Cephalosporin resistance mechanisms in *Escherichia coli* isolated from raw chicken imported into the UK

Hiran Dhanji 1*, Niamh M. Murphy 2, Michel Doumith 1, Sema Durmus 2, Susanne Surman Lee 2, Russell Hope 1, Neil Woodford 1 and David M. Livermore 1

1Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK; 2Food, Water and Environmental Microbiology Network—London Laboratory, 61 Colindale Avenue, London NW9 5EQ, UK

*Corresponding author. Tel: +44-(0)20-8327-7236; Fax: +44-(0)20-8327-6264; E-mail: hiran.dhanji@hpa.org.uk

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Objectives: We characterized mechanisms of resistance to oxyimino-cephalosporins in *Escherichia coli* isolated from raw chicken meat imported into the UK from South America, to ascertain whether this foodstuff contributes to the dissemination in the UK of extended-spectrum β-lactamase (ESBL)-producing *E. coli* belonging to the international uropathogenic ST131 clone.

Methods: Sampling and collection of imported raw chicken meat was performed in accordance with regulatory guidelines by the London Port Health Authority at Tilbury. *E. coli* strains producing ESBLs were isolated based on growth within the zones of cefpodoxime (10 μg) discs. MICs were determined by agar dilution and interpreted using BSAC/EUCAST breakpoints. PCR was used to determine the phylogenetic groups of *E. coli*, to detect ESBL genes and to determine the incompatibility groups of plasmids encoding CTX-M enzymes. The molecular environments surrounding *bla<sub>CTX-M</sub>* were determined by DNA sequencing and PCR mapping.

Results: A total of 141 oxyimino-cephalosporin-resistant *E. coli* were isolated from 62 of 210 batches of imported raw chicken sampled. Thirty percent of these isolates produced group 2 CTX-M ESBLs, 27% produced group 8 CTX-M ESBLs, 42% produced CMY-type AmpC enzymes and 1% produced a group 2 CTX-M along with a CMY enzyme; none produced CTX-M-15 ESBL and none belonged to the ST131 clone. In contrast to human clinical ESBL *E. coli*, 90% of isolates were susceptible to ciprofloxacin and 74% to all aminoglycosides.

Conclusions: Raw chicken imported into the UK from South America commonly carries ESBL-producing *E. coli*, but is not a significant source for the ST131 clone or for the CTX-M-15 ESBL.

Keywords: CTX-M β-lactamases, AmpC β-lactamases, ST131, IS10, ISCR1

Introduction

*Escherichia coli* with CTX-M extended-spectrum β-lactamases (ESBLs) have become frequent as clinical isolates since 2003 in the UK, most often being recovered from urinary tract infections, but sometimes from bacteraemias. 1 Most have the CTX-M-15 enzyme encoded by multidrug resistance IncFII plasmids and many belong to the international O25b:H4-ST131 lineage, which belongs to phylogenetic group B2. 2 The source for the dissemination of this clone in the UK is unknown, but potentially might involve a food source within the global market. To investigate this possibility, we examined the antibiotic susceptibility profiles, phylogenetic groups, ESBL genes, molecular environment and plasmid types of 141 oxyimino-cephalosporin-resistant *E. coli* isolated from raw chicken meat imported into the UK from South America (Argentina, Brazil and Chile), which is one of the largest non-EU sources of raw poultry meat for the UK.

Materials and methods

Source of *E. coli isolates*

The London Port Health Authority at Tilbury sampled 210 batches of non-EU raw chicken meat imported into the UK during August to October 2008. The sampling was in accordance with Local Authorities Coordinators of Regulatory Services (LACORS) guidelines 2006 (http://www.lacors.gov.uk/lacors/ContentDetails.aspx?id=6966), which specify the policies and methodology for the sampling and collection of imported foods for microbiological testing. The samples were then tested by the Food, Water and Environmental Microbiology Network—
London Laboratory at the HPA in Colindale. E. coli were isolated from all 210 batches using selective tryptone bile agar containing 75 mg/L 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (BCIG agar, Oxoid, Hampshire, UK).

