Comparative in-vitro activity of cefaclor against urinary tract isolates of *Escherichia coli*

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Insusceptibility levels of cefaclor and other commonly prescribed antibiotics were determined for 489 consecutive hospital and community-associated urinary tract isolates of *Escherichia coli* from the Nottingham area of the UK. Significant resistance (MIC of ≥8 mg/L) to cefaclor was found to be uncommon in the UK, with insusceptibility percentages as low as 1.5% and 1.4% amongst hospital and community isolates, respectively. When compared with other antimicrobials used commonly for treating urinary tract infection, only ciprofloxacin showed greater activity, though cefaclor showed significantly greater in-vitro activity than cephalaxin, ampicillin and trimethoprim. Only seven isolates were insusceptible to cefaclor at a concentration of 8 mg/L. Each of these isolates produced a β-lactamase, but it is unlikely that β-lactamase production was the sole reason for insusceptibility since these isolates were also insusceptible to co-amoxiclav. Cefaclor compared extremely well with co-amoxiclav against ampicillin-insusceptible isolates, with none of the pharmacokinetic difficulties and considerations associated with the use of β-lactam:β-lactamase inhibitor combinations. Cefaclor appears to be a useful cost-effective alternative to current therapeutic options, particularly for long-term low-dose treatment of recurrent urinary tract infection in those geographical areas where the likelihood of resistance to other possible agents is becoming unacceptably high.

**Introduction**

Cefaclor is an orally administered second generation cephalosporin, available since 1979, that has been used widely throughout the world for the management of bacterial infections of the respiratory and urinary tracts, the skin and associated structures (Preston, 1993). Despite its ready availability, cefaclor remained effective clinically after its first decade of use in the USA and other countries (Moellering & Waldvogel, 1988), and continued to show good activity over this period against a range of common pathogens including *Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus* and *Escherichia coli* (Preston, 1993). More recently, an advanced modified release cefaclor formulation (cefaclor MR) has become available for use as a twice-a-day antibiotic and shows good potential for treating infection of the respiratory tract, the urinary tract and skin (Thornsberry, 1992).

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It has been shown recently that low-dose cefaclor is an effective long-term prophylactic treatment for recurrent urinary tract infection (Brumfitt & Hamilton-Miller, 1995). This is a serious condition that can affect significantly the quality of life of the patient (Brumfitt & Hamilton-Miller, 1995). Trimethoprim has been a very effective prophylactic treatment of recurrent urinary tract infection (Brumfitt & Hamilton-Miller, 1990), but while long-term control is not usually affected by breakthrough infections caused by organisms mutating to trimethoprim resistance during treatment (Pearson et al., 1979), the effectiveness of trimethoprim has become compromised in recent years (Brumfitt & Hamilton-Miller, 1995). This is because of a general increase in the number of resistant strains in the community (Towner, 1992), and hence a greater risk that a patient would be carrying a resistant strain at the commencement of treatment or would acquire one rapidly from the environment. In the light of this previous experience, this paper presents the results of a comparative re-evaluation of cefaclor susceptibility levels, in comparison with other commonly prescribed antibiotics, against hospital and community-associated urinary tract isolates of *E. coli* from the Nottingham area, and examines mechanisms of cefaclor resistance in those strains found to be insusceptible.

**Materials and methods**

**Bacteria**

In total, 489 consecutive isolates of *E. coli* (129 hospital isolates and 360 community and outpatient isolates) from infected ($\geq 10^5$ cfu/mL) urine samples were included in the study. Species identification was initially on the basis of a series of in-house biochemical and other tests, derived from standard methods (Barrow & Feltham, 1993), and was confirmed in equivocal cases by means of the API 20E system (bioMérieux).

**Susceptibility tests**

Initial breakpoint susceptibilities were determined by multipoint inoculation on to freshly prepared Isosensitest agar plates (Oxoid) containing 5% lysed horse blood and breakpoint concentrations of either cefaclor 8 mg/L, cephalexin 8 mg/L, ampicillin 8 mg/L, trimethoprim 2 mg/L or ciprofloxacin 4 mg/L. When required, full MIC values, including those of co-amoxiclav (amoxycillin + clavulanic acid) were determined by Etests (AB Biodisk).

**β-Lactamase characterization**

Where necessary, strains were tested for β-lactamase production by preparing crude enzyme extracts (Carter et al., 1989) and testing (1:1) in microtitre plates with a solution (1 mg/mL) of the chromogenic cephalosporin nitrocefin (Oxoid). Strains that produced β-lactamase in this test were then screened for production of TEM-family enzymes by means of the rapid PCR-based test described below.

