Emergence of Extended-Spectrum-β-Lactamase CTX-M-2-Producing Salmonella enterica Serovars Schwarzengrund and Agona in Poultry Farms

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he emergence and spread of antimicrobial resistance among Salmonella serovars originating from food-producing animals and their immediate environment is a major public health problem, because Salmonella is one of the most common causes of human food-borne illness (1). In this study, we describe for the first time the presence of extended-spectrum beta-lactamase (ESBL) CTX-M-2-producing Salmonella enterica isolates belonging to serotypes Schwarzengrund and Agona in poultry farms. From 2008 to 2009, 93 Salmonella spp. were isolated from commercial poultry (i.e., chicken, turkey, and tinamou) and related sources (poultry farm floor, drag swab of the rearing facility, carcasses, and eggs) in farms of five Brazilian states. Strains were identified by using conventional biochemical tests and serotyped on the basis of somatic O and H flagellar antigens by the agglutination test, according to the Kaufmann-White scheme for Salmonella serotyping. ESBL phenotypes were detected using the double-disc synergy test (DDST) and cefotaxime-ceftazidime-clavulanic acid Etest ESBL strips (bioMérieux, Marcy l’Étoile, France), and genes encoding ESBLs were confirmed by PCR and sequencing (2). Plasmids were extracted by the alkaline lysis method (3), and their sizes were estimated using a standard curve constructed from plasmids of known molecular sizes from Escherichia coli strain 39R861 (4). ESBL gene-carrying plasmids, classified according to their incompatibility group (5), were transformed into E. coli TOP10 (6). Finally, the clonal relationship among ESBL-positive strains was determined by pulsed-field gel electrophoresis (PFGE) of XbaI-digested DNA by following the standardized PulseNet protocol for Salmonella species (http://www.cdc.gov/pulsenet/protocols.htm).

Thirteen Salmonella enterica isolates (14%) that were grouped into two major PFGE clusters (A and B), belonging to serotypes Schwarzengrund and Agona, respectively, were found to produce extended-spectrum β-lactamase CTX-M-2 (GenBank accession no. KC633129). The isolates were recovered from poultry-rearing environments and foodstuffs in poultry farms in the states of

TABLE 1 Epidemiological and microbiological characteristics of CTX-M-2-producing Salmonella enterica isolates in poultry farms

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Serovar</th>
<th>Poultry</th>
<th>Specimen*</th>
<th>Yr/state†</th>
<th>PFGE pulotype‡</th>
<th>MIC (µg/ml)§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Amp</td>
</tr>
<tr>
<td>S721A</td>
<td>Schwarzengrund</td>
<td>Turkey</td>
<td>Drag swab</td>
<td>2008/SC</td>
<td>A</td>
<td>&gt;256 32</td>
</tr>
<tr>
<td>S721B</td>
<td>Schwarzengrund</td>
<td>Turkey</td>
<td>Drag swab</td>
<td>2008/SC</td>
<td>A</td>
<td>&gt;256 64</td>
</tr>
<tr>
<td>S778</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A</td>
<td>&gt;256 16</td>
</tr>
<tr>
<td>S783</td>
<td>Untyped</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A</td>
<td>&gt;256 32</td>
</tr>
<tr>
<td>S779</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A1</td>
<td>&gt;256 16</td>
</tr>
<tr>
<td>S776</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A2</td>
<td>&gt;256 16</td>
</tr>
<tr>
<td>S780a</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A2</td>
<td>&gt;256 16</td>
</tr>
<tr>
<td>S782</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A2</td>
<td>&gt;256 32</td>
</tr>
<tr>
<td>S784a</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A2</td>
<td>&gt;256 32</td>
</tr>
<tr>
<td>S785c</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/PR</td>
<td>A2</td>
<td>&gt;256 32</td>
</tr>
<tr>
<td>S791</td>
<td>Schwarzengrund</td>
<td>Chicken</td>
<td>Drag swab</td>
<td>2008/SC</td>
<td>A3</td>
<td>&gt;256 32</td>
</tr>
<tr>
<td>S769c</td>
<td>Agona</td>
<td>Turkey</td>
<td>Carnass</td>
<td>2008/SC</td>
<td>B</td>
<td>&gt;256 16</td>
</tr>
<tr>
<td>S770c</td>
<td>Agona</td>
<td>Chicken</td>
<td>Eggs</td>
<td>2008/SC</td>
<td>B1</td>
<td>&gt;256 24</td>
</tr>
</tbody>
</table>

*Dr g swabs were from the rearing facility. 
†PR, Paraná State; SC, Santa Catarina State. 
‡PFGE patterns were analyzed using the Dice similarity coefficient and the unweighted-pair group method using the average linkage cluster method (BioNumerics software; Applied Maths, Kortrijk, Belgium). PFGE clusters A and B were assigned based on a <90% similarity of banding patterns. For each PFGE profile, banding patterns with >90% similarity were assigned a numeric subprofile designation. 
§MICs were determined by the Etest and/or agar dilution method (15, 16). Amp, ampicillin; Caz, ceftazidime; Cro, ceftriaxone; Ctx, cefotaxime; Cft, ceftiofur; Cpm, cefepime; Nal, nalidixic acid; Cip, ciprofloxacin; Enol, enrofloxacin; Tet, tetracycline; Str, streptomycin; Sut, sulfamethoxazole-trimethoprim. ESBL-positive Salmonella isolates were found to be sensitive to kanamycin, gentamicin, amikacin, chloramphenicol, amoxicillin-clavulanic acid, cefotixin, imipenem, and meropenem, as determined by Kirby-Bauer susceptibility testing (15). 
Transformants were obtained with 38.5% of the ESBL-producing strains. The cefotaxime MICs for all transformed E. coli TOP10 strains exhibited a 5-fold increase. 
Flagellar antigens were absent or could not be typed.

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Santa Catarina and Paraná in southern Brazil (Table 1). The blaCTX-M-2 genotype was associated with the presence of an IncP plasmid of approximately 40 kb, which was successfully transformed into the recipient TOP10 E. coli strain (7).

CTX-M β-lactamases have been widely distributed in South America at least since 1989 and possibly before appearing in Europe (8). Enteropathogens such as Vibrio cholerae and Salmonella spp. were among the first microorganisms found to carry the blaCTX-M-2 gene (9, 10). In Brazil, there has been a paucity of studies on the molecular identification of ESBL-encoding genes in Salmonella spp.

Previous studies have shown the production of CTX-M-8 and CTX-M-9 by Enteropathogens such as Vibrio cholerae and Salmonella enterica (11), whereas the production of CTX-M-2 has been documented, so far, in Salmonella enterica serotypes of animal origin. Since food-producing animals and their immediate environment can become an important reservoir and potential vehicle for ESBL-producing isolates from a tertiary care hospital: first report of blaSHV-12, blaSHV-11, blaSHV-38, and blaCTX-M-15 in Brazil. Microb. Drug Resist. 17:7–16.


