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# Detection of *Salmonella enterica* Isolates Producing CTX-M Cephalosporinase in U.S. Livestock Populations

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**We report the detection of *Salmonella* carrying $bla_{CTX-M}$ in U.S. livestock populations. We identified 12 of 2,034 (0.6%) *Salmonella* isolates originating from turkeys, horses, and pigs from at least 6 U.S. states, all carrying $bla_{CTX-M-1}$, many on a pandemic sequence type 1 IncN plasmid.**

We have previously reported commensal *Escherichia coli* isolated from U.S. livestock harboring $bla_{CTX-M}$ on conjugative plasmids (15, 29) and subsequently hypothesized that *Salmonella* spp. bearing $bla_{CTX-M}$ are present but unrecognized in U.S. livestock populations, posing a potential public health risk. Thus, our objective was to identify and characterize *Salmonella* carrying $bla_{CTX-M}$ among veterinary diagnostic submissions to the USDA APHIS National Veterinary Services Laboratories (NVSL).

To accomplish this, we screened 2,034 clinical *Salmonella* isolates submitted for serotyping to the NVSL between October 2010 and June 2011 (Table 1). *Salmonella* isolates that had been previously serotyped were tested in June 2011 by streaking to Mueller-Hinton agar containing 8 μg/ml cefepime. We identified a total of 12 (0.6%) *Salmonella* isolates carrying $bla_{CTX-M}$ on transferable plasmids (Table 2). We did not detect other classes of β-lactamase resistance genes, including CMY, TEM, SHV, and OXA (11, 14, 22), by PCR. MICs for these *Salmonella* isolates generally displayed the expected phenotype (Fig. 1). Southern blot hybridization (10, 13, 20) using a $bla_{CTX-M}$ probe indicated the localization of the gene on the plasmids (Fig. 2).

We found that 6 of 88 (6.8%) turkey isolates carried $bla_{CTX-M}$. Among these, one *Salmonella enterica* serovar Bredeney isolate was received December 2010 and carried $bla_{CTX-M-1}$ on an IncN

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**TABLE 1** *Salmonella* clinical isolates screened[a]

| *S. enterica* serovar | No. of represented states | No. of isolates screened by species | | | | | | Total no. of isolates |
|---|---|---|---|---|---|---|---|---|
| | | Cattle | Chickens | Horses | Swine | Turkeys | Other[b] | |
| Typhimurium var. 5− | 31 | 26 | 5 | 7 | 215 | 1 | 17 | 271 |
| Agona | 16 | 25 | 1 | 4 | 111 | 6 | 2 | 149 |
| Dublin | 21 | 116 | 0 | 0 | 2 | 0 | 3 | 121 |
| Typhimurium | 24 | 30 | 4 | 9 | 54 | 0 | 15 | 112 |
| Cerro | 16 | 100 | 1 | 1 | 5 | 0 | 0 | 107 |
| Derby | 16 | 1 | 0 | 0 | 98 | 1 | 3 | 103 |
| Montevideo | 20 | 61 | 0 | 3 | 6 | 4 | 6 | 80 |
| Infantis | 15 | 4 | 1 | 13 | 55 | 0 | 2 | 75 |
| Heidelberg | 16 | 8 | 1 | 1 | 53 | 10 | 1 | 74 |
| Newport | 21 | 31 | 0 | 17 | 4 | 0 | 18 | 70 |
| Anatum | 13 | 14 | 0 | 11 | 33 | 0 | 5 | 63 |
| Senftenberg | 13 | 1 | 2 | 2 | 34 | 15 | 8 | 62 |
| Enteritidis | 13 | 3 | 42 | 2 | 1 | 0 | 8 | 56 |
| Kentucky | 21 | 30 | 9 | 2 | 3 | 0 | 2 | 46 |
| Mbandaka | 13 | 17 | 2 | 1 | 12 | 1 | 4 | 37 |
| Worthington | 16 | 0 | 1 | 1 | 30 | 0 | 0 | 32 |
| Ouakam | 7 | 0 | 1 | 0 | 8 | 16 | 1 | 26 |
| Bredeney | 5 | 4 | 0 | 2 | 7 | 3 | 0 | 16 |
| Rough O:d:e,n,z15 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| All other serotypes[c] | 38 | 110 | 13 | 68 | 208 | 31 | 103 | 533 |
| Total | 41 | 581 | 83 | 144 | 940 | 88 | 198 | 2,034 |

