Molecular epidemiology of multiresistant *Escherichia coli* isolates from community-onset urinary tract infections in Cornwall, England

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**Objectives:** To study the clonality of gentamicin-resistant, extended-spectrum β-lactamase (ESBL)-negative and ESBL-producing *Escherichia coli* isolated from community-onset urinary tract infections (UTIs) in Cornwall.

**Methods:** Isolates were identified by API, susceptibilities were determined by local disc testing, and MICs were determined at the reference laboratory, both interpreted using BSAC guidelines. *bla*<sub>CTX-M</sub> genes were sought by PCR, and isolates were compared by PFGE.

**Results:** In the years 2004 and 2005, 69 *E. coli* were submitted by Truro (Cornwall) laboratory for reference laboratory testing: these included 14 gentamicin-resistant, ESBL-negative isolates; 45 with group 1 CTX-M enzymes; seven with group 9 CTX-M enzymes; and three with non-CTX-M ESBLs. By PFGE, nine gentamicin-resistant, ESBL-negative *E. coli* were distinct (<85% similarity) from all the ESBL producers, but three were related to producers of group 1 CTX-M enzymes, and two isolates were related to a non-CTX-M ESBL producer. An outbreak strain was identified, represented by 11 gentamicin-resistant and one gentamicin-susceptible isolates, all with group 1 CTX-M enzymes, and two gentamicin-resistant, ESBL-negative isolates. This was distinct by PFGE from nationally distributed CTX-M-producing strains. Five of nine patients infected with this strain had been on the same ward in a local hospital; four presented with community-onset UTIs; one inpatient developed a hospital-acquired bacteraemia. Of the other four patients presenting with community-onset UTIs, three were admitted to different hospitals and the fourth had only attended an outpatient clinic.

**Conclusions:** Community-onset, ESBL-producing and non-producing *E. coli* were diverse. Two ESBL-negative isolates were closely related to a local CTX-M-producing outbreak strain, suggesting gain or loss of a *bla*<sub>CTX-M</sub>-carrying plasmid. An outbreak strain was linked with prior hospital admission and appeared not to represent genuine community acquisition.

Keywords: cephalosporins, ESBLs, resistance, Enterobacteriaceae

**Introduction**

Multiresistant *Escherichia coli* strains from community-onset infections are an increasing public health concern, especially those with CTX-M extended-spectrum β-lactamases (ESBLs). In the UK, ~90% of CTX-M-producing *E. coli* produce group 1 enzymes (mainly CTX-M-15) and PFGE analysis of a national collection has defined five major strains. The local epidemiology of *E. coli* producing CTX-M enzymes may, however, differ from this national picture with, for example, dominance of different strains, or higher prevalence of those with group 9 CTX-M enzymes.

In Cornwall (the south-west peninsula of England), concern was raised over the emergence of gentamicin-resistant *E. coli* isolates recovered from urines of patients attending general practice. Gentamicin is rarely prescribed in such settings. Several, but not all of these resistant isolates were ESBL producers.

We therefore studied the clonality of gentamicin-resistant, ESBL-negative isolates from urines of patients attending general practitioners (GPs) in Cornwall and compared them with...
CTX-M-producing *E. coli* in Cornwall

ESBL-producing *E. coli*, many also gentamicin-resistant and also isolated from GP urines.

Materials and methods

**Bacterial isolates and initial susceptibility testing**

Most *E. coli* isolates included in this study were associated with community-onset urinary tract infections (UTIs) in patients attending GPs’ surgeries in Cornwall during 2004 and 2005. One isolate was from a case of hospital-acquired bacteremia (onset >48 h after admission).

The isolates were identified as *E. coli* (API system; bioMerieux, Basingstoke, UK), and their antibiotic susceptibilities were determined by disc testing using BSAC criteria in the Department of Clinical Microbiology, Royal Cornwall Hospital. Fourteen gentamicin-resistant, ESBL-negative isolates and 55 ESBL-producing isolates, variably gentamicin-resistant, were referred to the Health Protection Agency’s Centre for Infections for further investigation. Multiple isolates were referred from some patients.

