Rapid and widespread dissemination of multidrug-resistant \textit{bla}_{CMY-2} \textit{Salmonella} Typhimurium in Mexico

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\textbf{Objectives:} We describe the emergence and dissemination of multidrug-resistant (MDR) \textit{Salmonella} Typhimurium in humans, retail meat and food animals from Yucatan, Mexico.

\textbf{Methods:} \textit{Salmonella} Typhimurium isolates were collected through an active surveillance system and tested for susceptibility to 12 antimicrobial agents. Isolates that were non-susceptible to ceftriaxone were tested with 10 additional antimicrobials and assayed by PCR for the presence of CMY, CTX-M, SHV, TEM and OXA \textit{\beta}-lactamase genes. Plasmid-borne phenotypes were identified by transfer to susceptible \textit{Escherichia coli}. Isolates from humans, retail meat and food animals were compared by PFGE to determine genetic relatedness.

\textbf{Results:} MDR \textit{Salmonella} Typhimurium containing a plasmid-mediated \textit{bla}_{CMY-2} AmpC \textit{\beta}-lactamase rose from 0\% (0/27) during 2000 and 2001 to 75\% (63/84) in 2004 and 2005 (\textit{P} < 0.0001). MDR \textit{bla}_{CMY-2} \textit{Salmonella} Typhimurium (\textit{n} = 115) was most common in ill children (44.3\%) and pork or swine intestine (36.5\%). In several cities, MDR \textit{bla}_{CMY-2} \textit{Salmonella} Typhimurium from retail meat or swine intestine exhibited PFGE patterns and antibiograms indistinguishable from those in strains recovered from hospitalized children. The \textit{CMY} gene was transferred to \textit{E. coli} by electroporation, along with resistance to three to six other antimicrobials. Children with MDR \textit{bla}_{CMY-2} \textit{Salmonella} Typhimurium infection (\textit{n} = 39) had a higher frequency of systemic infection (13\% versus 0\%), mortality (8\% versus 0\%) and hospital re-admission due to protracted diarrhoea (28\% versus 17\%) than children with non-MDR-\textit{Salmonella} Typhimurium (\textit{n} = 24), although the difference was not statistically significant.

\textbf{Conclusions:} The rapid and widespread dissemination of MDR \textit{bla}_{CMY-2} \textit{Salmonella} Typhimurium in Mexico calls for urgent interventions to contain this potentially fatal pathogen.

Keywords: swine, humans, PFGE, mortality, developing countries

\textbf{Introduction}

During the last two decades, \textit{Salmonella enterica} subsp. \textit{enterica}, in particular, serovar Typhimurium, has become increasingly resistant to antimicrobial compounds worldwide. During the 1980s, \textit{Salmonella} Typhimurium definitive phage type (DT) 104 with resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT resistance type) emerged in the UK and subsequently disseminated throughout Europe and North America. By the 1990s, \textit{Salmonella} Typhimurium (both DT 104 and non-DT 104) had acquired additional resistance to trimethoprim–sulfamethoxazole, ciprofloxacin and extended-spectrum cephalosporins (ESCs).1 Although the ACSSuT phenotype, which is linked to a complex class I integron contained within Salmonella Genomic Island I, was the most prevalent multidrug-resistant (MDR) \textit{Salmonella} phenotype during the 1980s and 1990s,1 the recent emergence of MDR \textit{Salmonella} with AmpC-like ESC resistance represents a new challenge. In North America, this ESC resistance is mediated by a plasmid-borne CMY-2 \textit{\beta}-lactamase and has spread horizontally to different serotypes. These plasmids frequently harbour multiple resistance determinants to other antimicrobial classes.2,3
The emergence of MDR, ESC-resistant *Salmonella* is particularly worrisome, because clinicians are left with very few options for effectively treating severe infections. Furthermore, previous studies have shown that antibiotic-resistant *Salmonella Typhimurium* is associated with an increased frequency of bloodstream infection and mortality.4,5 This study is one of the few to describe the emergence and widespread dissemination of MDR *Salmonella Typhimurium* in a developing country such as Mexico, as well as the outcome of affected children.