[a] Summary of results for 2,034 *Salmonella* clinical isolates originally submitted to the NVSL for serotyping between October 2010 and June 2011 that were screened for the $bla_{CTX-M}$ phenotype using selective media.
[b] Includes isolates submitted from wild animals, zoo animals, cats, dogs, goats, and sheep and isolates of unknown origin.
[c] Includes 134 additional serotypes that were present in this isolate set.

**TABLE 2** *Salmonella* isolates carrying $bla_{CTX-M}$[a]

| Isolate no. | Isolate ID | Serotype | Serogroup | Source | State | Date received (day/mo/yr) | PRT[b] |
|---|---|---|---|---|---|---|---|
| 1 | 11-13049 | Anatum | E | Equine | TX | 11/26/2010 | N |
| 2 | 11-13094 | Rough O:d:e,n,z15 | E | Swine | MN | 11/26/2010 | N, I1 |
| 3 | 11-13665 | Bredeney | B | Turkey | UNK[c] | 12/16/2010 | N |
| 4 | 11-13933 | Anatum | E | Equine | TX | 12/21/2010 | I1 |
| 5 | 11-104 | Ouakam | D | Turkey | AR | 1/6/2011 | N |
| 6 | 11-2362 | Ouakam | D | Turkey | MO | 3/7/2011 | N |
| 7 | 11-2945 | Anatum | E | Equine | TX | 3/25/2011 | na[d] |
| 8 | 11-2946 | Anatum | E | Equine | TX | 3/25/2011 | I1, HI1 |
| 9 | 11-3665 | Ouakam | D | Turkey | IN | 4/12/2011 | N |
| 10 | 11-4809 | Anatum | E | Equine | TX | 4/29/2011 | I1 |
| 11 | 11-4872 | Ouakam | D | Turkey | MO | 5/3/2011 | N |
| 12 | 11-6604 | Ouakam | D | Turkey | AR | 5/31/2011 | N |
| 13 | 11-9696[e] | Ouakam | D | Turkey | NC | 8/15/2011 | N |

[a] Summary of results for *Salmonella* isolates carrying $bla_{CTX-M}$ identified from clinical isolates originally submitted to the NVSL for serotyping between October 2010 and June 2011.
[b] PRT, plasmid replicon type. All IncN plasmids were identified as ST1 on pMLST. None of the IncI1 plasmids matched a reported ST.
[c] Isolate 11-13665 state of origin unknown because the submission form was incomplete.
[d] Isolate 11-2945 plasmid replicon type could not be identified using our typing procedure.
[e] Isolate 11-9696 was not part of the original isolate set used for this study but was identified upon targeted screening of *S.* Ouakam isolates following completion of the original screening of 2,034 isolates.

plasmid. The remaining five turkey isolates were all *Salmonella enterica* serovar Ouakam (Fig. 3) and were received between March and May 2011, originating from three U.S. states (Arkansas, Missouri, Indiana) that also carried $bla_{CTX-M-1}$ on IncN plasmids. We screened an additional 48 *S.* Ouakam isolates submitted between January 2009 and October 2010 in an attempt to determine if earlier *S.* Ouakam isolates contained this gene, although none were identified. However, one additional *S.* Ouakam isolate, received in August 2011 from a turkey clinical diagnostic submission originating from North Carolina, carried $bla_{CTX-M-1}$.

