Emergence of multidrug-resistant *Salmonella enterica* serotype 4, [5], 12:i:- involving human cases in Canada: results from the Canadian Integrated Program on Antimicrobial Resistance Surveillance (CIPARS), 2003–10

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**Objectives:** Over the last decade, a marked increase in *Salmonella enterica* serotype 4, [5], 12:i:- with a core resistance to ampicillin, streptomycin, sulphonamides and tetracycline (ASSuT) has been observed in Europe. This study describes the emergence and characterization of isolates of multidrug-resistant *Salmonella* 4, [5], 12:i:- in Canada.

**Methods:** Human clinical isolates of *Salmonella* 4, [5], 12:i:- were identified by provincial laboratories from 2003 to 2010. Serotyping and phage typing were performed by standardized methodologies. MIC values were determined using broth microdilution. PCR was used to determine the presence of resistance genes. Multilocus sequence typing was performed on a selected number of isolates.

**Results:** A total of 26 251 *Salmonella* were submitted as part of the Canadian Integrated Program on Antibiotic Resistance Surveillance (CIPARS). Of these, *Salmonella* 4, [5], 12:i:- accounted for a total of 766 isolates (2.9%), and the number increased significantly from 42 (1.4%) in 2003 to 164 (4.8%) in 2010. The ASSuT+ phenotype was observed in 11.9% (n = 91) of *Salmonella* 4, [5], 12:i:- isolates and increased from two isolates in 2003 to 35 isolates in 2010. Two sequence types (STs) were observed. ST34 was mainly associated with the ASSuT isolates (n = 24; 38%), which contained *bla*TEM, strA-strB, tet(B) and sul2. ST19 was more likely to be associated with the ACSSuT phenotype and contained *bla*TEM, floR, strA-strB, sul2 and tet(A) or *bla*PSE-1, floR, aadA2, sul1 and tet(G).

**Conclusions:** The prevalence of *Salmonella* 4, [5], 12:i:- has significantly increased from 2003 to 2010 and it is now the fifth most common serotype reported in Canada causing human disease. Similar antimicrobial resistance patterns, phage types and STs have been observed in Europe.

**Keywords:** MDR, *Salmonella enterica*, resistance

**Introduction**

Multidrug resistance (MDR) to antimicrobial agents is a growing concern with *Salmonella* as it limits treatment options for invasive disease and could lead to the transfer of resistance to other pathogenic organisms. The first *Salmonella* 4, [5], 12:i:- was identified in the late 1980s from poultry in Portugal and between 1998 and 2004 it was the fourth most common serovar in Spain.1,2 Phage type U302 with a resistance profile of ACSuGSTTm (see Table 1 for abbreviations) was the predominant resistance type. Since that time, *Salmonella* 4, [5], 12:i:- has become a predominant serotype in many countries including Italy, the UK and France; and outbreaks have been reported in Luxembourg, Austria, Ireland, Germany and the Netherlands.3 However, there appeared to be a shift in phage types and
resistance patterns in the Salmonella 4,[5],12:i:- in these countries as a majority of isolates had a multidrug-resistant phenotype of ASSuT with a phage type of DT193/DT120 predominating.6 As of 2007, Salmonella 4,[5],12:i:- has become the fifth most common serovar in Canada.9 This report describes the emergence and characteristics of MDR present in this serovar in Canada.

**Methods**

**Study design**

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was established in 2003 (see http://www.phac-aspc.gc.ca/cipars-picas/surv-eng.php for details). Salmonella isolates identified from Alberta, British Columbia, Ontario and Quebec were submitted from days 1–15 of each month, whereas all other provinces submitted all Salmonella isolates.

**Phenotypic characterization**

Serotyping was conducted using the standard methodologies used at each provincial laboratory. Phage typing was conducted as previously described.6 Antimicrobial susceptibilities to 15 antibiotics were determined using the broth microdilution method (Sensititre Automated Microbiology System; Trek Diagnostic Systems Ltd, Westlake, OH, USA) using established CLSI breakpoints7 or interpretive criteria harmonized with the National Antimicrobial Resistance Monitoring System when no formal breakpoints were available.