Antibiotic susceptibility testing and PCR testing

Five E. coli colonies from each batch sample were tested for resistance to cefpodoxime using 10 μg discs according to the BSAC standardized disc susceptibility testing method.3 MICs were determined for resistant isolates by agar dilution.5 PCR was used to seek antibiotic resistance genes, including blaCTX-M and those encoding acquired AmpC enzymes.6 PCR was also used to assign isolates to phylogenetic groups.7 Isolates belonging to phylogenetic group B2 were screened by real-time PCR to identify members of the ST131 clone, based on detecting a combination of two single nucleotide polymorphisms that are unique to ST131 within the pabB gene.8

Genetic environment of blaCTX-M and plasmid conjugation studies

PCR mapping and DNA sequencing were performed on an ABI Genetic Analyser capillary platform 3130XL (Applied Biosystems, CA, USA) and were used to identify DNA sequences upstream of group 2 or 8 blaCTX-M genes using forward primers IS10for (5′-CTA TGG CGT AGG AGG CCT AGG CCG TAG G-3′) or ISRCRF (5′-ATG TCG CTG GCA AGG AA-3′) with reverse primers CTX-M-2rev (5′-TCA GAA ACC GTG GGT TAC GTT TC-3′) or CTX-M-8rev (5′-TTA ACC GTC GGT GAT GAC TTT C-3′). The cycling conditions were: initial denaturation for 5 min at 95°C; 30 cycles of 20 s at 95°C, 40 s at 60°C and 3 min at 72°C; and then 5 min at 72°C. When ISRCR was found upstream of blaCTX-M, two further PCRs were performed using the Expand High Fidelity PCR system (Roche, Burgess Hill, UK) with primers Sul1F (5′-GCC CTC TCC GAT GAG ATG CA-3′) or Int1F (5′-TCC AGA ATT ACC TGT ACC AGA CG-3′) together with primer CTX-M-2rev or CTX-M-8rev to search for sul1-type integron structures.

Plasmids encoding CTX-M ESBLs were transferred by conjugal plate mating to E. coli K12 J53-2 (rifR). Transconjugants were selected on agar plates containing 250 mg/L rifampicin+2 mg/L cefotaxime (Sigma, Poole, UK). Plasmids from transconjugants were extracted by alkaline lysis and rep typed by PCR.9

Results

Oxyimino-cephalosporin resistance mechanisms

Cefpodoxime susceptibility was tested for 1050 E. coli isolates selected from the 210 batches of chicken meat. Fifteen of these batches were from Argentina, 50 from Chile and 145 from Brazil. One hundred and forty-one isolates (13.4%) from 62 (29.5%) batches were resistant. Among these, 42 from 32 batches harboured genes encoding group 2 CTX-M ESBLs, 38 from 29 batches harbouring group 8 CTX-M enzymes and 59 from 36 batches had CMY-type AmpC enzymes; two separate batches yielded single isolates with both a group 2 CTX-M ESBL and a CMY-type AmpC enzyme (Table 1). None of the E. coli isolates had a group 1 CTX-M ESBL, such as CTX-M-15. Overall, 37/210 batches yielded at least one E. coli with each of CTX-M-2, CTX-M-8 or CMY-type AmpC enzymes. Forty-two of 44 isolates producing group 2 CTX-M and 34/38 isolates producing group 8 CTX-M ESBLs were from Brazil. Of the two remaining isolates producing group 2 CTX-M ESBLs, one each was from Argentina and Chile, and two each of the remaining four producing group 8 CTX-M ESBLs were also from these two countries.

Antibiotic susceptibilities

All the isolates producing group 2 (n=42) or group 8 (n=38) CTX-M enzymes were resistant to cefotaxime, whereas MICs were lower for ceftazidime (Table 2). Synergy arose between these cephalosporins and clavulanic acid. All these 80 isolates were susceptible to amikacin (MIC 1–4 mg/L) and tobramycin (MIC 0.5–4 mg/L).