**Investigation in the reference laboratories**

MICs were determined and interpreted using BSAC methodology, and *blaCTX-M* genes were sought by multiplex PCR. Selected isolates were compared by PFGE of XhoI-digested genomic DNA, and banding patterns were analysed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

**Investigation of an outbreak strain**

The clinical notes for selected patients were analysed to determine any obvious common factors associated with acquisition of a PFGE-defined outbreak strain.

**Results and discussion**

**Reference investigations and recognition of a ‘Cornish outbreak’ strain**

During 2004–2005, the reference laboratories received 69 *E. coli* isolates from Truro (Cornwall) laboratory for inclusion in this study. These included: 14 gentamicin-resistant, ESBL-negative isolates; 45 with group 1 CTX-M enzymes; seven with group 9 CTX-M enzymes and three with non-CTX-M ESBLs.

All 14 ESBL-negative isolates, and 16 ESBL-producing isolates dating from 2004, were compared by PFGE (Figure 1). Nine gentamicin-resistant, ESBL-negative isolates were distinct (<85% similarity) from the ESBL producers, but three were related to isolates with CTX-M group 1 enzymes, and two isolates from one patient were related to an isolate with a non-CTX-M ESBL.

One major outbreak strain was identified, and comprised 11 gentamicin-resistant and one gentamicin-susceptible CTX-M group 1 producers and two gentamicin-resistant, ESBL-negative isolates (12 of these are shown in Figure 1); the 14 isolates were from nine patients. This ‘Cornish outbreak’ strain was distinct by PFGE from five nationally distributed CTX-M-producing strains, including the UK epidemic strain A (Figure 1), which accounted for only three of the 69 *E. coli* isolates referred from Truro laboratory.

Geometric mean MICs for the ‘Cornish outbreak’ strain are shown in Table 1. CTX-M ESBL-producing isolates of this strain were resistant to penicillins, all cephalosporins tested (but with potentiation by clavulanate), and also to ciprofloxacin, gentamicin and tobramycin. Isolates of the outbreak strain were susceptible to piperacillin/tazobactam, amikacin and carbapenems.

Two gentamicin-resistant, ESBL-negative isolates (unrelated by PFGE and distinct from the ‘Cornish outbreak’ strain) were highly resistant to the veterinary aminoglycoside apramycin (MICs > 512 mg/L), which is consistent with AAC(3)-IV activity; the range of apramycin MICs for all other isolates was 2–32 mg/L.

**Analysis of case notes**

Recognition of the ‘Cornish outbreak’ strain was unexpected and prompted analysis of clinical notes for the nine patients (5 male and 4 female) affected to determine any obvious common factors that might be associated with its acquisition. The average age of these patients was 80 years (range, 57–93 years).

One patient developed a hospital-acquired bacteremia (defined as onset >48 h after admission) with the ‘Cornish outbreak’ strain while an inpatient on ward G of a local hospital (RCH) in March to May 2004 during a cluster of infections on this ward caused by ESBL-producing *E. coli*. This blood culture isolate was the only saved representative from the ward cluster. The other eight patients infected by the ‘Cornish outbreak’ strain had presented at various GP surgeries, separated by up to 30 miles. The strain was isolated from urine in eight patients, from sputum in three, from a peg site in one, and two patients were widely colonized. Despite the community-onset of their UTIs, seven of these eight patients had prior, and in some instances multiple, hospital inpatient admissions, and multiple transfers between wards. The eighth only attended an outpatient clinic. The average time after hospital discharge before presentation with a UTI at their GP’s surgery was 39 days (range 6–89 days). Four of the eight GP patients had been inpatients on ward G of RCH during March–May 2004. Another had been in other wards at RCH during this period, though not on ward G. Two other patients had been on ward L of a second hospital (CRH), but there was no temporal overlap; one had been an inpatient from December 2003 to March 2004; the other was transferred from ward G of RCH in April 2004 and was discharged in May 2004. Six of the nine affected patients died. Mortality was not directly attributable to the ESBL-producing strain in five cases, but the sixth died with ESBL-producing *E. coli* septicemia, implying causality.

