Change in the prevalence of extended-spectrum-
β-lactamase-producing Escherichia coli in Japan by
clonal spread

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Received 23 July 2008; returned 5 September 2008; revised 14 October 2008; accepted 14 October 2008

Introduction: In the early 2000s, there was a rapid increase in extended-spectrum β-lactamase (ESBL)-
producing Escherichia coli in hospital settings throughout Japan. The reasons for this rapid increase
are unclear.

Methods: Between 2002 and 2003, 142 clinical isolates of E. coli suspected of producing ESBL were
obtained from 37 hospitals and commercial clinical laboratories in geographically distinct regions
throughout Japan. They were tested for ESBL types and further subtyped for serogroups, fimH single
nucleotide polymorphism, pulsed-field gel electrophoresis patterns and multilocus sequence type
(MLST). Representative isolates were also subjected to plasmid analysis.

Results: Of 142 E. coli isolates suspected of producing ESBL, 130 were confirmed as harbouring
blaCTX-M by PCR analysis and sequencing. Of these, 84 (65%) harboured CTX-M-9-group
blaCTX-M. Two serogroups O25 and O86 accounted for 41% of the 130 blaCTX-M-positive E. coli. All O86 serogroup
strains belonged to ST38 by MLST and they formed 18% of all the blaCTX-M-positive E. coli. Serogroup
O25 strains belonged to ST131 and ST73, and formed 21% and 1% of blaCTX-M-positive E. coli, respect-
ively. Seven characterized plasmids carrying blaCTX-M genes belonged to three distinct incompatibility
groups: IncF, IncN and IncI1.

Conclusions: In this study, clonally related strains of E. coli accounted for a large proportion of
blaCTX-M-positive E. coli. This high proportion of clonal groups identified in different regions of Japan
suggests their recent spread by mechanisms other than healthcare-associated transmission. These
observations imply that restricting antimicrobial use in human clinical settings may have limited
impact on the spread of ESBL-producing E. coli.

Keywords: E. coli, ESBLs, CTX-M

Introduction

The incidence of hospital and community-acquired extraintestin-
al infections caused by multidrug-resistant Escherichia coli is
increasing worldwide. The oxynimocephalosporins, such as
cefotaxime, ceftriaxone and cefotaxime, have potent activity
against E. coli clinical isolates resistant to other β-lactam
agents. However, even these antimicrobial agents have come to
be challenged by the emergence of strains that produce
extended-spectrum β-lactamases (ESBLs).1

ESBLs confer resistance by hydrolysing oxynimoc-
cephalosporins but not cephamycins (e.g. cefoxitin, cefotetan) or
carbapenems (e.g. imipenem, meropenem). Most ESBLs can be
classified into three main groups: TEM, SHV and CTX-M.2,3
Worldwide, ESBL-producing organisms are most frequently
found among the members of the family Enterobacteriaceae,
especially E. coli and Klebsiella pneumoniae. There are now
more than 60 different variants of CTX-M-type ESBLs, which
 can be further classified into five different subgroups based on
 their amino acid sequences: CTX-M-1, CTX-M-2, CTX-M-8,
CTX-M-9 and CTX-M-25 groups. Of these, CTX-M-1, CTX-M-2 and CTX-M-9 groups are the most common. Community-acquired extraintestinal infections caused by *E. coli* and hospital-acquired *K. pneumoniae* strains harbouring plasmid-encoded *bla*CTX-M have been increasingly reported worldwide, from both developed and developing countries in the last decade. They have become the most prevalent type of ESBL-producing isolates belonging to the family Enterobacteriaceae during the past 5 years. In Japan, ESBL-producing *E. coli* were rarely isolated until the late 1990s, although strains producing FEC-1 and Toho-1, which were subsequently classified under the CTX-M-type ESBLs, were identified earlier. A report of nosocomial spread of Toho-1-like β-lactamase-producing *E. coli* and a preliminary survey in 1997–98 showed that *E. coli* strains producing CTX-M-2 were the predominant ESBL-producing *E. coli* strains in Japan. However, in the early 2000s, the dominant CTX-M group shifted from CTX-M-2 to CTX-M-9 among the *E. coli* isolates from clinical facilities. This change in the prevalence of the CTX-M group occurred nationwide in a relatively short period and cannot be solely explained by local and multiple person-to-person nosocomial transmissions. Thus, we characterized these *E. coli* isolates further to determine a possible explanation for this rapid emergence of CTX-M-9-group *bla*CTX-M-positive *E. coli* in hospital settings all over Japan.