Turkey production in the United States has consolidated such that there are few large centralized hatcheries that supply day-old turkey poults to grower-finisher operations throughout the United States. Ceftiofur is approved for the control of mortality in day-old turkey poults, and turkey grower-finisher operations can order poults from hatcheries that have been treated with ceftiofur prior to shipment. Turkey poults are maintained in high-population-density environments conducive to the exchange of enteric flora throughout the production system. The mass application of ceftiofur to large populations of day-old poults at the hatchery prior to shipment could provide the selection pressure required to support the emergence, dissemination, and maintenance of a ceftiofur-resistant strain of *Salmonella* at a central hatchery. The shipment of poults from the hatchery to grower-finisher operations in multiple states could then disseminate the resistant strain over a wide geographic area.

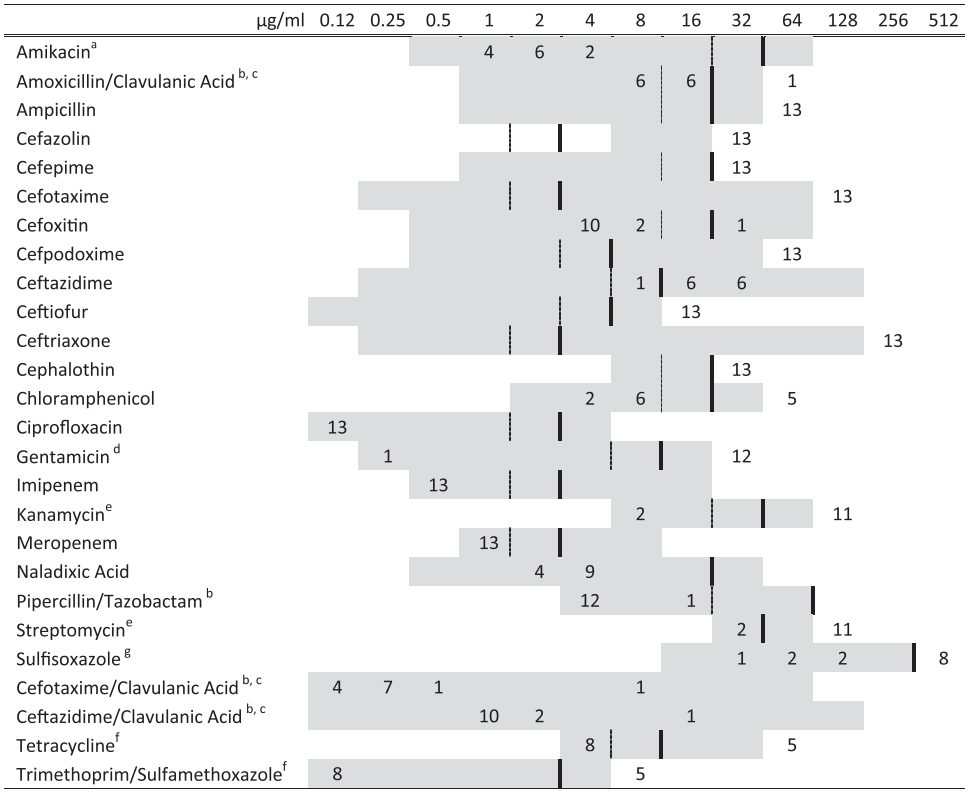


**FIG 1** MICs of 26 antimicrobial drugs for 13 *Salmonella* clinical isolates containing the $bla_{CTX-M}$ genetic element (numbers of isolates are shown in the body of the figure). Broken lines represent susceptible breakpoints, and solid lines represent resistant breakpoints when available. Corresponding to the concentration listed at the top of each column (μg/ml), the included range of each antimicrobial is shown in gray. a, amikacin MICs were not determined for isolate 11-9696. b, clavulanic acid and tazobactam were included at fixed concentrations of 4 μg/ml. c, isolate 11-13665 (*S.* Bredeney) was resistant to cefoxitin and the β-lactamase inhibitors. d, isolate 11-13094 (*Salmonella* with rough serotype O:d:e,n,z15) was susceptible to gentamicin. e, isolates 11-13094 (*Salmonella* with rough serotype O:d:e,n,z15) and 11-104 (*S.* Ouakam) were susceptible to kanamycin and streptomycin. f, the 5 *S.* Anatum isolates were resistant to chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. g, the 5 isolates susceptible to sulfisoxazole were the *S.* Ouakam isolates except 11-9696.