β-Lactamase-positive strains were also characterized by analytical isoelectric focusing (IEF) on cellulose acetate membranes (Eley, Ambler & Greenwood, 1983) as described previously (Carter et al., 1989).
**In-vitro activity of cefaclor**

**PCR-based test for TEM-family β-lactamases**

Total DNA was extracted from each *E. coli* isolate by emulsifying a small loopful of overnight growth from an agar plate in 100 μL of sterile H2O contained in a microfuge tube, overlaying with sterile mineral oil (Sigma), heating to 98°C for 15 min in a DB2A Dri Block (Techne), followed by cooling and diluting into 900 μL of sterile H2O. The resulting crude DNA extract was centrifuged for a few seconds at 12,000 g in a microcentrifuge.

The supernatant was then used directly in PCR multiplex reactions in which two sets of primers, for TEM-family β-lactamase genes and a conserved sequence coding for 16S rRNA, respectively, were used simultaneously in the same tube. The primers used are listed in Table I and were custom-synthesized (Oswel DNA Service, Southampton). The specificity of these primer sets for their targets has been demonstrated previously (Geha *et al.*, 1994; Tenover *et al.*, 1994). PCR mixes were amplified with the following set of conditions: 4 min at 94°C, followed by 30 cycles of 45 sec at 94°C, 45 sec at 67°C and 1 min at 72°C, followed by 2 min at 72°C. Each PCR mix contained (in a final volume of 25 μL): 2.5 pmol of each of the four primers; 0.2 mM dNTPs (Boehringer Mannheim); 1.5 mM MgCl2; 0.6 U Taq polymerase (Boehringer Mannheim); and 4 μL of bacterial DNA extract, prepared as above. The end-product was electrophoresed on an agarose 1.5% gel, then stained with ethidium bromide and examined with UV-transillumination for the presence of the specific PCR end-products.

**Results**

**Insusceptibility tests**

Figure 1 shows the combined insusceptibility breakpoint percentages for the strains of *E. coli* included in the study. When hospital isolates were compared with the group of community and outpatient isolates, the respective percentage insusceptibilities were as follows: cefaclor, 1.5%:1.4%; cephalexin, 14.0%:4.7%; ampicillin 47.3%:45.0%; trimethoprim, 23.3%:17.2%; and ciprofloxacin, 0%:0.6%. The MICs of cefaclor for the strains from different origins, as determined by Etests, are shown in Figure 2. MIC₉₀ values of cefaclor were 2 and 1.5 mg/L for hospital and community/outpatient isolates respectively (range 0.125–96 mg/L).

**Cefaclor-insusceptible isolates**

The seven isolates (Table II) insusceptible to a cefaclor breakpoint concentration of 8 mg/L were also insusceptible to ampicillin and cephalexin, but were all susceptible to ciprofloxacin. Three of the seven isolates were insusceptible to trimethoprim. All seven of the cefaclor-insusceptible isolates produced β-lactamase, and three of these produced the specific 526-bp PCR reaction product characteristic of TEM-family β-lactamas and an enzyme of pI ca. 5.5 following isoelectric focusing on cellulose acetate membranes. The remaining four isolates were negative in the PCR test and produced β-lactamases with pI values of 6.9, 7.4, 5.95 and 5.95, respectively. Only three of the seven isolates had a cefaclor MIC of >8 mg/L when tested by the Etest method (MICs of 24, 32 and 96 mg/L, respectively), and these three isolates were also relatively insusceptible to co-amoxiclav (amoxycillin + clavulanic acid) by the Etest method (Table II).
Table I. Primers used in PCR amplification experiments to detect TEM-family β-lactamase genes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Target</th>
<th>Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>5'-GGAATTCAAA/GGAATTGACGGGGGC</td>
<td>Conserved 16S rRNA sequence</td>
<td>479-bp</td>
<td>Geha et al. (1994)</td>
</tr>
<tr>
<td>Y</td>
<td>5'-CGGGATCCAGGCCCGGGAACGTATTCAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp1</td>
<td>5'-TGGGTGCACGAGTGGGTTAC</td>
<td>TEM-family β-lactamase genes</td>
<td>526-bp</td>
<td>Tenover et al. (1994)</td>
</tr>
<tr>
<td>Amp2</td>
<td>5'-TTATCCGCCTCCATCCAGTC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II. Characteristics of E. coli strains susceptible to a cefaclor breakpoint concentration of 8 mg/L

<table>
<thead>
<tr>
<th>Strain number</th>
<th>MIC (mg/L)</th>
<th>Breakpoint susceptibilities (mg/L)</th>
<th>β-Lactamase production</th>
<th>pI value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ccl</td>
<td>CoA</td>
<td>Tmp 2</td>
<td>Amp 8</td>
</tr>
<tr>
<td>80</td>
<td>8</td>
<td>2 + 1*</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>81</td>
<td>24</td>
<td>16 + 8</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>84</td>
<td>96</td>
<td>16 + 8</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>116</td>
<td>32</td>
<td>128 + 64</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>157</td>
<td>8</td>
<td>12 + 6</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>349</td>
<td>8</td>
<td>12 + 6</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>388</td>
<td>8</td>
<td>12 + 6</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

Amp, Ampicillin; Ccl, cefaclor; Cip, ciprofloxacin; Clx, cephalexin; CoA, co-amoxiclav; Tmp, trimethoprim.
R, resistant; S, susceptible.
*Amoxycillin + clavulanic acid.
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Figure 1. Percentages of E. coli strains insusceptible to the antibiotic breakpoints shown, subdivided according to origin (Amp, ampicillin; Ccl, cefaclor; Cip, ciprofloxacin; Clx, cephalaxin; Tmp, Trimethoprim). □, Hospital strains; ☐, community strains.