### Materials and methods

We report the results obtained from 2000 to 2005 for an integrated surveillance system of the food chain in Yucatan, Mexico, that included samples from humans, retail meat and food-animal intestines.3 Sampling of meat and food-animal intestines was designed to reflect regional consumption of each meat product. Data on disease severity and outcome were obtained from the hospital records of children admitted for *Salmonella Typhimurium* infection. The study was approved by the Hospital General O’Horan Internal Review Board and written informed consent was obtained from all parents or guardians. Statistical testing of differences in proportions was conducted using the *χ*2 test; *P* values less than 0.05 were considered significant.

Methods for isolation, identification, susceptibility testing, serotyping and PFGE of *Salmonella* have been described previously.6 ESC-resistant *Salmonella Typhimurium* isolates were subjected to PFGE analysis and examined for the presence of CTX-M, CMY and CMY-2 gene was identified in all of these. DNA sequences were determined and used to screen the GenBank database using the BLAST algorithm available at the National Center of Biotechnology Information’s web site (http://www.ncbi.nlm.nih.gov/BLAST/). Transfer experiments were carried out on a subset of 4 isolates, which were selected from a total of 48 isolates on the basis of distinct plasmid RFLP patterns using *Pst*I. Plasmid DNA was introduced by electroporation into *Escherichia coli* DH10B (EP-Max 10B) (Invitrogen, Carlsbad, CA, USA), and transformants were selected using ceftriaxone (4.0 mg/L). Transformed host cells were tested for antimicrobial susceptibility as before and assayed for the acquisition of CMY using PCR.

### Results

The number of human, retail meat and food-animal intestine samples positive for *Salmonella Typhimurium* and MDR *Salmonella Typhimurium* is shown in Table 1. A total of 2431 *Salmonella* were recovered from 7206 samples, of which 177 (7.3%) isolates were serovar Typhimurium. Of the 177 *Salmonella Typhimurium* isolates, 115 (65%) were ESC-resistant. Most of these were recovered from ill children (44.3%) and from pork or swine intestine (36.5%). The prevalence of MDR *Salmonella Typhimurium* among all *Salmonella* Typhimurium isolates rose from 0% (0/27) during 2000 and 2001 to 75% (63/84) in 2004 and 2005 (*P < 0.0001*, *χ*2 for trend).

The ceftriaxone MIC50 and MIC90 for these isolates were 64 and 128 mg/L, respectively (range 16 to >128 mg/L). In addition to the ACSSuT phenotype, these isolates were non-susceptible to piperacillin, ticarcillin, cefoxitin, ceftazidime, cefotaxime, ceftiofur and aztreonam and did not show increased susceptibility in the presence of clavulanic acid. An important percentage of these strains was also non-susceptible to amikacin.

Of the 115 ESC-resistant isolates, 113 were available for molecular testing. The *blaCMY-2* gene was identified in all of these.

### Table 1. Prevalence of *Salmonella Typhimurium* and MDR *blaCMY-2* Salmonella Typhimurium in humans, retail meat and food animals from Yucatan, Mexico, 2000–05

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of isolates</th>
<th>prevalence (%)</th>
<th>no.</th>
<th>%</th>
<th>no.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with diarrhoea6</td>
<td>279</td>
<td>16.6</td>
<td>71</td>
<td>25.4</td>
<td>45</td>
<td>16.1</td>
</tr>
<tr>
<td>Asymptomatic children</td>
<td>369</td>
<td>10.0</td>
<td>22</td>
<td>6.0</td>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td>Retail chicken</td>
<td>357</td>
<td>46.0</td>
<td>16</td>
<td>4.5</td>
<td>14</td>
<td>3.9</td>
</tr>
<tr>
<td>Retail pork</td>
<td>637</td>
<td>63.3</td>
<td>29</td>
<td>4.6</td>
<td>22</td>
<td>3.5</td>
</tr>
<tr>
<td>Retail beef</td>
<td>336</td>
<td>60.5</td>
<td>5</td>
<td>1.5</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>Chicken intestine</td>
<td>105</td>
<td>25.6</td>
<td>1</td>
<td>1.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Swine intestine</td>
<td>270</td>
<td>74.3</td>
<td>27</td>
<td>10.0</td>
<td>20</td>
<td>7.4</td>
</tr>
<tr>
<td>Bovine intestine</td>
<td>72</td>
<td>56.8</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Strains referred from other hospitals</td>
<td>6</td>
<td>—</td>
<td>6</td>
<td>—</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>2431</td>
<td>—</td>
<td>177</td>
<td>—</td>
<td>115</td>
<td>—</td>
</tr>
</tbody>
</table>