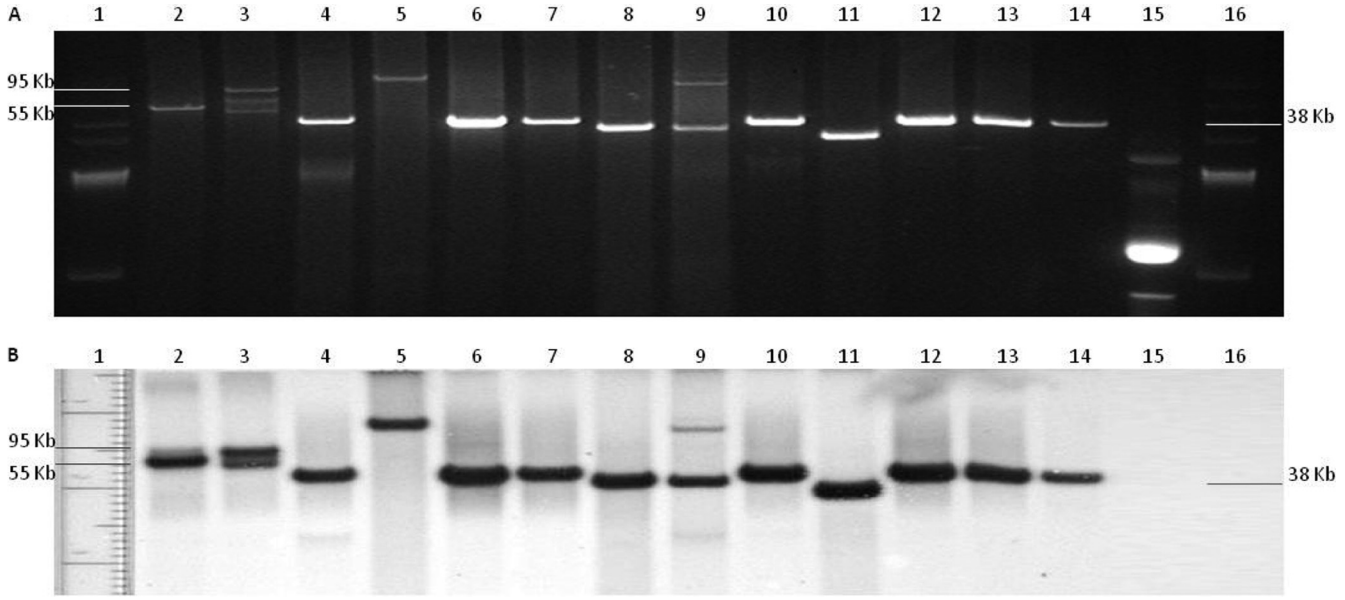


**FIG 2** (A) Plasmid content of 13 *Salmonella* clinical isolates containing the $bla_{CTX-M}$ genetic element originally submitted to the NVSL for serotyping. Lanes: 1, BAC-Tracker supercoiled DNA ladder; 2, 11-13049; 3, 11-13094; 4, 11-13665; 5, 11-13933; 6, 11-104; 7, 11-2362; 8, 11-2945; 9, 11-2946; 10, 11-3665; 11, 11-4809; 12, 11-4872; 13, 11-6604; 14, 11-9696; 15, control plasmid DNA containing $bla_{CMY-2}$; 16, BAC-Tracker supercoiled DNA ladder. (B) Southern blot hybridization using CTX-M probe of plasmid content of 13 *Salmonella* clinical isolates containing the $bla_{CTX-M}$ genetic element originally submitted to the NVSL for serotyping. Lanes: 1, BAC-Tracker supercoiled DNA ladder; 2, 11-13049; 3, 11-13094; 4, 11-13665; 5, 11-13933; 6, 11-104; 7, 11-2362; 8, 11-2945; 9, 11-2946; 10, 11-3665; 11, 11-4809; 12, 11-4872; 13, 11-6604; 14, 11-9696; 15, control plasmid DNA containing $bla_{CMY-2}$; 16, BAC-Tracker supercoiled DNA ladder.

