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# Letters to the Editor

## *Escherichia coli* Producing CTX-M-1, -2, and -9 Group β-Lactamases in Organic Chicken Egg Production▽

A zoonotic contribution to the spread of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* at the community level has been proposed repeatedly (3), but information on the origin and frequency of ESBLs within different animal reservoirs is still limited. To improve the current understanding of the evolution and epidemiology of ESBLs in poultry farming, we investigated the occurrence of ESBL-producing *E. coli* in flocks of chicken egg layers reared in Danish organic systems, where low flock density, restricted antimicrobial use, and access to outdoor areas are regulated at the European level (4). From June to August 2009, we visited four randomly selected healthy layer flocks (flocks A, B, C, and D), reared at independent farms with no history of antimicrobial and biocide use. *E. coli* bacteria with reduced susceptibility to cefotaxime were recovered from flocks B and C using sock samples enriched in MacConkey broth supplemented with cefotaxime (2 μg/ml) (1). PCR analysis (6, 7) followed by partial sequencing revealed the presence of $bla_{\text{TEM-1}}$ ($n = 7$), $bla_{\text{CTX-M-1}}$ group (compatible with $bla_{\text{CTX-M-1/61}}$) ($n = 9$), $bla_{\text{CTX-M-2}}$ group (compatible with $bla_{\text{CTX-M-2/20/44}}$) ($n = 3$), and $bla_{\text{CTX-M-9}}$ group (compatible with $bla_{\text{CTX-M-14/17}}$) ($n = 4$) genes (http://www.ncbi.nlm.nih.gov/ and http://www.lahey.org/Studies/) in randomly selected colonies (Table 1). We revisited farms B and C after 10 and 11 weeks, respectively, aiming to quantify the prevalence of CTX-M-positive chickens and the concentration of CTX-M-producing *E. coli* in feces. The study population remained unchanged, since no chickens had entered

TABLE 1. Genetic and phenotypic traits of CTX-M-producing *Escherichia coli* from organic layers

| Farm | Strain designation[a] | Chicken age (wk) | PFGE type[b] | β-Lactamase genes[c] | ClaI plasmid RFLP[d] | BglII plasmid RFLP[d] | Plasmid replicon type[d] | Resistance cotransferred by the plasmid[e] |
|---|---|---|---|---|---|---|---|---|
| B | **S1** | 48 | B | CTX-M-9 group (14/17) | 2 | b | N | None |
| B | **S2**[1]* | 48 | M | CTX-M-9 group (14/17) | 2 | b | N | None |
| B | S3[1] | 48 | M | CTX-M-9 group (14/17) | – | – | – | – |
| B | **S4**[2]* | 51 | M | CTX-M-9 group (14/17) | 2 | b | N | None |
| B | **S5**[2] | 51 | I | CTX-M-1 group (1/61), TEM-1 | 3 | c | I1 | STR, SUL, TET, TMP |
| B | S6[3] | 51 | I | CTX-M-1 group (1/61), TEM-1 | – | – | – | – |
| B | S7[3] | 51 | I | CTX-M-1 group (1/61), TEM-1 | – | – | – | – |
| B | S8[4] | 51 | I | CTX-M-1 group (1/61), TEM-1 | – | – | – | – |
| B | S9[4] | 51 | I | CTX-M-1 group (1/61), TEM-1 | – | – | – | – |
| B | **S10**[5] | 51 | I | CTX-M-1 group (1/61), TEM-1 | 3 | c | I1 | STR, SUL, TET, TMP |
| B | S11[5] | 51 | I | CTX-M-1 group (1/61), TEM-1 | – | – | – | – |
| B | **S12**[6] | 51 | J | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **S13**[6] | 51 | K | CTX-M-2 group (2/20/44) | – | – | – | SUL, TET, TMP |
| B | **S14**[6] | 51 | L | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **F1** | 61 | A | CTX-M-2 group (2/20/44), TEM-1 | NI | NI | Untypeable | SUL, TET, TMP |
| B | **F2** | 61 | D | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **F3** | 61 | C | CTX-M-1 group (1/61) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **F4** | 61 | E | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **F5** | 61 | N | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **F6** | 61 | O | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **F7** | 61 | NT | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| B | **CS1** | 61 | H | CTX-M-2 group (2/20/44) | – | – | – | SUL, TET, TMP |
| B | **CS2** | 61 | G | CTX-M-2 group (2/20/44) | NI | NI | Untypeable | SUL, TET, TMP |
| C | **S1**[7] | 66 | P | CTX-M-1 group (1/61) | 1 | a | I1 | SUL, TET |
| C | S2[7] | 66 | P | CTX-M-1 group (1/61) | – | – | – | – |
| C | **F1** | 77 | D | CTX-M-1 group (1/61) | 1 | a | I1 | SUL, TET |
| C | **F2** | 77 | F | CTX-M-1 group (1/61), TEM-1 | 1 | a | I1 | SUL, TET, TMP |
| C | F3 | 77 | F | CTX-M-1 group (1/61), TEM-1 | – | – | – | – |
| C | **CS1** | 77 | NT | CTX-M-1 group (1/61) | 1 | a | I1 | SUL, TET |
| C | **CS2** | 77 | F | CTX-M-1 group (1/61), TEM-1 | 1 | a | I1 | SUL, TET, TMP |