**Genotypic characterization**

All isolates that displayed a core antimicrobial resistance phenotype to ampicillin, streptomycin, sulphonamides and tetracycline (ASSuT) were characterized. PCR was performed on all isolates with a core resistance phenotype of ASSuT and ACCSuT as previously described.6,9 Multiplex PCR to detect extended-spectrum β-lactamase genes (for TEM, SHV, CTX and CMY-2) was conducted on all isolates with a ceftiofur MIC of ≥8 mg/L.10 Multilocus sequence typing (MLST) was performed on a subset of isolates based on unique phage types and antimicrobial susceptibility patterns according to the method described by Harbottle et al.11 Data were submitted to the MLST database web site to determine sequence types (STs; http://mlst.ucc.ie/mlst/dbs/Senterica).

**Statistical analysis**

Multivariate analysis using PROC LOGISTIC (SAS version 9.3; SAS Institute Inc., USA) was conducted to compare the presence of Salmonella 4,[5],12:i:- and antimicrobial resistance across years and provinces.

**Results and discussion**

A total of 26,251 human-derived Salmonella were submitted to CIPARS from 2003 to 2010. During this period, 766 (2.9%) Salmonella 4,[5],12:i:- were identified with the annual number of cases as follows: 2003 (n = 42), 2004 (n = 46), 2005 (n = 63), 2006 (n = 57), 2007 (n = 85), 2008 (n = 124), 2009 (n = 186) and 2010 (n = 163). There was a significant increase in the number of cases from 2003 to 2010 (P < 0.001). The number of invasive (blood) infections was 27 (3.5%), which is slightly lower than the rate of 5.3% reported for Salmonella 4,[5],12:i:- in the USA.12 None of the bloodstream infections reported in this study was considered to be multidrug resistant (resistance to three or more classes of antibiotics). The regional distribution over the time period was as follows: western provinces (British Columbia, Alberta, Saskatchewan and Manitoba; 55.2%, n = 423), central provinces (Ontario and Quebec; 38.4%, n = 294) and eastern provinces (Nova Scotia, New Brunswick, Newfoundland and Prince Edward Island; 6.4%, n = 49). A dramatic increase occurred in the number of cases since 2006 in western Canada compared with the other regions of Canada (Figure 1).

Of the 766 Salmonella 4,[5],12:i:- submitted for antimicrobial susceptibility testing, the majority of isolates (n = 456; 59.5%) remained susceptible to all antimicrobials tested. Quinolone resistance was rare, with only 13 isolates resistant to nalidixic acid and one isolate resistant to ciprofloxacin. Of concern was that the single ciprofloxacin-resistant isolate was also resistant to all the other antimicrobial resistance classes tested (Table 1). In addition, there were 119 (15.5%) isolates that had a multidrug-resistant phenotype, and the majority of the multidrug-resistant isolates (n = 91; 76.5%) had a core R-type of ASSuT. The geographical distribution of these isolates was as follows: western provinces (n = 26), central provinces (n = 58) and eastern provinces (n = 7). The first case of the ASSuT phenotype occurred in June, 2004 in British Columbia. There has been a significant increase in the number of ASSuT+ Salmonella 4,[5],12:i:- cases since 2003 (P = 0.0234). The majority of cases have been reported from Quebec (n = 34; 37.4%), followed by Ontario (n = 24; 26.4%) and then British Columbia (n = 12; 13.2%). Quebec was more likely to have cases of ASSuT+ Salmonella 4,[5],12:i:- compared with Ontario (P < 0.001), while Alberta and Manitoba where less likely to have cases than Ontario (P = 0.0197 and P = 0.0059, respectively). Despite the rapid increase in Salmonella 4,[5],12:i:- cases in the west (Figure 1), this does not seem to be associated with an increase in the ASSuT+ phenotype. Interestingly, in 2009 and 2010, when the most ASSuT+ cases were observed, Quebec had significantly more cases (P < 0.001), with 62.8% (n = 22) of the ASSuT+ cases in Canada identified in this province.

