Resistance to third-generation cephalosporins in human non-typhoidal Salmonella enterica isolates from England and Wales, 2010–12

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Objectives: To identify the mechanism(s) underlying cefotaxime resistance in 118 of 21641 (0.55%) non-typhoidal Salmonella enterica isolates collected from humans throughout England and Wales from January 2010 to September 2012.

Methods: Non-duplicate isolates (n = 118) resistant to cefotaxime (MICs >1 mg/L) were screened by PCR for genes encoding CTX-M extended-spectrum β-lactamases (ESBLs) and associated ISEcpl-like elements, and for genes encoding acquired AmpC, SHV, TEM, VEB, PER and GES β-lactamases. Sequencing was used to identify specific alleles in selected isolates. Carbapenem resistance was sought by ertapenem disc screening.

Results: Seventy-nine isolates (0.37% of all referred S. enterica) produced ESBLs, 37 isolates (0.17%) produced CMY-type AmpC enzymes, and 1 isolate had both enzyme types; the mechanism of cefotaxime resistance in 3 isolates could not be identified. Group 1 CTX-M genes were identified in 57 isolates belonging to 22 serotypes, with CTX-M-1 (n = 11), -15 (n = 9) and -55/57 (n = 8) the most prevalent alleles among the 29 (51%) investigated. CTX-M-2 (n = 5), -14 (n = 5), -8 (n = 1) and -65 (n = 1) were also identified. TEM-52 was identified in two isolates and SHV-12 in seven isolates. There was no evidence of carbapenem resistance. ESBL and AmpC genes were detected in both domestically acquired and travel-associated salmonellae. Eighty-nine isolates (75%) were multidrug resistant (resistant to at least three antimicrobial classes) and 42 (36%) had decreased susceptibility to ciprofloxacin (MICs 0.25–1 mg/L), with a further 13 (11%) isolates resistant (MICs >1 mg/L).

Conclusions: The prevalence of CTX-M and acquired AmpC genes in human non-typhoidal S. enterica from England and Wales is still low, but has increased from 0.03% in 2001–03 to 0.49% in 2010–12. Resistance to third-generation cephalosporins requires monitoring as it may reduce therapeutic options.

Keywords: ESBLs, AmpCs, surveillance

Introduction

Non-typhoidal Salmonella enterica (NTS) frequently cause mild gastrointestinal infections that normally resolve without the need for antimicrobials. However, invasive infections can occur in vulnerable patients, in whom treatment with a fluoroquinolone or third-generation cephalosporin can be life-saving. Resistance to third-generation cephalosporins is increasing in Salmonella spp., and is mainly due to the production of acquired AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs).1 The increased occurrence of these enzymes in Salmonella spp., coupled with decreased susceptibility to quinolones, compromises the use of these drugs and is a serious public health issue.1,2 The prevalence of cephalosporin resistance was very low (0.04%) in clinical NTS isolates collected in England and Wales between 1992 and 2003, with only 14 CTX-M and 9 AmpC enzymes detected over the 11 year period.1,4 At present, carbapenem resistance in S. enterica is extremely rare, although isolates expressing different acquired carbapenemases have been reported.5–7 This study aimed to determine the prevalence of cephalosporin resistance in NTS isolates collected throughout England and Wales from January 2010 to September 2012. We
also sought to identify the underlying ESBL and AmpC genes, and screened cephalosporin-resistant isolates for carbapenem resistance.

