisms conferring resistance to third-generation cephalosporins.

Materials and methods

Bacterial isolates

Two groups of E. coli isolates were studied. The first group consisted of a collection of 30 international isolates with well-defined, often multiple,
resistance mechanisms to third-generation cephalosporins. The presence of specific β-lactamases had previously been established by PCR and sequencing, and comprised: TEM-1 (2 isolates), hyperexpression of chromosomal AmpC (1 isolate), CTX-M (12 isolates), CTX-M/SHV (3 isolates), CMY-2 (3 isolates), CMY-2/CTX-M-1 (1 isolate), OXA (1 isolate), CTX-M/OXA-2 (1 isolate), TEM-1/CTX-M-1/SHV/OXA-2 (1 isolate), TEM/SHV/CTX-M (1 isolate), TEM/SHV/CTX-M/CMY/OXA-2 (1 isolate), TEM-1/SHV/OXA-2 (1 isolate), TEM/SHV/CTX-M-1 (1 isolate) and TEM/SHV/CTX-M-1/CTX-M-2/OXA-2 (1 isolate). The second group comprised 325 cefpodoxime-resistant E. coli isolates referred to the Welsh Specialist Antimicrobial Chemotherapy Unit (SACU) between September 2007 and September 2008 by microbiology laboratories across Wales as part of an ongoing Antimicrobial Resistance Surveillance Programme.

**MICs**

MICs in the range from 0.008 to 128 mg/L were determined on Iso-Sensitest agar (Oxoid, Basingstoke, UK) according to BSAC standard methods.9 MICs of mecillinam (Leo-Pharma, Princes Risborough, UK) were obtained for all isolates and, in addition, the international collection had MICs determined for ciprofloxacin (Claris Life Sciences Ltd, Crewe, UK), nitrofurantoin (Sigma-Aldrich, Poole, UK), gentamicin sulphate (Sigma-Aldrich), cefotaxime (Sigma-Aldrich), meropenem (AstraZeneca, Macclesfield, UK), piperacillin/tazobactam (8:1 ratio) (Sigma, Poole, UK), amoxicillin (Sigma), trimethoprim (Sigma) and co-amoxiclav (4:1 ratio) (Sigma). Inocula were prepared in saline to achieve 10⁷ cfu/mL (0.5 McFarland standard), then diluted 1 in 10 using sterile saline. A multipoint inoculator was used to dispense 1 μL of diluted inoculum to achieve 10⁴ cfu/mL per plate. Plates were incubated in air at 37°C for 18 h. A fully susceptible E. coli (NCTC 12241) and a β-lactamase-producing E. coli (NCTC 11560) were used as controls.

**Results**

The susceptibility results for the 30 international strains of E. coli with defined mechanisms of resistance to third-generation cephalosporins are summarized in Table 1. All isolates tested were susceptible to mecillinam (BSAC urinary breakpoint: resistance, MIC >8 mg/L) and meropenem. All isolates were resistant to amoxicillin and co-amoxiclav, 13.3% were susceptible in vitro for cefotaxime (Sigma-Aldrich, Poole, UK), gentamicin sulphate (Sigma-Aldrich), cefotaxime (Sigma-Aldrich), meropenem (AstraZeneca, Macclesfield, UK), piperacillin/tazobactam (8:1 ratio) (Sigma, Poole, UK), amoxicillin (Sigma), trimethoprim (Sigma) and co-amoxiclav (4:1 ratio) (Sigma). Inocula were prepared in saline to achieve 10⁷ cfu/mL (0.5 McFarland standard), then diluted 1 in 10 using sterile saline. A multipoint inoculator was used to dispense 1 μL of diluted inoculum to achieve 10⁴ cfu/mL per plate. Plates were incubated in air at 37°C for 18 h. A fully susceptible E. coli (NCTC 12241) and a β-lactamase-producing E. coli (NCTC 11560) were used as controls.

### Table 1. MICs for 30 E. coli isolates with well-defined mechanisms of resistance to third-generation cephalosporins

<table>
<thead>
<tr>
<th>Antimicrobial tested</th>
<th>MIC₅₀ (mg/L)</th>
<th>MIC₉₀ (mg/L)</th>
<th>Percentage susceptibility (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mecillinam</td>
<td>0.5</td>
<td>4</td>
<td>100 (30/30)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.03</td>
<td>0.06</td>
<td>100 (30/30)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>13.3 (4/30)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>0 (0/30)</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>0 (0/30)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>16</td>
<td>128</td>
<td>53.3 (16/30)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
<td>128</td>
<td>40 (12/30)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>16</td>
<td>64</td>
<td>76.7 (23/30)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>30 (9/30)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.5</td>
<td>64</td>
<td>53.3 (16/30)</td>
</tr>
</tbody>
</table>

Breakpoints used: mecillinam resistance, MIC >8 mg/L; meropenem resistance, MIC >8 mg/L; cefotaxime resistance, MIC >2 mg/L; amoxicillin resistance, MIC >16 mg/L; co-amoxicillin resistance, MIC >16 mg/L; piperacillin/tazobactam resistance, MIC >16 mg/L; ciprofloxacin resistance, MIC >1 mg/L; nitrofurantoin resistance, MIC >32 mg/L; trimethoprim resistance, MIC >2 mg/L; gentamicin resistance, MIC >4 mg/L.13

![Figure 1. Population distribution of mecillinam MICs for 325 cefpodoxime-resistant E. coli isolates.](https://academic.oup.com/jac/article-abstract/65/1/79/726791/0.02-0.03-0.06-0.13-0.25-0.5-1-2-4-8-16-32-64-128-256?download=18jan2019)
Mecillinam activity against cephalosporin-resistant E. coli

Discussion
With the increasing isolation of clinically significant antibiotic-resistant E. coli from patient specimens, the issue of choosing appropriate antibiotic therapy becomes more difficult. This is especially so if, as is shown here and is commonly found, there is resistance to multiple classes of antibiotics. Oral therapy in the community or intravenous/oral switch in hospitals becomes problematic where no effective oral agent exists. Choosing the correct antibiotic therapy is essential to optimize the clinical outcome of infection. On the other hand, the inappropriate intravenous administration of broad-spectrum antibiotics, such as carbapenems, potentially increases the incidence of drug side effects, Clostridium difficile infection and healthcare-associated costs. Our results show that mecillinam has excellent in vitro activity against a range of E. coli exhibiting β-lactamase activity, some with the production of multiple β-lactamases. This activity against international strains is also reflected locally in a large number of E. coli isolates tested that were resistant to third-generation cephalosporins.

Pivmecillinam is widely used in Scandinavian countries where there is broad experience of its good safety profile and clinical use in the treatment of acute uncomplicated urinary tract infections (including in pregnancy), suggesting its efficacy against all urinary tract infection pathogens. Surveillance has shown that resistance to this agent in E. coli from community sources is low and has not increased in these countries despite the widespread use of pivmecillinam for >20 years. Previous reports suggest that ESBLs show activity against mecillinam in vitro, as seen when using a heavy inoculum. This would imply that mecillinam should not be used for severe infections. However, given the prevalence of faecal carriage of ESBL-producing E. coli, the increasing number of infections in the community with multiply antibiotic-resistant E. coli, our current paucity of oral antimicrobial agents with activity against these organisms and the slow development of new agents, it is time to further evaluate the clinical utility of mecillinam in the treatment of infections caused by such organisms.

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Transparency declarations
None to declare.

References