The majority of isolates with an R-type of ASSuT+ had the ASSuT phenotype (n = 63; 68.5%). These isolates comprised six different phage types, as well as a number of isolates that were untypeable or atypical (Table 1). Of the 24 (38%) isolates that were sequence-typed, all belonged to ST34. Isolates with the same R-type, phage types and STs have been described in many European countries.1 Recent studies for this R-type have been elucidated and found to be localized to the chromosome of Salmonella 4,[5],12:i:-.13 In 2008
<table>
<thead>
<tr>
<th>Resistance type</th>
<th>Phage type(s)</th>
<th>Province(s) where identified</th>
<th>Year(s) observed</th>
<th>ST(s)</th>
<th>Antimicrobial resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASSuT (63)</td>
<td>35 (9), 120 (3), 179 (4), 193 (31), 193a (1), 195 (1), atypical (8), untypable (2), ND (4)</td>
<td>BC (4), AB (2), SK (2), MB (1), ON (20), QC (29), NB (1), NS (2), PEI (2)</td>
<td>2004 (3), 2005 (7), 2006 (3), 2007 (6), 2008 (6), 2009 (11), 2010 (27)</td>
<td>ST34 (24)</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (B) (62)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ANSSuT (3)</td>
<td>untypable (2), U311 (1)</td>
<td>BC (2), SK</td>
<td>2007, 2008, 2009</td>
<td>ST34 (3)</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (B) (3)</td>
</tr>
<tr>
<td>AKSSuT (1)</td>
<td>193</td>
<td>AB</td>
<td>2007</td>
<td>ST34</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (B)</td>
</tr>
<tr>
<td>AGNSSuT (1)</td>
<td>UT6</td>
<td>AB</td>
<td>2010</td>
<td>ST34</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (B)</td>
</tr>
<tr>
<td>ACSSuT (5)</td>
<td>104 (2), atypical, 35, 104a</td>
<td>BC (3), MB, ON</td>
<td>2003, 2004, 2005, 2008, 2009</td>
<td>ST19 (3), ST34 (1)</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (B) + floR (2); bla&lt;sub&gt;PSE-1&lt;/sub&gt; +aadA2 + sul1 + tet (G) + floR (3)</td>
</tr>
<tr>
<td>ACKSSuT (6)</td>
<td>104, 302 (4), 110b</td>
<td>QC (4), NB, ON</td>
<td>2006, 2009, 2010 (4)</td>
<td>ST19 (4)</td>
<td>bla&lt;sub&gt;PSE-1&lt;/sub&gt; + strA-strB + sul2 + tet (A) + sul2 + floR (5); bla&lt;sub&gt;PSE-1&lt;/sub&gt; +aadA2 + sul1 + tet (G) + floR (1)</td>
</tr>
<tr>
<td>AcACSSuT (1)</td>
<td>191</td>
<td>SK</td>
<td>2006</td>
<td>ST19</td>
<td>strA-strB + sul2 + tet (A) + floR</td>
</tr>
<tr>
<td>ACKSSuTTm (2)</td>
<td>UT1, 110b</td>
<td>BC, QC</td>
<td>2005, 2010</td>
<td>ST19 (2)</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; +aadA2 + sul1 + tet (G) + floR; bla&lt;sub&gt;PSE-1&lt;/sub&gt; +aadA2 + sul1 + tet (A) + floR</td>
</tr>
<tr>
<td>AcACxCSSuT (1)</td>
<td>191</td>
<td>SK</td>
<td>2006</td>
<td>ST19</td>
<td>strA-strB + sul2 + tet (A) + floR</td>
</tr>
<tr>
<td>AcACxCGCSSuT (2)</td>
<td>193 (2)</td>
<td>NF, ON</td>
<td>2008, 2010</td>
<td>ST34 (2)</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (A) + floR + bla&lt;sub&gt;CTX-M-55&lt;/sub&gt;; bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (A) + floR + bla&lt;sub&gt;CTX-M-57&lt;/sub&gt;</td>
</tr>
<tr>
<td>AcACKSSTTm (1)</td>
<td>atypical</td>
<td>AB</td>
<td>2005</td>
<td>ST19</td>
<td>strA-strB + sul2 + tet (A) + floR</td>
</tr>
<tr>
<td>AGCNSSuTTm (2)</td>
<td>UT5, UT1</td>
<td>MB, ON</td>
<td>2004 (2)</td>
<td>ST19 (2)</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; +strA-strB + sul2 + tet (A) + cmfA; bla&lt;sub&gt;TEM&lt;/sub&gt; +strA-strB + sul2 + tet (B) + floR</td>
</tr>
<tr>
<td>ACGKNSSuTTm (1)</td>
<td>atypical</td>
<td>BC</td>
<td>2008</td>
<td>ST34</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + strA-strB + sul2 + tet (B) + floR</td>
</tr>
<tr>
<td>AcACECfCSSuT (1)</td>
<td>193</td>
<td>BC</td>
<td>2010</td>
<td>ST34</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; +strA-strB + tet (B) + floR + bla&lt;sub&gt;CMY-2&lt;/sub&gt;</td>
</tr>
<tr>
<td>AcACECfCpCGSSTmT (1)</td>
<td>191</td>
<td>SK</td>
<td>2003</td>
<td>ST19</td>
<td>strA-strB + sul2 + tet (A) + floR + bla&lt;sub&gt;CMY-2&lt;/sub&gt;</td>
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</tbody>
</table>