Ampicillin-insusceptible isolates

It was of interest to compare the in-vitro activities of cefaclor and co-amoxiclav (amoxycillin + clavulanic acid) against the 223 isolates that were insusceptible to ampicillin. Figure 3 shows the MICs of cefaclor and co-amoxiclav (as determined by Etests) against the ampicillin-insusceptible isolates. The MIC\textsubscript{90} values were 2

Figure 2. MICs of cefaclor, as determined by Etests, for the 489 isolates of E. coli included in the study. □, Hospital strains; ☐, community strains.
Figure 3. MICs of cefaclor (■) and co-amoxiclav (□), as determined by Etests, for the 223 ampicillin-insusceptible *E. coli* isolates included in the study. Co-amoxiclav is 2:1 amoxycillin: clavulanic acid. Amoxycillin concentrations are shown.

vs 2 mg/L for cefaclor (range 0.19–96) and 12 + 6 vs 12 + 6 mg/L for co-amoxiclav (range 0.75 + 0.38–≥ 256 + 128). Thirty of the ampicillin-insusceptible isolates failed to produce a β-lactamase, and for these isolates, the MIC₉₀ values were 2 mg/L (range 1–2) and 16 + 8 mg/L (range 2 + 1–16 + 8) for cefaclor and co-amoxiclav, respectively.

**Discussion**

A study performed in the USA between 1988 and 1990 (i.e., after widespread usage of cefaclor for 10 years) found that cefaclor retained microbiological activity against more than 95% of strains isolated from uncomplicated urinary infections, including 97.9% of the *E. coli* strains isolated (Hamilton-Miller *et al.*, 1992). The present study, performed 5 years later, of isolates from the Nottingham area, found that significant resistance to cefaclor amongst isolates of the most common urinary tract pathogen *E. coli*, remains uncommon in the UK, with insusceptibility percentages of 1.5% and 1.4% amongst hospital and community isolates respectively. In comparison with other antimicrobials used commonly for treating urinary tract infection (Figure 1), only ciprofloxacin showed greater activity at the breakpoint concentrations used, and cefaclor showed significantly greater in-vitro activity than cephalaxin, ampicillin and trimethoprim.

Only seven of the 489 isolates investigated were found to be insusceptible to a cefaclor breakpoint concentration of 8 mg/L (Figure 2). Each of these isolates produced a β-lactamase (three of the seven strains produced a TEM-family β-lactamase), but it is unlikely that β-lactamase production was the sole reason for insusceptibility since these isolates were also relatively insusceptible to co-amoxiclav. In addition, cefaclor showed excellent activity, with an MIC₉₀ value of 2 mg/L, against ampicillin-insusceptible
isolates that produced $\beta$-lactamase. The access of most $\beta$-lactams, including cefaclor, to target enzymes located in the cytoplasmic membranes of Gram-negative bacteria is usually controlled to a greater or lesser extent by the presence and character of the bacterial outer membrane (Thornsberry, 1992). It therefore seems probable that cefaclor permeability considerations may play a part in the overall level of insusceptibility observed amongst these seven resistant strains, but this aspect was not examined further in the present study. It is worth noting that cefaclor compared well with co-amoxiclav against ampicillin-insusceptible isolates (Figure 3), with none of the pharmacokinetic difficulties and considerations associated with the use of $\beta$-lactam:$\beta$-lactamase inhibitor combinations.

In conclusion, cefaclor has already been shown to be an effective agent for long-term low-dose treatment of recurrent urinary tract infection, with a low likelihood of breakthrough infections caused by the emergence of resistance during the course of therapy (Brumfitt & Hamilton-Miller, 1995). Previous treatments for recurrent urinary tract infection have become compromised in recent years by the increasing likelihood of initial infection with a resistant strain before the commencement of therapy. The present study has demonstrated that significant resistance to cefaclor remains extremely uncommon, particularly in comparison with trimethoprim and ampicillin, amongst urinary isolates of *E. coli* in the UK, and that the chance of initial infection with a cefaclor-resistant organism is correspondingly low. Cefaclor therefore appears to be a useful cost-effective alternative to current therapeutic strategies in geographical areas where the likelihood of resistance to other possible agents is becoming unacceptably high.

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**References**


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