First Report of Extended-Spectrum-β-Lactamase-Producing Salmonella enterica Serovar Kentucky Isolated from Poultry in Ireland

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Therapy of invasive human salmonellosis is complicated by increasing antimicrobial resistance. Food animals are the principal source of infection with nontyphoid Salmonella. We report the emergence of broad-spectrum-cephalosporin resistance in Salmonella enterica serovar Kentucky in poultry in Ireland.

Increasing antimicrobial resistance among isolates of Salmonella from food animals is a significant public health concern because of the potential to cause human infection. Broad-spectrum cephalosporins, together with fluoroquinolones, were until recently considered reliable agents for empirical therapy of invasive salmonellosis. Cephalosporin-resistant Salmonella enterica bacteria that disseminate in food animals therefore have the potential to cause human infection, for example, cephalosporin-resistant Salmonella enterica serovar Virchow (poultry in France) and Salmonella enterica serovar Newport (cattle in the United States) (1, 3, 5, 12).

Prior to 2008, resistance to cephalosporins was not reported for isolates of Salmonella from food animals in Ireland. Cephalosporin resistance has been detected in isolates from humans associated with travel outside Ireland. From January 2000 to September 2008, we analyzed 925 isolates of Salmonella enterica serovar Kentucky of human (n = 60) and animal (n = 865) origin, with all isolates being susceptible to cephalosporins. Between October 2008 and March 2009, 7 of 115 S. Kentucky isolates were resistant to cefotaxime and ceftazidime (5, 7).

A number of human cases of S. Kentucky infection have occurred during most years in Ireland, and human infection has also been documented to occur elsewhere (5, 7). Collard and colleagues in 2007 (9) reported on SMPC production in human isolates of S. Kentucky bacteria that were co-resistant to ciprofloxacin and cotrimoxazole. Other studies have discussed the importance of emerging serovars of Salmonella to human health (7, 9, 17, 21).

Identification and serotyping of isolates were performed by standard methods. Testing of susceptibility to 14 antimicrobial agents was performed by Clinical and Laboratory Standards Institute (CLSI) disk diffusion methods (8). Cephalosporin-resistant isolates were assessed for extended-spectrum-β-lactamase (ESBL) production by using cephalosporine (30 µg) and by using cephalosporine plus clavulanic acid (10 µg/1 µg) and ESBL Etests with ceftazidime, cefotaxime, and cefepime in each case with and without clavulanic acid (AB Biodisk, Solna, Sweden). AmpC production was suspected in the absence of clavulanic acid potentiation of cephalosporins and additional resistance to cefoxitin. Pulsed-field gel electrophoresis (PFGE) was performed by the PulseNet method with XbaI and BlnI, and the results were analyzed using BioNumerics software (Applied Maths, Saint-Martens-Latem, Belgium). Plasmids were prepared as previously described (20) and visualized on 0.7% Tris-acetate-EDTA gels by staining with 1 mg/ml ethidium bromide. Size was estimated using a standard curve constructed from plasmids from strains NCTC 50012 (72 MDa), NCTC 50001 (62 MDa), NCTC 50083 (126 MDa), and NCTC 50005 (26 MDa). Conjugaion was carried out using the method of Hasman et al. (13). A plasmid-free, amoxicillin-susceptible, and nalidixic acid- and rifampin-resistant Salmonella enterica serovar Dublin isolate was the recipient for mating experiments. Transconjugants were selected on LB agar plates containing cefoxitin (32 µg/ml) and nalidixic acid (50 µg/ml) or ampicillin (32 µg/ml) and nalidixic acid (50 µg/ml). Genomic DNA was extracted with a QIAamp DNA minikit (Qiagen, Inc., Valencia, CA). Absence of inhibitors of PCR was confirmed by amplification of 16S rRNA and the 23S rRNA spacer region (2). PCR amplifications with specific primers for blaCTX-M, blashaV, and blaCTX-M groups 1, 2, 8, 9, and 25; plasmid-mediated blaaugV (sequencing of positive amplifications by using primers specific for the entire blaCMY gene); Salmonella genomic island 1 (SGI1); and class 1 integrons were performed as previously described (11, 14, 15, 18, 22). Positive amplifications were sequenced by Sequiverse, Vaterstetten, Germany.