These data suggest that the ‘Cornish outbreak’ strain may have caused the cluster of infections on ward G at RCH during March–May 2004, although only one isolate was available for testing. The alarming finding of a CTX-M-producing *E. coli* strain presenting in the community, but that could be traced to a hospital source led to a rapid review of susceptibility testing of all ‘coliforms’ in the clinical laboratory, as advocated previously, and methodology for detecting ESBLs was in place within two months of these findings.

In summary, most gentamicin-resistant, ESBL-negative isolates from community-onset UTIs in Cornwall were diverse and were distinct from ESBL-producing *E. coli* from community-onset UTIs. However, two ESBL-negative, gentamicin-resistant isolates were closely related to a local CTX-M-producing and gentamicin-resistant outbreak strain, suggesting gain or loss of
Figure 1. Dendrogram (UPGMA, Dice) showing the relatedness of PFGE banding patterns for 30 E. coli isolates from Cornwall. Bold lines indicate clusters with >85% similarity. Symbols: +, CTX-M group 1 producer; +a, CTX-M group 9 producer; –b, ESBL (non-CTX-M) producer; –, non-ESBL producer. Isolates of the local outbreak strain and UK epidemic strain A are shaded light grey and dark grey, respectively.

ctx-m status GEN MIC (mg/L)

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the bla\textsubscript{CTX-M} gene or a plasmid carrying it. Most of the patients infected by this outbreak strain had hospital admissions in the three months prior to onset of their UTIs. This has been identified previously as a risk factor for infections caused by ESBL producers in non-hospitalized patients.\textsuperscript{6,7} Old age (>60 years), as seen in patients described here, is also a known risk factor.\textsuperscript{6}

Although manifesting in the community, many of the UTIs of these Cornish patients appeared to have their origins in local hospitals and did not represent genuine community acquisition. This study highlights weaknesses in the concept of a clear distinction between hospital- and community-acquired infections caused by CTX-M-producing and other multiresistant \textit{E. coli}.

Clearly, acquisition in hospital may be followed by a period of colonization, which may be prolonged in some instances, prior to infection. Screening for faecal colonization at discharge, especially during on-going hospital clusters with ESBL-producing strains, would identify those patients most at risk of developing subsequent community-onset infections with multiresistant strains.

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Transparency declarations

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References


Table 1. Susceptibilities of the ‘Cornish outbreak’ CTX-M-producing \textit{E. coli} strain compared with UK epidemic strain A\textsuperscript{1}

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>‘Cornish outbreak’ strain ((n = 12))\textsuperscript{a}</th>
<th>strain A\textsuperscript{1}</th>
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<tbody>
<tr>
<td>AMP</td>
<td>64</td>
<td>64</td>
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<tr>
<td>AMC</td>
<td>17</td>
<td>16</td>
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<tr>
<td>CTX</td>
<td>71.8</td>
<td>64</td>
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<tr>
<td>CTX + CLA</td>
<td>0.1</td>
<td>(&lt;0.06)</td>
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<tr>
<td>CAZ</td>
<td>26.9</td>
<td>8</td>
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<tr>
<td>CAZ + CLA</td>
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<tr>
<td>FEP</td>
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<td>2</td>
</tr>
<tr>
<td>FEP + CLA</td>
<td>0.1</td>
<td>(&lt;0.06)</td>
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<tr>
<td>FOX</td>
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<tr>
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<tr>
<td>MEM</td>
<td>0.1</td>
<td>(&lt;0.06)</td>
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<td>ETP</td>
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<tr>
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<td>8.2</td>
</tr>
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</table>

\textsuperscript{a}Two non-CTX-M-producing isolates of the outbreak strain were excluded from this analysis.