[a] Letters indicate the sample type. S, sock sample; F, fecal dropping; CS, cloacal swab. Isolates sharing the same superscript number were obtained from the same sample. *, indole-negative isolates confirmed as *E. coli* by *rpoB* sequencing. Designations in bold represent isolates used for conjugative plasmid transfer experiments.

[b] PFGE types were assigned by visual inspection of the macrorestriction profiles interpreted according to Tenover et al. (8). NT, nontypeable isolates.

[c] Beta-lactamase genes in the donors were characterized by PCR and sequencing. The specific genes compatible with the obtained sequences are reported in parentheses. Designations representing genes transferred by conjugation, as determined by PCR but not by sequencing, are underlined.

[d] Characterization was done on plasmids obtained from the transconjugants. –, not characterized; NI, not interpretable.

[e] Susceptibility to additional antimicrobials was determined for donors and transconjugants by the disk diffusion method. The antimicrobials tested included sulfonamides (SUL), streptomycin (STR), tetracycline (TET), and trimethoprim (TMP). Designations representing patterns transferred by conjugation are underlined. –, not characterized.

the farms in the interval between the two sampling points. At both farms, 2 (3%) out of 60 cloacal swabs collected from individual animals and processed as described previously (1) were positive for CTX-M-producing *E. coli*. Of 10 fresh fecal droppings collected from the floor on each farm, 7 (farm B) and 3 (farm C) samples yielded CTX-M-producing *E. coli* at concentrations ranging between $10^2$ and $10^3$ CFU per gram of feces, which accounted for $\leq$0.03% of total *E. coli*. By XbaI pulsed-field gel electrophoresis (PFGE) analysis, 30 CTX-M producers obtained from different sample types (sock samples, swabs, and fecal droppings) displayed 16 epidemiologically unrelated band patterns according to Tenover et al. (8). Two isolates were untypeable. Twenty strains representing distinct PFGE profiles and sample types carried $bla_{\text{CTX-M}}$ on transferable IncI1 ($n = 7$), IncN ($n = 3$), and untypeable ($n = 10$) plasmids, often containing additional determinants conferring resistance to tetracycline, sulfonamides, and trimethoprim, as demonstrated by conjugation and PCR-based replicon typing (PBRT) (2, 5) (Table 1). Restriction fragment length polymorphism (RFLP) analysis using ClaI and BglII showed that farm-specific IncI1 and IncN plasmids were widespread among different *E. coli* lineages in association with specific $bla_{\text{CTX-M}}$ genes (Table 1). All PBRT-untypeable plasmids, which demonstrated poor quality images by RFLP analysis, were approximately 149 kb in size.

To our knowledge, this is the first description of CTX-M-producing *E. coli* in the Danish chicken production. The factors leading to the origin and persistence of these resistance genes of high clinical relevance in organic poultry not exposed to antimicrobial agents remain unknown. Further research based on plasmid sequencing and gene characterization is needed to elucidate the nature of the nonantimicrobial resistance genes located on $bla_{\text{CTX-M}}$ gene-carrying plasmids and their possible role in favoring the spread and maintenance of such plasmids in the absence of antimicrobial selective pressure.

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