**Methods**

**Surveillance data and bacterial strains**

To estimate the prevalence of oxymino-cephalosporin resistance among the clinical isolates of *E. coli* in Japan, we reviewed data maintained by the Japan Nosocomial Infections Surveillance (JANIS), which includes participation of more than 200 hospitals across Japan. For microbiological characterization, a total of 142 clinical isolates of *E. coli* phenotypically positive for ESBL production were obtained between 2002 and 2003 from 37 hospitals throughout Japan. ESBL-producing *E. coli* strains were screened for oxymino-cephalosporin resistance and double-disc diffusion synergy tests as recommended by the CLSI (formerly the NCCLS).

**PCR and sequencing analysis**

To determine the genotype of ESBLs, we performed PCR using the TEM-, SHV-, CTX-M-1, CTX-M-2 and CTX-M-9 group-specific primers as reported previously. Sequencing was performed with PCR primers as follows: CTX-M-2sequenceF (5'-TTA ATG ATG ACT CAG AGC ATT C-3') and CTX-M-2sequenceR (5'-GAT ACC TCG CTC CAT TTA TGG T-3'); CTX-M-9sequenceF (5'-GAT TGA CCG TAT TGG GAG TTT G-3') and CTX-M-9sequenceR (5'-ATT TAC TTC CAT TAC GCC G-3'); CTX-M-14sequenceF (5'-GAT TGA CCG TAT TGG GAG TTT G-3') and CTX-M-14sequenceR (5'-TTG AAC TTT TGC TTT GGC ACG G-3'). PCR amplicons were purified with Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI, USA) and were subsequently sequenced with the appropriate primers.

**Serotyping**

Serotyping was performed with the *E. coli* antisera ‘SEIKEN’ Set 1 (Denka Seiken, Tokyo, Japan) for O antigen and Set 2 (Denka Seiken) for H antigen according to the manufacturer’s instructions.

**Antimicrobial susceptibility testing**

MICs were determined by the Etest (AB Biodisk, Solna, Sweden) method according to the manufacturer’s instructions. Resistance was interpreted based on the recommended breakpoints of the CLSI (formerly the NCCLS).

**Genotyping**

Genotypic analysis based on *fimH* single nucleotide polymorphism (SNP) was performed as reported previously, and multilocus sequence typing was performed according to the protocol described on the Max-Planck Institut für Infektionsbiologie web site. Pulsed-field gel electrophoresis (PFGE) analysis was performed as reported previously. A dendrogram was generated from the distance matrix by the unweighted pair-group method using arithmetic averages.

**Plasmid analysis**

Broth mating method was used for conjugation experiments with *E. coli* DH10B as described previously. Transconjugants were selected on Luria–Bertani agar plates containing 16 mg/L ceftaxime and 800 mg/L streptomycin. Plasmid DNA was purified from transconjugant cells with the PureYield Plasmid Miniprep System (Promega), according to the manufacturer’s instructions. Purified plasmid DNA was digested with *PstI* and *EcoRI* restriction enzymes (New England Biolabs, Beverly, MA, USA) for 2 h at 37°C. The digested plasmid DNA was electrophoresed in a 0.8% agarose gel for the restriction fragment length polymorphism (RFLP) analysis and hybridized with a digoxigenin-labelled DNA probe specific for CTX-M-2- or CTX-M-9-group *bla*CTX-M with the PCR DIG detection system (Roche Diagnostics, Indianapolis, IN, USA). The plasmids were also classified according to their incompatibility group by a PCR-based replicon-typing method.

**Statistical analysis**

Comparisons of proportions were made by χ² test with SPSS, version 14.0J for Windows (SPSS, Chicago, IL, USA).

