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

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The 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 Kauffmann-White scheme for *Salmonella* serotyping. ESBL phenotypes were detected using the double-disc synergy test (DDST) and cefotaxime-cefotaxime-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

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

Isolate	Serovar	Poultry	Specimen[a]	Yr/state[b]	PFGE pulsotype[c]	MIC (μg/ml)[d]											
						Amp	Caz	Cro	Ctx	Cft	Cpm	Nal	Cip	Eno	Tet	Str	Sut
S721A	Schwarzengrund	Turkey	Drag swab	2008/SC	A	>256	32	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S721B	Schwarzengrund	Turkey	Drag swab	2008/SC	A	>256	64	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S778	Schwarzengrund	Chicken	Drag swab	2008/PR	A	>256	16	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S783	Untyped[f]	Chicken	Drag swab	2008/PR	A	>256	32	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S779	Schwarzengrund	Chicken	Drag swab	2008/PR	A1	>256	16	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S776	Schwarzengrund	Chicken	Drag swab	2008/PR	A2	>256	16	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S780[e]	Schwarzengrund	Chicken	Drag swab	2008/PR	A2	>256	16	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S782	Schwarzengrund	Chicken	Drag swab	2008/PR	A2	>256	32	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S784[e]	Schwarzengrund	Chicken	Drag swab	2008/PR	A2	>256	32	>256	>256	>256	>512	>512	4	4	>128	>256	0.125
S785[e]	Schwarzengrund	Chicken	Drag swab	2008/PR	A2	>256	32	>256	>256	>256	>512	>512	4	4	>128	128	0.125
S791	Schwarzengrund	Chicken	Carcass	2008/SC	A3	>256	32	>256	>256	>256	>512	>512	4	2	>128	>256	0.125
S769[e]	Agona	Turkey	Carcass	2008/SC	B	>256	16	>256	>256	>256	>512	>512	8	16	>128	>256	>32
S770[e]	Agona	Chicken	Eggs	2008/SC	B1	>256	24	>256	>256	>256	128	>512	16	16	>64	>256	>32

[a] Drag swabs were from the rearing facility.
[b] PR, Paraná State; SC, Santa Catarina State.
[c] 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.
[d] 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; Eno, 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, cefoxitin, imipenem, and meropenem, as determined by Kirby-Bauer susceptibility testing (15).
[e] 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.
[f] Flagellar antigens were absent or could not be typed.

Santa Catarina and Paraná in southern Brazil (Table 1). The $bla_{CTX\text{-}M\text{-}2}$ 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 $bla_{CTX\text{-}M\text{-}2}$ 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 *S. enterica* (11), whereas the production of CTX-M-2 has been documented, so far, in *Salmonella enterica* serovar Typhimurium isolated from pediatric patients and poultry (12). Our results confirm the spread of the $bla_{CTX\text{-}M\text{-}2}$ gene in *S. enterica* isolates in poultry farms and demonstrate that emerging clinically relevant *Salmonella* serotypes (13, 14) have also acquired this plasmid gene. In fact, to the best of our knowledge, this is the first report of CTX-M-2-producing *S.* Schwarzengrund and Agona serotypes of animal origin.

Since food-producing animals and their immediate environment can become an important reservoir and potential vehicle for ESBL-producing *Salmonella enterica*, further studies are needed to clarify the reason why clinically relevant *Salmonella* serotypes producing ESBLs are emerging in poultry farms in Brazil.

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REFERENCES

1. **Sánchez-Vargas FM, Abu-El-Haija MA, Gómez-Duarte OG.** 2011. *Salmonella* infections: an update on epidemiology, management, and prevention. Travel Med. Infect. Dis. **9**:263–277.
2. **Tollentino FM, Polotto M, Nogueira ML, Lincopan N, Neves P, Mamizuka EM, Remeli GA, De Almeida MT, Rúbio FG, Nogueira MC.** 2011. High prevalence of $bla_{CTX\text{-}M}$ extended spectrum beta-lactamase genes in *Klebsiella pneumoniae* isolates from a tertiary care hospital: first report of $bla_{SHV\text{-}12}$, $bla_{SHV\text{-}31}$, $bla_{SHV\text{-}38}$, and $bla_{CTX\text{-}M\text{-}15}$ in Brazil. Microb. Drug Resist. **17**:7–16.
3. **Birboim HC, Doly J.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Cergole-Novella MC, Guth BE, Castanheira M, Carmo MS, Pignatari AC.** 2010. First description of $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$-producing *Escherichia coli* isolates. Microb. Drug Resist. **16**:177–184.
5. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005. Identification of plasmid by PCR-based replicon typing. J. Microbiol. Methods **63**:219–228.
6. **Sambrook J, Russell DW.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
7. **Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A.** 2006. Replicon typing of plasmids carrying CTX-M or CMY β-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. Antimicrob. Agents Chemother. **50**:3203–3206.
8. **Radice M, Power P, Di Conza J, Gutkind G.** 2002. Early dissemination of CTX-M-derived enzymes in South America. Antimicrob. Agents Chemother. **46**:602–604.
9. **Rossi A, Galas M, Binztein N, Rivas M, Caffer MI, Corso A, Radice M, Gutkind G.** 1993. Unusual multiresistant *Vibrio cholerae* O1 El Tor in Argentina. Lancet **342**:1172–1173.
10. **Rossi A, Lopardo H, Woloj M, Picandet AM, Mariño M, Galds M, Radice M, Gutkind G.** 1995. Non-typhoid *Salmonella* spp. resistant to cefotaxime. J. Antimicrob. Chemother. **36**:697–702.
11. **Peirano G, Agersø Y, Aarestrup FM, dos Reis EM, dos Prazeres Rodrigues D.** 2006. Occurrence of integrons and antimicrobial resistance genes among *Salmonella enterica* from Brazil. J. Antimicrob. Chemother. **58**:305–309.
12. **Fernandes SA, Paterson DL, Ghilardi-Rodrigues AC, Adams-Haduch JM, Tavechio AT, Doi Y.** 2009. CTX-M-2-producing *Salmonella Typhymurium* isolated from pediatric patients and poultry in Brazil. Microb. Drug Resist. **15**:317–321.
13. **Aarestrup FM, Hendriksen RS, Lockett J, Gay K, Teates K, McDermott PF, White DG, Hasman H, Sørensen G, Bangtrakulnonth A, Pornreongwong S, Pulsrikarn C, Angulo FJ, Gerner-Smidt P.** 2007. International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. Emerg. Infect. Dis. **13**:726–731.
14. **Antunes P, Mourão J, Pestana N, Peixe L.** 2011. Leakage of emerging clinically relevant multidrug-resistant *Salmonella* clones from pig farms. J. Antimicrob. Chemother. **66**:2028–2032.
15. **Clinical and Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing, twenty-first susceptibility informational supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
16. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, approved standard, 3rd ed. CLSI document M31-A3. Clinical and Laboratory Standards Institute, Wayne, PA.