**Methods**

**Selection and phenotypic characterization of Salmonella isolates**

Non-duplicate isolates (n=118) resistant to >1 mg/L cefotaxime were selected from all NTS isolates causing human salmonellosis in England and Wales between January 2010 and mid-September 2012 (n=216/41). Isolates were recovered from faeces (n=116), blood (n=1) and wound swab (n=1) samples. Resistance to antimicrobials was determined using breakpoint concentrations and methodology based on long-term studies within the Gastrointestinal Bacteria Reference Unit, Public Health England. Isolates were screened for carbapenem resistance as described by Lolans et al., with modifications. Briefly, suspensions (with turbidities equivalent to that of a 0.5 McFarland standard) in Iso-Sensitest broth were used to inoculate Mueller–Hinton agar plates, and a 10 μg meropenem disc (Oxoid, Basingstoke, UK) was added. Inhibition zone diameters <27 mm after 18 h of incubation at 37°C were considered resistant by comparison with positive control strains (not salmonellae) producing NDM-1, KPC, VIM, IMP and OXA-48 carbapenemases.

**Determination of β-lactamase genotypes**

Isolates were screened by PCR for the presence of CTX-M, AmpC, TEM, SHV, VEB, PER and GES β-lactamase genes (primer sequences in Table S1, available as Supplementary data at JAC Online). Where SHV or TEM enzymes were the sole mechanism identified to explain cefotaxime resistance, ESBL production was confirmed by the double-disc synergy test and the alleles were identified by sequencing in most cases. All group 2, group 8 and group 9 CTX-M genes were identified to allele level by sequencing. Group 1 CTX-M alleles and their upstream genetic environments were investigated by PCR and sequencing using primers specific for ISecp1-like and IS26-like elements (Table S1) in 29 (51% of CTX-M producers) isolates representing diverse serotypes. Isolates positive for CIT group genes were subsequently screened for CMY genes by PCR (Table S1). OXA-1-like, OXA-2-like, OXA-10-like and PSE-1 genes were sought among isolates for which no other cephalosporin resistance mechanism was detected (Table S1).

**Results and discussion**

One hundred and eighteen (0.55%) human NTS isolates collected in England and Wales from January 2010 to mid-September 2012 were resistant to cefotaxime (MICs >1 mg/L) (Table 1). This indicates a significant increase (P<0.0001 by χ² test with Yates correction) since the last prevalence study in 2003. However, this represents the 2010 European average among countries using low breakpoints: the Netherlands (0.3%) and Denmark (0.5%) (breakpoint >0.5 mg/L), and Ireland (3.1%) and France (4.3%) (breakpoint >2 mg/L). Resistance to third-generation cephalosporins in 2010–12 in England and Wales was due primarily to the production of CTX-M-type ESBLs and AmpC β-lactamases, which were found in 69 (58%) and 37 (31%) of cefotaxime-resistant isolates, respectively. The prevalence of these genes has increased from 0.03% (15/45 318) among human isolates from 2001 to 2003 to 0.49% (107/21 641) among isolates from 2010 to 2012. Where a travel history was known (74 of 118 isolates), ESBL and AmpC genes were associated with both domestically acquired (n=16, 22%) and travel-associated (n=58, 78%) NTS infections (Table 1).

**CTX-M genes in S. enterica**

The occurrence of β-lactamase genes in NTS serotypes is detailed in Table 1, and in Table S2 and Figure S1 (both available as Supplementary data at JAC Online). The most common resistance mechanisms detected were group 1 CTX-M genes, which were identified in 57 isolates, 29 of which were Salmonella Typhimurium of various phage types and were often associated with travel to Asia. The other 28 isolates represented 21 serotypes. PCR revealed that group 1 CTX-M genes were linked to an upstream ISecp1-like element in 27 of 29 isolates investigated, representing diverse serotypes. IS26 elements were sought, but not found, in the two remaining isolates, one of which contained CTX-M-15/28. The remaining group 1 CTX-M alleles sequenced comprised 11 CTX-M-1, 8 CTX-M-55/57 and 9 CTX-M-15.

CTX-M-1 is the most common food animal-associated CTX-M enzyme in European Union countries and is circulating throughout Europe on IncN plasmids among Escherichia coli and Salmonella spp. from human, animal and environmental sources. The transfer of resistance plasmids encoding group 1 CTX-M enzymes from E. coli to S. enterica has been demonstrated previously, and this may have contributed to the increased prevalence of these genes among NTS.