**Results**

**A temporal change in prevalence of *E. coli* resistance to ceftazidime and cefotaxime**

The JANIS, a sentinel hospital-based surveillance system established in Japan in 2000, shows that the prevalence of *E. coli* non-susceptible to cefotaxime increased from 2001 through 2006. In 2001, 3.8% and 0.7% of the *E. coli* isolates were non-susceptible to ceftazidime and cefotaxime, respectively. However, in 2004, the prevalence reversed, when ceftazidime-non-susceptible *E. coli* decreased to 1.1% and cefotaxime-non-susceptible isolates increased to 1.7%. Since then, the prevalence of *E. coli* non-susceptible to cefotaxime has continued to increase, reaching 4.6% in 2006. The prevalence of *E. coli* non-susceptible to ceftazidime remained virtually unchanged at 1.4% in the last 2 years of the surveillance.

**PCR classification of CTX-M-type β-lactamases and serotyping**

PCR analysis showed that 130 of the 142 *E. coli* isolates phenotypically positive for ESBL production harboured *bla*CTX-M. Among the 12 *bla*CTX-M-negative isolates, nine harboured...
three were positive for \(\text{bla}_{\text{TEM}}\); in one of these, the sequence of the PCR amplicon was identical to the gene that encodes TEM-28. The sequence of the \(\text{bla}_{\text{TEM}}\) PCR amplicon from the other two isolates was identical to that of the gene that encoded TEM-1, but the molecular mechanism for its resistance to oxymino-cephalosporin has not yet been determined.

Among the 130 \(\text{bla}_{\text{CTX-M}}\)-positive \(E.\ coli\) isolates 84 (65%) strains harboured \(\text{bla}\) genes from the CTX-M-9 group, 25 (19%) from the CTX-M-1 group and 21 (16%) from the CTX-M-2 group. The most predominant serogroups O25 and O86 comprised 29 (22%) and 24 (18%) of the 130 \(\text{bla}_{\text{CTX-M}}\)-positive isolates, respectively (Table 1). All of the O86 strains were H18, while three of the O25 isolates were non-motile and H untypeable. Others were H4. These two most prevalent \(E.\ coli\) serogroup isolates were, therefore, subjected to further genotypic analysis as outlined below.

Among the 130 \(\text{bla}_{\text{CTX-M}}\)-positive \(E.\ coli\) isolates, all 24 \(E.\ coli\) O86 isolates harboured CTX-M-9-group \(\text{bla}_{\text{CTX-M}}\), while only 60 (57%) of the non-O86 isolates harboured the same gene \((P < 0.001)\). CTX-M-9-group \(\text{bla}_{\text{CTX-M}}\) was found in 22 (76%) of the 29 O25 \(E.\ coli\) isolates and in 62 (61%) of non-O25 isolates \((P > 0.05)\).

### Antimicrobial susceptibility profiles

Tests for susceptibility to 11 antimicrobial agents were conducted for 25 unduplicated \(E.\ coli\) O86 and O25 isolates harbouring \(\text{bla}_{\text{CTX-M}}\) (Table 2). Duplication was defined as isolates belonging to the same O serogroup and CTX-M group from the same hospital. The drug susceptibility patterns between O25 and O86 isolates were distinct. All O86 isolates were resistant to sulfamethoxazole/trimethoprim, while O25 isolates were more frequently resistant to cefazidime, cefoxitin, ciprofloxacin and chloramphenicol. There was no imipenem and fosfomycin resistance in any O86 or O25 strains.

<table>
<thead>
<tr>
<th>O serogroup</th>
<th>CTX-M-9 group</th>
<th>CTX-M-1 group</th>
<th>CTX-M-2 group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>O25</td>
<td>22</td>
<td>7</td>
<td>1</td>
<td>29 (22.3%)</td>
</tr>
<tr>
<td>O86</td>
<td>24</td>
<td></td>
<td></td>
<td>24 (18.5%)</td>
</tr>
<tr>
<td>O1</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>13 (10.0%)</td>
</tr>
<tr>
<td>O166</td>
<td>3</td>
<td></td>
<td>1</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td>O146</td>
<td>2</td>
<td>1</td>
<td></td>
<td>3 (2.3%)</td>
</tr>
<tr>
<td>O153</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2 (1.5%)</td>
</tr>
<tr>
<td>O8</td>
<td></td>
<td>2</td>
<td></td>
<td>2 (1.5%)</td>
</tr>
<tr>
<td>O125</td>
<td></td>
<td>1</td>
<td></td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>O15</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>O18</td>
<td></td>
<td></td>
<td>1</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>O44</td>
<td></td>
<td>1</td>
<td></td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>OUT</td>
<td>22</td>
<td>15</td>
<td>12</td>
<td>49 (37.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>84 (64.6%)</td>
<td>25 (19.2%)</td>
<td>21 (16.2%)</td>
<td>130</td>
</tr>
</tbody>
</table>