*Calculation accounts for number of samples positive for *Salmonella*; many samples had more than one serotype.

*Percentage of *Salmonella Typhimurium* or MDR *blaCMY-2* Salmonella Typhimurium among all *Salmonella* isolates from each source.

*Five children presented systemic complications.
With the exception of a single isolate (CVM34474) that also carried TEM-1, none of the other assayed bla genes was detected. The CMY β-lactam resistance phenotype was transferred by electrottransformation of purified plasmid DNA to *E. coli* DH10 recipient, along with different patterns of resistance to sulfamethoxazole, chloramphenicol, tetracycline, gentamicin, trimethoprim–sulfamethoxazole and kanamycin. For transformant 35417-T, the MIC of ceftriaxone increased 8-fold relative to the wild-type donor strains. Plasmids from this strain type were also distinct in that trimethoprim–sulfamethoxazole, tetracycline and chloramphenicol resistances failed to transfer to the *E. coli* recipient.

PFGE analysis of the 113 MDR bla<sub>CMY-2</sub> *Salmonella* Typhimurium isolates revealed 21 clusters, each of which contained at least two strains with indistinguishable banding patterns. Most of the clusters contained isolates from identical or neighbouring cities. In 8 of the 21 clusters (clusters A–H), human isolates were found to be closely associated with those from swine intestine or retail meat. (Figure 1).

Clinical charts were available for 63 children with *Salmonella* Typhimurium infection. Children with MDR bla<sub>CMY-2</sub> *Salmonella* Typhimurium infection (*n* = 39) had a higher frequency of systemic infection (12.8% versus 0%, *P* = 0.15), mortality (7.7% versus 0%, *P* = 0.28) and hospital re-admission due to protracted diarrhea (28% versus 16.6%, *P* = 0.46) than children with non-MDR bla<sub>CMY-2</sub> *Salmonella* Typhimurium (*n* = 24); however, we could not demonstrate statistical significance because of small sample size. Of the children with MDR bla<sub>CMY-2</sub> *Salmonella* Typhimurium infection, two presented severe thrombocytopenia (2000 and 18 000 platelets/mm<sup>2</sup>, respectively), one of whom died from a cerebral haemorrhage; two other infants died from bloodstream infection.

**Discussion**

This study documents the emergence and rapid dissemination of MDR bla<sub>CMY-2</sub> *Salmonella* Typhimurium in food animals and humans from Yucatan, Mexico. MDR bla<sub>CMY-2</sub> *Salmonella* Typhimurium has become the predominant phenotype of serovar Typhimurium in the state, currently accounting for 75% of all

**Figure 1.** PFGE patterns for MDR bla<sub>CMY-2</sub> *Salmonella* Typhimurium from humans, retail meat and food animals in Mexico from 2002 to 2005. Isolates were collected from humans with systemic infection (HS), humans with symptomatic enteric infection (HE), humans with asymptomatic enteric infection (HA), chicken meat (CM), pork (PM), beef (BM) and swine intestine (SI) from 30 cities throughout the state of Yucatan. Non-susceptibility to antimicrobials, indicated by a black box, was present for ampicillin (AMP), cefoxitin (FOX), ceftriaxone (CRO), chloramphenicol (CHL), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfisoxazole (SSS), tetracycline (TET), trimethoprim/sulfamethoxazole (SXT) and ceftiofur (TIO). Clusters A–H, highlighted in boxes, indicate strains from humans and retail meat and/or swine intestine with indistinguishable PFGE patterns. Clusters B, E, F and G contain four of the five isolates from systemic infections. The fifth extraintestinal isolate (35 733) was closely related (97%) to strains from cluster C that included isolates from human enteric infection (32 090) and beef (35 423). Clusters 1–13, marked to the left of the dendrogram with numbered lines, indicate isolates of human or animal origin only. Most of the clusters contain isolates from identical or neighbouring cities. NA, not applicable.
MDR Salmonella Typhimurium bla\textsubscript{CMY-2} in Mexico