The 59 isolates with CMY enzymes were resistant to cefotaxime and 45 were also resistant to ceftazidime, with no synergy with clavulanic acid. All 59 were susceptible to amikacin and tobramycin (MICs 0.5–4 mg/L), and 49 to gentamicin (Table 2). The two isolates producing both CTX-M group 2 and CMY enzymes were resistant to cefotaxime, but only one was clearly resistant to ceftazidime; neither showed synergy between these agents and clavulanic acid.

All 141 cefpodoxime-resistant isolates were susceptible to ertapenem, imipenem and meropenem (MICs 0.06–0.125 mg/L), tigecycline (MICs ≤0.25 mg/L) and colistin (MICs ≤0.5 mg/L), and most (>90%) also to ciprofloxacin.

Phylogenetic groups

Phylogenetic group D was dominant among the isolates producing CTX-M ESBLs, with group B1 dominant among those with CMY-type enzymes (Table 1). Only five isolates (three with group 2 CTX-M enzymes and two with CMY-type enzymes) belonged to the extraintestinal virulent phylogenetic group B2. None belonged to the O25b:H4-ST131 clone.

<table>
<thead>
<tr>
<th>Resistance gene(s) detected</th>
<th>CTX-M-2</th>
<th>CTX-M-8</th>
<th>CMY-type AmpC</th>
<th>CTX-M-2 + CMY-type AmpC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phylogenetic group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (n=21)</td>
<td>5</td>
<td>15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>B1 (n=65)</td>
<td>14</td>
<td>9</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>B2 (n=5)</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>D (n=50)</td>
<td>20</td>
<td>14</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. Phylogenetic groups and major resistance mechanisms of 141 oxyimino-cephalosporin-resistant E. coli isolated from raw chicken.
Genetic environment of bla_{CTX-M}

bla_{CTX-M} alleles were sequenced for all ESBL-producing isolates. Isolates producing group 2 CTX-M ESBLs (n=44) harboured bla_{CTX-M-2}, whereas isolates producing group 8 CTX-M ESBLs (n=38) harboured bla_{CTX-M-8}. The genetic environment upstream of bla_{CTX-M} was determined for 33 isolates, comprising: (i) five carrying each of bla_{CTX-M-2} and bla_{CTX-M-8} from each of phylogenetic groups A, B1 and D; and (ii) all of the three phylogenetic group B2 isolates producing CTX-M-2 enzymes (Table 1).

The genetic environments upstream of bla_{CTX-M-8} in the 15 selected isolates all comprised an IS\textsubscript{10} element immediately adjacent to the bla gene. This genetic organization was previously reported in Brazil.\textsuperscript{9} Further PCR screening with primers IS\textsubscript{10}for + CTX-M-8rev (product ~2 kb) confirmed the presence of this IS\textsubscript{10}-bla_{CTX-M-8} arrangement in all the 38 isolates harbouring bla_{CTX-M-8}. Similarly, an IS\textsubscript{CR1} element was found immediately upstream of a bla_{CTX-M-2} allele in all 18 selected isolates. This arrangement was confirmed by PCR with primers IS\textsubscript{CR1}F + CTX-M-2rev (product ~2.9 kb) for all 44 isolates harbouring bla_{CTX-M-2}. No amplification was obtained for IS\textsubscript{CR1}-CTX-M-2-positive isolates with primers Sul1F + CTX-M-2rev or Int1F + CTX-M-2rev, suggesting that the group 2 CTX-M gene was not associated with sul1-type integron structures, as previously described.\textsuperscript{5}