OUT, O-antigen untypeable.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>(E.\ coli) O86</th>
<th>(E.\ coli) O25</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>11 (68.8%)</td>
<td>8 (88.9%)</td>
<td>0.258</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>0 (0%)</td>
<td>3 (33.3%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>0 (0%)</td>
<td>2 (22.2%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>—</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0 (0%)</td>
<td>3 (33.3%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1 (6.3%)</td>
<td>4 (44.4%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sulfamethoxazole/trimethoprim</td>
<td>16 (100.0%)</td>
<td>4 (44.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>14 (87.5%)</td>
<td>6 (66.75%)</td>
<td>0.211</td>
</tr>
<tr>
<td>Amikacin</td>
<td>4 (25.0%)</td>
<td>0 (0%)</td>
<td>0.102</td>
</tr>
<tr>
<td>Minocycline</td>
<td>2 (12.5%)</td>
<td>1 (11.1%)</td>
<td>0.918</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>
Genotyping of E. coli O86 and O25 isolates

The fimH-sequence-based SNP analysis of all 53 E. coli O86 and O25 isolates revealed five distinct genotypes (designated A, B, C, D and E; Figure 1). All CTX-M-9-group blaCTX-M-positive E. coli O86 isolates (n = 24) belonged to a single fimH sequence type (cluster A). By multilocus sequence type (MLST), they were all found to belong to ST38. In contrast, 29 E. coli O25 isolates fell into four distinct fimH SNP types (cluster B, C, D and E). By MLST analysis, the fimH cluster C, D and E strains fell into ST131, while cluster B isolates belonged to ST73. By PFGE analysis of selected O86 and O25 isolates, the O86 isolates had similar electrophoretic banding patterns, but the O25 isolates showed more diverse patterns (Figure 2).

Sequence analysis of blaCTX-M

Half of 46 CTX-M-9-group blaCTX-M-positive E. coli O86 and O25 isolates harboured blaCTX-M-9 that was 100% identical in

![Figure 1](https://academic.oup.com/jac/article-abstract/63/1/72/704669)

**Figure 1.** Neighbour-joining trees constructed from the fimH sequences and multilocus sequence type (MLST) of blaCTX-M-positive E. coli O86 and O25. Isolates with single asterisk were subjected to PFGE analysis. Isolates with double asterisk were subjected to both PFGE and MLST analyses. Five fimH SNP clusters designated as A–E were identified.
sequence; the rest had a variant \(\text{bla}_{\text{CTX-M-9}}\) sequence designated as \(\text{bla}_{\text{CTX-M-14}}\). All but one of the \(E.\ coli\) O86 isolates harboured the prototype \(\text{bla}_{\text{CTX-M-9}}\), and all O25 \(E.\ coli\) isolates positive for CTX-M-9-group ESBL harboured the variant \(\text{bla}_{\text{CTX-M-14}}\). The enzyme CTX-M-14 differs from CTX-M-9 by only one amino acid at position 234 (Ala → Val) and two silent mutations at nucleotide positions 372 (A → G) and 570 (G → A). However, the nucleotide sequence of a 20–30 bp downstream region of \(\text{bla}_{\text{CTX-M}}\) differed completely between \(\text{bla}_{\text{CTX-M-9}}\) and \(\text{bla}_{\text{CTX-M-14}}\) genes (data not shown).