Pulsed-field gel electrophoresis (PFGE) analysis of the *S.* Ouakam isolates (Fig. 3) with XbaI (18) finds that they are similar but do not represent a single epidemic clone. Resistant *Salmonella* that disseminated clonally at the serotype level, but not the pulsotype level, have occurred previously, including *S.* Newport carrying $bla_{CMY-2}$ on an IncA/C plasmid (6, 17) and *Salmonella* serovar Typhimurium DT104 expressing the characteristic ACSSuT resistance phenotype (resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) (1, 5).

Of the 940 swine submissions, a single isolate (0.1%) was identified to be a *Salmonella* isolate, with a rough O:d:enz15 serotype, carrying $bla_{CTX-M-1}$. As with turkey poults, ceftiofur is also commonly applied to large groups of piglets at weaning when they are processed into nurseries where they are maintained in a population-dense environment conducive to the spread of enteric pathogens. This mass application, typically with the extended-activity formulation of ceftiofur, may provide the selection pressure required for the emergence of a ceftiofur-resistant *Salmonella* strain in a population of pigs.

We found 5 of 143 (3.5%) equine isolates, all originating from Texas, were *Salmonella enterica* serovar Anatum (Fig. 3) carrying $bla_{CTX-M-1}$ on multiple plasmid replicon types (Table