CTX-M-2 alleles were identified in five isolates, including 3/6 Salmonella Heidelberg isolates with the same multidrug resistance type (ASMxu). CTX-M-2 has previously been described in Salmonella Virchow isolates common to poultry and humans in Europe, and CTX-M-14, which has previously been described in Spanish NTS, was identified in five isolates from diverse serotypes, one of which was associated with travel to Spain. Multiple cefotaxime resistance mechanisms were identified in three isolates, including one Salmonella Concord isolate that expressed a group 1 CTX-M gene in combination with TEM and SHV-12 and resembled a multidrug-resistant (MDR; resistant to at least three antimicrobial classes) strain associated with Ethiopian adoptees that was previously identified in England and Wales (Table S2 and Figure S1). One Salmonella Enteritidis isolate contained CTX-M-8 and one Salmonella Infantis isolate contained a group 9 CTX-M-65 gene. To our knowledge, this is the first description of either gene in S. enterica.

**Other β-lactamases in S. enterica**

TEM-type genes were identified as the sole resistance mechanism potentially explaining cefotaxime resistance in three isolates, which were confirmed as ESBL producers by the double-disc synergy test. The most common European TEM ESBL, blaTEM-S2, was identified in two of these isolates, which were investigated by sequencing. SHV-12 was found in seven isolates in the present study and was the sole ESBL in six isolates, including both Salmonella Virchow isolates. This ESBL was first found in human NTS isolates from Africa and has since occurred in NTS from Europe, the USA and, more recently, India.
Table 1. Genotypic, phenotypic and epidemiological features of third-generation cephalosporin-resistant non-typhoidal S. enterica

<table>
<thead>
<tr>
<th>Genotypic features</th>
<th>Antimicrobial resistance</th>
<th>Foreign travel history</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactamase group(s)</td>
<td>n</td>
<td>alleles (n)</td>
</tr>
<tr>
<td>Group 1 CTX-M</td>
<td>56</td>
<td>CTX-M-1 (11), CTX-M-15 (9), CTX-M-15/28(^a) (1), CTX-M-55/57(^b) (8)</td>
</tr>
<tr>
<td>Group 1 CTX-M and SHV</td>
<td>1</td>
<td>SHV-12 (1)</td>
</tr>
<tr>
<td>Group 2 CTX-M</td>
<td>4</td>
<td>CTX-M-2 (4)</td>
</tr>
<tr>
<td>Groups 2 and 9 CTX-M</td>
<td>1</td>
<td>CTX-M-2 (1)</td>
</tr>
<tr>
<td>Group 8 CTX-M</td>
<td>1</td>
<td>CTX-M-8 (1)</td>
</tr>
<tr>
<td>Group 9 CTX-M</td>
<td>1</td>
<td>CTX-M-14 (1), CTX-M-65 (1)</td>
</tr>
<tr>
<td>Group 9 CTX-M and CMY</td>
<td>1</td>
<td>CTX-M-14 (1)</td>
</tr>
<tr>
<td>SHV</td>
<td>7</td>
<td>SHV-12 (6)</td>
</tr>
<tr>
<td>TEM</td>
<td>3</td>
<td>TEM-52 (2)</td>
</tr>
<tr>
<td>CMY</td>
<td>36</td>
<td>ND</td>
</tr>
<tr>
<td>None found</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>All isolates</td>
<td>118</td>
<td>NA</td>
</tr>
</tbody>
</table>

ND, not determined; NA, not applicable.
\(^a\)Not possible to differentiate between these two alleles with the DNA sequence obtained.
\(^b\)Share identical DNA sequences.
Thirty-seven isolates were positive for CMY-type AmpC enzymes by PCR. Similar to the situation reported in the USA, CMY enzymes were found in a wide range of S. enterica serotypes. The mechanism(s) of cefotaxime resistance in three isolates could not be identified, although two of them were phenotypically positive for an ESBL by the double-disc synergy test. None of the isolates produced PCR products with primers specific for VEB, PER, GES, OXA-1-like, OXA-2-like, OXA-10-like or PSE-1 genes.