Conjugation studies and plasmid rep types

CTX-M plasmids from the 33 isolates from which the bla_{CTX-M} genes were sequenced were transferred by conjugation to E. coli J53, with the presence of bla_{CTX-M} confirmed by PCR. Conjugation frequencies ranged from $10^{-6}$ to $10^{-7}$ transconjugants per donor cell. These low frequencies may relate to the large plasmid sizes, exceeding 95 kb, which were detected in the transconjugants. The transconjugants exhibited typical CTX-M phenotypes, with resistance to penicillins and oxyimino-cephalosporins, in particular ceftaxime, but remained susceptible to aminoglycosides, even when the corresponding donors were gentamicin resistant. The two donor isolates that produced CMY enzymes in addition to group 2 CTX-M ESBLs did not co-transfer AmpC activity, and transfers were not attempted for the isolates that had only CMY-type enzymes.

PCR rep typing using the transconjugants showed that the bla_{CTX-M-2} plasmids from 18 donors were type IncFIA, whereas the bla_{CTX-M-8} plasmids derived from 15 donors were type IncI1. PCR confirmed that the remaining isolates with group 2 (n=26) and group 8 (n=23) CTX-M ESBLs also had IncFIA and IncI1 plasmids, respectively.

Discussion

We have shown that raw chicken imported into the UK from South America is a reservoir for E. coli with CTX-M-type ESBLs of groups 2 and 8, which are extremely rare among UK human clinical isolates.\textsuperscript{1} It is not a significant reservoir either for E. coli belonging to the O25b:H4-ST131 lineage or for plasmids encoding CTX-M-15 ESBL, which is dominant in the UK.\textsuperscript{5} Warren et al.\textsuperscript{10} reported similar findings to those presented here using raw chicken meat purchased from UK retail outlets. However, (i) they initially selected isolates on medium supplemented with ciprofloxacin, which may have underestimated ESBL.

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Table 2. Distribution of MICs for oxyimino-cephalosporin-resistant E. coli isolated from imported raw chicken

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>bla gene</th>
<th>Number of isolates with the following MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.125</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>CTX-M-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTX-M-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY+CTX-M-2</td>
<td></td>
</tr>
<tr>
<td>Ceftiazide</td>
<td>CTX-M-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTX-M-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY+CTX-M-2</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CTX-M-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTX-M-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY+CTX-M-2</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>CTX-M-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTX-M-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMY+CTX-M-2</td>
<td></td>
</tr>
</tbody>
</table>

Underlined numbers indicate susceptible by current BSAC recommendations.
\textsuperscript{a}Numbers indicate intermediate breakpoints by current BSAC recommendations.
E. coli with CTX-M and AmpC enzymes in raw chicken

producers, since >90% of the ESBL-producing isolates in this study were susceptible to ciprofloxacin, and (ii) they studied only 10 purchased meat samples from South America, whereas we studied 210 samples.

Most E. coli isolates producing CTX-M-2 and -8 ESBLs were not multiresistant to other antibiotic classes, although 46% belonged to virulent phylogenetic groups B2 and D. We consistently found that group 2 CTX-M enzymes were carried by IncFIA plasmids and group 8 CTX-M enzymes by IncI plasmids, with IS10 and ISCR1 elements located immediately upstream of bla_{CTX-M-8} and bla_{CTX-M-2} genes, respectively. Both of these insertion sequences have been reported to facilitate the mobilization of bla_{CTX-M} genes. Even though bla_{CTX-M-2} and bla_{CTX-M-8} are harboured on widely disseminated plasmid types and are associated with mobile IS elements, they have not yet become widely disseminated among clinical human isolates in the UK. However, they cannot be dismissed as a clinical concern, because CTX-M-2 is the dominant ESBL among clinical Enterobacteriaceae in South America.

In conclusion, we have shown that imported raw chicken does not seem to be a source for the successful dissemination of the ST131 clone harbouring bla_{CTX-M-15} in the UK; however, it has the potential to act as a source for faecal colonization with oxyimino-cephalosporin-resistant E. coli.

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Transparency declarations
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References