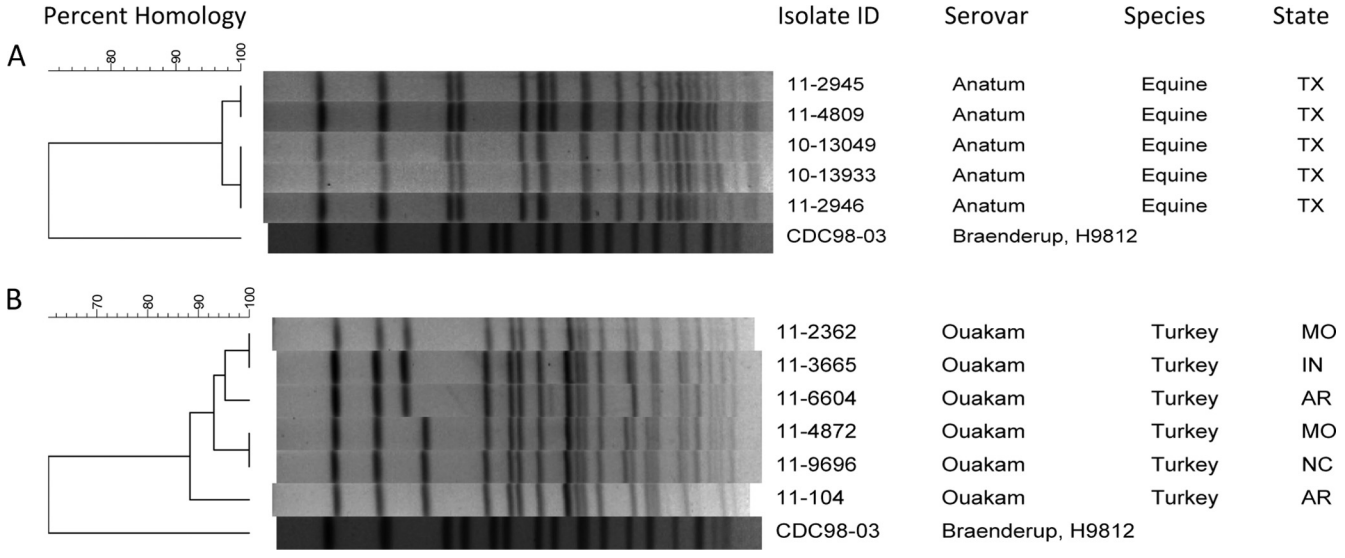


**FIG 3** (A) Dendrographic analysis of XbaI PFGE data for five *Salmonella* serovar Anatum clinical isolates containing the $bla_{CTX-M}$ genetic element. (B) Dendrographic analysis of XbaI PFGE data for six *Salmonella* serovar Ouakam clinical isolates containing the $bla_{CTX-M}$ genetic element.

**TABLE 3** pMLST allele variants of IncI1 plasmids from *Salmonella* spp. harboring $bla_{CTX\text{-}M\text{-}1}$

| Isolate ID | Allele variant | | | | |
|---|---|---|---|---|---|
| | *rep1* | *ardA* | *trbA-pndC* | *sogS* | *pilL* |
| 11-13094 | 1 | 4 | 3 | 6 | 3 |
| 11-13933 | 1 | 4 | UN[a] | 9 | 3 |
| 11-2946 | 1 | 4 | UN | 9 | 3 |
| 11-4809 | 1 | 2 | UN | 9 | 3 |

[a] UN, the allele variant has not been characterized in the pMLST database.

2). The PFGE similarity (>97%) of these isolates received over a relatively short time period suggests a common source exposure, as might occur at an equine event or facility. Ceftiofur is approved for the treatment of lower respiratory tract infections in horses, but the extended-activity formulation is applied at some breeding facilities to populations of mares as a postbreeding intrauterine infusion to improve conception rates (12). This application of ceftiofur therapy to mares while transiently housed in a population-dense environment may provide the population-level selection pressure required to support the emergence and spread of a ceftiofur-resistant strain of *Salmonella*.

None of the 581 cattle, 83 chicken, or 198 other source isolates were found to carry $bla_{CTX\text{-}M}$. This result suggests that if *Salmonella* isolates carrying $bla_{CTX\text{-}M}$ are present in cattle or chicken populations in the United States, either they do not produce clinical disease sufficient to initiate a diagnostic investigation, including microbiological culture and serotyping at the NVSL, or their frequency in these populations is below the detection limits of our study.

Using plasmid multilocus sequence typing (MLST) (3, 4), we identified three unique I1 plasmid sequence types (ST) (Table 3). We also identified ST 1 IncN plasmids bearing $bla_{CTX\text{-}M\text{-}1}$ in multiple *Salmonella* strains representing 4 serotypes from 3 animal species from diverse geographic origins. Sequence type 1 IncN plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ have been previously reported to be epidemic in humans, livestock, and food in Europe (16). The pandemic dissemination of multiresistant organisms, including *S.* Typhimurium DT104 (5, 28) and *E. coli* B2-O25:H4-ST131 (9, 26), has been previously reported. However, pandemic plasmid dissemination independent of clonal spread of organisms has not been reported.

*Salmonella* bearing $bla_{CTX\text{-}M}$ have not been previously reported in livestock in the United States, although they have been recovered from multiple livestock species in Europe and Asia (2, 19, 27). In addition, *Salmonella* bearing $bla_{CTX\text{-}M}$ have been recovered sporadically from human cases of salmonellosis in the United States (23–25). The presence of these resistant isolates in U.S. livestock populations is important because most human salmonellosis cases in the United States result from food-borne zoonotic transmission in animal products (21). Empirical therapy of resistant infections by physicians without the benefit of microbiological culture and susceptibility results may result in treatment failure and thus increased health care costs (8) and higher risk of death (7).

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