In isolates positive for the CTX-M-2-group ESBL gene, which were all \(E.\ coli\) O25, four and three isolates harboured \(\text{bla}_{\text{CTX-M-35}}\) and \(\text{bla}_{\text{CTX-M-2}}\), respectively (GenBank accession number AB176534). The enzyme encoded by \(\text{bla}_{\text{CTX-M-35}}\) differs from that encoded by \(\text{bla}_{\text{CTX-M-2}}\) also by only one amino acid substitution at position 170 (Pro → Ser).

**Plasmid analysis**

Eight isolates from five \(\text{fimH}\) sequence type clusters were subjected to conjugation experiments and seven transconjugants were obtained. Transconjugants were not obtained from isolates belonging to \(\text{fimH}\) sequence type B. Among seven plasmids purified from the transconjugants, four carrying CTX-M-9-group \(\text{bla}_{\text{CTX-M}}\) belonged to the IncF group, two carrying CTX-M-2-group \(\text{bla}_{\text{CTX-M}}\) belonged to the IncN group and one carrying \(\text{bla}_{\text{CTX-M-14}}\) belonged to the IncI1-group plasmids according to the PCR-based replicon typing. Restriction fragment length polymorphism analysis of the plasmid DNA showed different patterns and digoxigenin-labelled \(\text{bla}_{\text{CTX-M}}\) probe also hybridized to bands of different molecular weight in all seven plasmids (data not shown).

**Epidemiological information on the \(E.\ coli\) O86 and O25 isolates**

Epidemiological and clinical information on the 53 \(E.\ coli\) O86 and O25 isolates harbouring \(\text{bla}_{\text{CTX-M}}\) are summarized in Table 3. Twenty-four \(E.\ coli\) O86:H18 isolates, which belonged to a single \(\text{fimH}\) cluster A, ST38 by MLST analysis and having a similar PFGE band pattern with higher than 90% similarity, were isolated from 14 different hospitals and a commercial laboratory in seven different Prefectures. These Prefectures are located in geographically distant regions of Japan. However, 11 (46%) of these were isolated from six hospitals located in one Prefecture, Ishikawa Prefecture, over a 1 year period, which suggests a regional outbreak. Two \(E.\ coli\) O25:H- isolates belonging to \(\text{fimH}\) cluster B were isolated from two different patients in one hospital, and two \(E.\ coli\) O25:H4 isolates belonging to \(\text{fimH}\) cluster D were isolated from different patients.
in another hospital, which suggests nosocomial transmissions. Four *E. coli* O25:H4 isolates and one *E. coli* O25:HNM isolate, which belonged to a single *fimH* cluster C and ST131, were isolated from four different hospitals in three Prefectures. Twenty *E. coli* O25:H4 isolates in *fimH* cluster E were obtained from three hospitals located in three different Prefectures. Of these, 15 (75%) were isolated during an outbreak in a haematology unit. Four (20%) isolates with *blaCTX-M-35* were isolated from different patients admitted to a single hospital.

### Discussion

CTX-M-9-group ESBL-producing *E. coli* were not common in Japan prior to 2000. According to the JANIS, *E. coli* isolates non-susceptible to cefotaxime increased by more than 6-fold between 2001 and 2006. In this study, we found a cluster of *E. coli* O86 isolates, obtained from recognized outbreaks as well as from sporadic cases from hospitals all across Japan. These isolates were highly clonal, as evidenced by the multiple subtyping tests we used. This single clonal group accounted for 18% of all the *blaCTX-M* positive *E. coli* isolates obtained during 2002–03. Its high clonality suggests that this epidemic strain spread throughout Japan relatively recently in a short period. As far as we know, this is the first documented report of a clonal spread of *E. coli* O86:H18-ST38 harbouring CTX-M-9-group *blaCTX-M* anywhere.

On the other hand, *E. coli* O25:H-4-ST131 has been already recognized as an emerging intercontinental clonal group expressing CTX-M-type ESBL. In our study, we also found a cluster of O25 isolates but they were composed of multiple lineages—two different MLST groups (ST131 and ST73), four distinct *fimH* SNP genotypes, diverse PFGE patterns and two different flagella types (H4 and HNM). This suggests that *E. coli* O25 serogroup strains may have been circulating in Japan for a longer period. *E. coli* O25 is increasingly recognized in recent decades and is now the second to third most frequent serogroup among clinical *E. coli* isolates in Japan.