None of the isolates was considered resistant to carbenapenem by the ertapenem disc screen used.

**Resistance to non-β-lactam antibiotics**

Eighty-nine (75%) cefotaxime-resistant isolates representing 25 serotypes were MDR (Tables 1 and S2). NTS isolates were resistant to sulphonamides (MICs $>64$ mg/L; 72%), tetracycline (MICs $>8$ mg/L; 68%), gentamicin (MICs $>4$ mg/L; 35%), amikacin (MICs $>4$ mg/L; 2%), kanamycin (MICs $>16$ mg/L; 18%), neomycin (MICs $>8$ mg/L; 16%), streptomycin (MICs $>16$ mg/L; 92%), nalidixic acid (MICs $>16$ mg/L; 31%), chloramphenicol (MICs $>8$ mg/L; 39%), trimethoprim (MICs $>2$ mg/L; 25%), furazolidone (MICs $>8$ mg/L; 11%) and colistin (MICs $>8$ mg/L; 4%).

Forty-two NTS (36%) had decreased susceptibility to ciprofloxacin (MICs 0.25–1 mg/L), with 22 of these retaining susceptibility to nalidixic acid (MICs $\leq 16$ mg/L), indicating the likely presence of a plasmid-mediated quinolone resistance determinant, based on the findings of a previous study. Thirteen NTS (11%) were resistant to ciprofloxacin (MICs $>1$ mg/L) including four of five Salmonella Kentucky isolates, three of which were associated with travel to Egypt. These isolates are likely to belong to the ciprofloxacin-resistant ST198-X1 international clone, which originated in Egypt.

Three ciprofloxacin-resistant isolates were Salmonella Agona associated with travel to Thailand or the Asian continent (Table S2). Co-resistance to fluoroquinolones and extended-spectrum cephalosporins is already a major public health problem in Asia, where 9.3% of NTS isolates sampled from 2003 to 2005 had dual resistance to ciprofloxacin (MICs $>0.125$ mg/L) and ceftriaxone (MICs 2–8 mg/L). In the present study, dual resistance was found in 0.25% of UK NTS isolates. Where travel history was recorded, 58% of these isolates had ties to Asia. The high rate (25%) of reduced susceptibility to ceftriaxone in Salmonella Typhimurium throughout Asia was not evident in England and Wales (0.8%).

In this study, all 13 isolates associated with travel to Thailand were resistant to >5 antimicrobial classes. Salmonella Typhimurium isolates (88% MDR) were significantly more resistant than Salmonella Enteritidis isolates (35% MDR) (P=0.0004 by Fisher’s exact test). Thirty-four percent of all NTS had penta-resistance type ACSSuT, including 16 (39%) Salmonella Typhimurium, 15 of which had a group 1 CTX-M gene. Twenty-seven (23%) NTS also had decreased susceptibility or resistance to ciprofloxacin (ACSSuTcP), including 12 (29%) Salmonella Typhimurium.

**Conclusions**

In conclusion, resistance to third-generation cephalosporins among NTS remains rare. Our data support the travel-associated spread of resistant strains to the UK from locally endemic areas. However, the epidemiology of resistance is clearly complex and may involve the spread of multidrug resistance plasmids expressing ESBLs and AmpCs between enterobacterial strains and species. The most commonly identified ESBL and AmpC genes in this study have all been identified among E. coli and Salmonella from food animals in Europe, and control measures to limit the dissemination of these strains through the food chain are necessary.

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**Transparency declarations**

None to declare.

**Supplementary data**

Table S1, Table S2 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**

8. Walker RA, Lindsay E, Woodward MJ et al. Variation in clonality and enterica-resistance genes among multiresistant Salmonella enterica