The results are summarized in Table 1. Plasmid-mediated β-lactamase gene blaCMY-2 was detected in 3 isolates and blashaV,12 in 4. blaCMY-2 has been reported to occur in Salmonella but has not previously been reported to occur in S. Kentucky isolates from poultry. blashaV,12 has been reported to occur in S. Kentucky from a human isolate (7) but not in that from poultry. All isolates contained two plasmids of ca. 4.9 and ca. 130 kb. In all cases, cephalosporin resistance was readily transferable, with cotransfer of both the ca. 4.9-kb and the ca. 130-kb plasmids in every case. blashaV was detected by PCR in the ca. 4.9-kb plasmid but not in the ca. 130-kb plasmid in isolates with the SHV phenotype, while blaCMY was detected in the ca. 130-kb plasmid but not in the ca. 4.9-kb plasmid in each case with and without clavulanic acid (AB Biodisk, Solna, Sweden). AmpC production was suspected in the absence of clavulanic acid potentiation of cephalosporins and additional resistance to cefoxitin. Pulsed-field gel electrophoresis (PFGE) was performed by the PulseNet method with XbaI and BlnI, and the results were analyzed using BioNumerics software (Applied Maths, Saint-Martens-Latem, Belgium). Plasmids were prepared as previously described (20) and visualized on 0.7% Tris-acetate-EDTA gels by staining with 1 mg/ml ethidium bromide. Size was estimated using a standard curve constructed from plasmids from strains NCTC 50012 (72 MDa), NCTC 50001 (62 MDa), NCTC 50083 (126 MDa), and NCTC 50005 (26 MDa). Conjugaion was carried out using the method of Hasman et al. (13). A plasmid-free, amoxicillin-susceptible, and nalidixic acid- and rifampin-resistant Salmonella enterica serovar Dublin isolate was the recipient for mating experiments. Transconjugants were selected on LB agar plates containing cefoxitin (32 µg/ml) and nalidixic acid (50 µg/ml) or ampicillin (32 µg/ml) and nalidixic acid (50 µg/ml). Genomic DNA was extracted with a QIAamp DNA minikit (Qiagen, Inc., Valencia, CA). Absence of inhibitors of PCR was confirmed by amplification of 16S rRNA and the 23S rRNA spacer region (2). PCR amplifications with specific primers for blaCTX-M, blashaV, and blaCTX-M groups 1, 2, 8, 9, and 25; plasmid-mediated blaaugV (sequencing of positive amplifications by using primers specific for the entire blaCMY gene); Salmonella genomic island 1 (SGI1); and class 1 integrons were performed as previously described (11, 14, 15, 18, 22). Positive amplifications were sequenced by Sequiverse, Vaterstetten, Germany.

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isolates with the AmpC phenotype. The associations of \( bla_{SHV-12} \) with the ca. 4.9-kb plasmid from some isolates but not others and of \( bla_{CMY-2} \) with the ca. 130-kb plasmid from some isolates warrant further analysis, such as PCR-based replicon typing (PBRT) with subsequent sequencing, which will allow us to establish the relatedness of the plasmids identified. The *Salmonella* genomic island (SGI-11-K) was not present, in contrast to previous reports (15). A class 1 integron was detected in 1 of 3 CMY-2-positive isolates and in all 4 of the SHV-12-positive isolates.

The isolates were 92% similar on XbaI PFGE, with sets of 3 (type D) and 2 (type C) isolates indistinguishable. PFGE with BlnI provided limited additional discrimination between the isolates. Four isolates were from different broiler farms, with 2 isolates (different PFGE profiles) from the same broiler farm. One isolate could not be traced. All the broiler farms are stocked from one breeder farm. Comparison of PFGE patterns with archived stock did not show additional discrimination between the isolates identified. One isolate could not be traced. All the broiler farms are stocked from one breeder farm. Comparison of PFGE patterns with archived stock did not show additional discrimination between the isolates identified.