In addition, all the *E. coli* O25:H4-ST131 isolates in our study harboured CTX-M-2-group *blaCTX-M* or *blaCTX-M-14*, which are the dominant CTX-M types in Asia including Japan. Most of the previously reported *E. coli* O25:H4-ST131 strains are CTX-M-15 producers. CTX-M-15 is a member of the CTX-M-1 group, which is dominant in European countries. In addition, plasmid analyses in this study revealed that even among the clonal *E. coli* strains, *blaCTX-M* genes were carried by different plasmids. Thus, *E. coli* O25:H4-ST131 strains could have multiple reservoirs from which they spread to different regions of the world acquiring different plasmids carrying different *blaCTX-M* types.

Clonal spread of other antimicrobial-resistant *E. coli* strains causing community- and hospital-acquired extraintestinal infections has been reported previously. Clusters of multidrug-resistant community-acquired *E. coli* O15:K52:H1 and O78:H10 extraintestinal infections have been reported in the 1990s in the UK and continental Europe. A clonally related group of multidrug-resistant uropathogenic *E. coli* called CgA has been reported throughout the USA and some parts of Europe, as well as from animals and the environment in the USA. The nationwide and intercontinental spread, as well as sporadic occurrences of unrelated clusters of community-acquired

### Table 3. Epidemiological, bacterial and genotypic characteristics of the 53 CTX-M type ESBL-positive *E. coli* O86 and O25 isolates

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Serotype; MLST</th>
<th>Year</th>
<th>Location</th>
<th>Specimen type</th>
<th>CTX-M-type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>fimH</strong></td>
<td><strong>Serotype; MLST</strong></td>
<td><strong>Year</strong></td>
<td><strong>Location</strong></td>
<td><strong>Specimen type</strong></td>
<td><strong>CTX-M-type</strong></td>
</tr>
<tr>
<td>A 24</td>
<td>O86:H18; ST38</td>
<td>2002, 2003</td>
<td>14 hospitals and a commercial laboratory/7 prefectures</td>
<td>urine (7), blood (5), endotracheal aspirate (2), vaginal secretion (1), faeces (1), ascites (1), unknown (1)</td>
<td>CTX-M-9 (23), CTX-M-14 (1)</td>
</tr>
<tr>
<td>B 2</td>
<td>O25:H-4; ST773</td>
<td>2002</td>
<td>1 hospital</td>
<td>unknown (2)</td>
<td>CTX-M-14 (2)</td>
</tr>
<tr>
<td>D 2</td>
<td>O25:H4; ST131</td>
<td>2002</td>
<td>1 hospital</td>
<td>faeces (1), sputum (1), urine (3), blood (1)</td>
<td>CTX-M-14 (2)</td>
</tr>
</tbody>
</table>
infections caused by ESBL-producing E. coli strains belonging to identical clonal lineages suggest that such strains are spread not just by person-to-person transmission or in hospital settings, but by some widely distributed contaminated ingestible products. Indeed, CTX-M-type ESBL-producing E. coli isolates have been isolated from food animals, usually from chickens but also from calves.\textsuperscript{28–31} This further observation supports the idea that the use of antimicrobial agents as growth promoters in animal food or as veterinary medicines contributes to the initial selection of these resistant organisms.

Our findings that a single clonal strain can abruptly change the prevalence of infections with ESBL-producing E. coli in different regions of a country suggest that restricting antimicrobial use in human clinical settings may have minimal impact on the spread of ESBL-producing E. coli that cause healthcare-associated infections in Japan and elsewhere. The control of antimicrobial resistance may require monitoring of antimicrobial use and surveillance of drug-resistant pathogens in the veterinary environment.

Acknowledgements

We thank Dr Lee W. Riley, School of Public Health, University of California, Berkeley, for critical review of the manuscript; all medical institutions for submitting the bacterial strains and clinical information; Kumiko Kai, Yumiko Yoshimura and Yoshie Taki for technical assistance.

Funding

This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (H18-Shinkou-011).

Transparency declarations

None to declare.

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Clonal spread of drug-resistant *E. coli*