<sup>a</sup>A, ampicillin; Ac, amoxicillin/clavulanic acid; C, chloramphenicol; Ce, cefoxitin; Cf, ceftriaxone; Cx, ceftriaxone; Cp, ciprofloxacin; G, gentamicin; K, kanamycin; N, nalidixic acid; S, streptomycin; Su, sulphonamides; T, tetracycline; Tm, trimethoprim/sulfamethoxazole.

<sup>b</sup>BC, British Columbia; AB, Alberta; SK, Saskatchewan; MB, Manitoba; ON, Ontario; QC, Quebec; NB, New Brunswick; NS, Nova Scotia; PEI, Prince Edward Island; NF, Newfoundland.

<sup>c</sup>One isolate was not viable and could not be further tested.

Mulvey et al., 1984

Table 1. Antimicrobial resistance patterns and other characteristics of the isolates in this study.
in Canada, it was rare to find Salmonella 4,[5],12:i:- in pigs on farms, with only one (1.7%) multidrug-resistant isolate reported, and none was found in abattoir surveillance, but four of eight isolates were identified in clinical isolates from pigs.14

There were 23 isolates identified with a core ACSSuT+ with 11 different R-types observed (Table 1). Using PCR, considerable variation was observed in the genotypes of the resistance genes analysed. MLST data on the unique R-types for these isolates revealed a second clonal group ST19, which comprised 15 isolates with a minimal R-type of ACSSuT (Table 1). ST19 varies from ST34 by only a single nucleotide change in the dnaV locus, which suggests that the two antimicrobial types are closely related. However, the majority of isolates that were multidrug resistant including resistance to chloramphenicol fell into this cluster. The antimicrobial resistance genes we identified in the ACSSuT pattern were different from those in the ASSuT ST34 genotype (Table 1).

Finally, the emergence of extended-spectrum β-lactamase resistance is a growing concern in Salmonella as it limits the use of frontline antimicrobials to treat severe infections. In this study, four isolates were resistant to ceftriaxone, a third-generation cephalosporin used in the agri-food industry. Of these, two isolates were identified that contained CTX-M genes (bla<sub>CTX-M-55</sub> and bla<sub>CTX-M-57</sub>), both of which had the R-type AmCfCxCGSSuT (Table 1), but these were identified from different provinces and years. In addition, two isolates harboured a bla<sub>CMY-2</sub> gene, one had an R-type of AcACeCfxCSSuT (PT193), while the other was AcACeCfxCpCGSSuTTm (PT191). Both of these isolates were identified from different provinces and years (Table 1). The bla<sub>CTX-M-55</sub> was previously identified in Salmonella 4,[5],12:i:- from the USA in 2007, and interestingly it was isolated from a patient with no travel history.15 bla<sub>CTX-M-57</sub> was first described in an isolate of Salmonella Typhimurium from a patient in the UK with a previous travel history to Thailand.16 To our knowledge, this is the first report of bla<sub>CMY-2</sub> identified in Salmonella 4,[5],12:i:-. bla<sub>CMY-2</sub> IS widespread in Canada, being found in Escherichia coli from water, animal and human sources and is particularly common in Salmonella Heidelberg from both chicken and retail poultry meat and human cases.17,18

Conclusions

The number of Salmonella 4,[5],12:i:- infections continues to increase in Canada, with an increasing proportion being found to be multidrug resistant. The isolates can be stratified into two groups; (i) the ASSuT phenotype associated with ST34, which has been described in many European Union countries; and (ii) the ACSSuT phenotype, which has been associated with ST19, has different molecular antimicrobial resistance mechanisms from ST34, and appears to have emerged in Spain. Further studies are required to determine whether these cases are associated with travel or related to food and animal sources in Canada, as linkages to zoonotic transmission, specifically in pigs, have been reported in the European Union for both STs.19

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Transparency declarations

None to declare.

References

9 Guerra B, Soto SM, Arguelles JM et al. Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent Salmonella

Figure 1. Graph depicting the number of cases of Salmonella 4,[5],12:i:- identified in Canada stratified by region. A description of the groupings of the provinces by region is located in the Results and discussion section.


1986