The simultaneous emergence of broad-spectrum-cephalosporin resistance associated with two distinct plasmid-encoded mechanisms (ESBL–SHV-12 and AmpC–CMY-2) in poultry in Ireland as described in this paper is a cause for concern. Previous experience indicates that such resistant *Salmonella* isolates in food animals are to be associated with human disease (4). The route by which these resistant mechanisms gained access to the multiple poultry farms involved is unclear, but possibilities include colonization of stock received from breeder flocks and/or inadequate biosecurity.

It is important to highlight that cephalosporins are not licensed for use in poultry production in Ireland; however, amoxicillin is used for the control of clostridia and bacterial enteritis and therefore may generate a selective pressure for possession and retention of a \( \beta \)-lactamase (4, 6, 16), as it is widely hypothesized that the introduction and persistence of \( \beta \)-lactamase-producing *Salmonella* on farms may be sustained due to selective pressure related to antimicrobial prescribing (16). However, it has been demonstrated that the acquisition and persistence of plasmid-mediated \( \beta \)-lactamase in *Salmonella* from commensal intestinal flora can occur even in the absence of selective antimicrobial pressure (10, 19). The exact mechanisms of dissemination and maintenance of resistance plasmids in the absence of selective pressure merit further investigation.

In conclusion, regular monitoring for *Salmonella* in food animals must include adequate antimicrobial susceptibility testing of isolates to ensure that new threats, such as broad-spectrum-cephalosporin resistance, are recognized and addressed before they are widely disseminated. In the context of poultry farming, breeder flocks, from which other farms are stocked, require especially close monitoring because of their potential to act as foci of dissemination of antimicrobial resistance.

**REFERENCES**


**TABLE 1. Results**

<table>
<thead>
<tr>
<th>Farm</th>
<th>Isolate</th>
<th>Date isolated</th>
<th>Specimen</th>
<th>Antibiogram result</th>
<th>PFGE type</th>
<th>Beta-lactamase</th>
<th>Class 1 integron(s)</th>
<th>Variable region(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>08-1060</td>
<td>7 October 2008</td>
<td>Whole chicken</td>
<td>ACazCtxFoxCppdA</td>
<td>A A</td>
<td>CMY-2</td>
<td>ND</td>
<td>aadB, sat</td>
</tr>
<tr>
<td>B</td>
<td>08-1259</td>
<td>25 November 2008</td>
<td>Chicken neck skin</td>
<td>ACsuTcazCtxCppdBB</td>
<td>B B</td>
<td>SHV-12</td>
<td>763, 638</td>
<td>sat</td>
</tr>
<tr>
<td>C</td>
<td>09-031</td>
<td>16 January 2009</td>
<td>Poultry broiler dust</td>
<td>ACsuTcazCtxCppdC</td>
<td>D C</td>
<td>SHV-12</td>
<td>638</td>
<td>sat</td>
</tr>
<tr>
<td>D</td>
<td>09-060</td>
<td>3 February 2009</td>
<td>Whole chicken</td>
<td>ACsuTcazCtxCppdC</td>
<td>D C</td>
<td>SHV-12</td>
<td>638</td>
<td>sat</td>
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<tr>
<td>E</td>
<td>09-135</td>
<td>18 February 2009</td>
<td>Poultry broiler dust</td>
<td>ACazCtxFoxCppdD</td>
<td>D E</td>
<td>CMY-2</td>
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<td>aadB</td>
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<td>6 March 2009</td>
<td>Whole chicken</td>
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<td>D E</td>
<td>CMY-2</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* For all strains, the SGI was absent, plasmids of ~4.9 and ~130 kb were present, and resistance transfer was observed.
* The values shown indicate the presence of both the 763-bp and the 638-bp variable regions, the 638-bp variable region only, or the 763-bp variable region only. ND, class 1 integron not detected.