Salmonella Typhimurium isolates; it is resistant to at least eight antimicrobials and has caused severe enteric and systemic infections in children, three of whom died. Molecular analysis enabled us to presumptively identify swine as the major source of these strains and to determine that ESC-resistance is mediated by a \textit{bla}\textsubscript{CMY-2} gene harboured on different plasmids.

Among food animals, the \textit{bla}\textsubscript{CMY-2} phenotype was only found in swine intestines, suggesting that the main selection pressure for ESC-resistant Salmonella Typhimurium in Yucatan originated and persists in swine production. Its presence in pork, beef, and chicken meat is likely due to cross-contamination during slaughter or at retail. Although we made no effort to mobilize them by conjugation, our transformation experiments show that the \textit{bla}\textsubscript{CMY-2} gene resides on plasmids with differing resistance gene content and that they are able to replicate in \textit{E. coli}. The extent to which CMY dissemination has been mediated by other genera is not known.

In North America, MDR \textit{bla}\textsubscript{CMY-2} salmonellae first emerged in Salmonella Typhimurium and later as part of the multiple resistance genotype in food animal and food-borne isolates of Salmonella Newport. In the USA, \textit{bla}\textsubscript{CMY-2} Salmonella has been primarily associated with cattle and beef, and in Canada, with turkey.\textsuperscript{2,3,9} Differences in animal reservoirs could be related to food-animal production practices, including the amount and type of antimicrobials used in each country. In both Mexico and the USA, \textit{bla}\textsubscript{CMY-2} Salmonella appears to have established itself in an animal reservoir before spreading to humans.

\textit{bla}\textsubscript{CMY-2} Salmonella is infrequent in countries outside North America, where ESC resistance is more commonly mediated by molecular class A \beta-lactamases such as TEM, SHV, OXA and CTX.\textsuperscript{10} Possible explanations for this low frequency could be differences in the prevalence of specific serovars among countries, the lack of systematic screening for AmpC-type \beta-lactamase genes in the veterinary sector and cefhalosporin usage. Some investigators attribute the emergence of CMY-2 to the use of ceftriaxone, a third-generation cephalosporin used exclusively in food animals.\textsuperscript{2,3} Although there are no reliable data in Mexico or the USA on the quantity of third-generation cefhalosporins used, these compounds are readily available in veterinary and medical pharmacies throughout Yucatan and can be purchased without prescription (M. Zaidi, unpublished results).

It is noteworthy that systemic Salmonella Typhimurium infection and mortality were only seen in children with MDR \textit{bla}\textsubscript{CMY-2} strains. Moreover, the occurrence of severe thrombocytopenia with haemorrhagic syndrome, a very uncommon presentation of non-typhoidal salmonellosis, should be alerted to all physicians. Although we were unable to demonstrate a statistically significant association between MDR and increased severity of disease, our findings show a clinically significant trend that supports the growing body of literature that associates MDR Salmonella with increased morbidity.\textsuperscript{4,5}

In conclusion, our findings highlight the rapid dissemination of MDR CMY-2-producing Salmonella in Mexico, a developing country that currently lacks the infrastructure for effective containment measures. Policy makers need to implement a series of urgent interventions to prevent and control selection of, and environmental contamination with, MDR Salmonella throughout the food chain. Foremost measures include tighter regulations for antimicrobial use and the establishment of an effective surveillance programme to monitor the public health impact of this emerging pathogen.

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Transparency declarations

None to declare.

References