Frequent Occurrence of Extended-Spectrum Beta-Lactamase- and Transferable AmpC Beta-Lactamase-Producing *Escherichia coli* on Domestic Chicken Meat in Sweden

Stefan Börjesson,^a Maria Egervärn,^b Mats Lindblad,^b Stina Englund^a

National Veterinary Institute (SVA), Uppsala, Sweden^a; National Food Agency, Uppsala, Sweden^b

Forty-four percent of Swedish chicken meat fillets were contaminated with extended-spectrum or transferable AmpC beta-lactamase-producing *Escherichia coli* strains. Isolates from Swedish chicken meat and broilers were closely related to isolates from chicken meat imported into Sweden; these results indicate a common source of the contamination.

The increasing trend of resistance to broad-spectrum cephalosporins in *Enterobacteriaceae* in human and animal settings is of great concern, due to the fact that cephalosporins are deemed critically important in human health care (1–4). The emergence of this type of resistance is mainly linked to the spread of genes encoding extended-spectrum beta-lactamases (ESBLs) and/or transferable AmpC beta-lactamases (pAmpC) (5). Poultry, primarily broilers, have been suggested as a source for these types of genes and/or the resistant bacteria (6–8). In Sweden, broilers are rarely treated with antibiotics and no usage of cephalosporins was registered in 2010 (9). It was therefore surprising when 34% of the Swedish broilers were identified as carrying ESBL- or pAmpC-producing *E. coli* in 2010 (9).

A potential swift route for transmission of ESBL- and pAmpC-producing *Escherichia coli* from broilers to humans can be through contaminated chicken meat and meat products. As a response to the current situation in broiler production in Sweden, this study investigated whether domestically produced chicken meat contained ESBL- and/or pAmpC-producing *E. coli* and characterized potential isolates phenotypically and genotypically. In addition, the relatedness of pAmpC-producing *E. coli* isolated from Swedish chicken meat to isolates from Swedish broilers in a previous study was also determined in order to establish whether the contamination originated from broilers. This part of the study was limited to bla_{CMY-2} *E. coli* only, since earlier studies have described this gene variant to be almost universal in the Swedish broiler population (9, 10). Furthermore, these isolates were compared with isolates from imported chicken meat by using pulsed-field gel electrophoresis (PFGE), as identical PFGE patterns can indicate a potential common source.

Over a 10-week period from September to November 2010, 100 frozen chicken meat fillets intended for sale on the Swedish market were collected in collaboration with the Swedish Poultry Meat Association. The samples selected each represented a unique batch, and the number of samples was distributed between the largest processing facilities in proportion to the volume of broilers slaughtered. A 25-g portion of each sample was placed in 225 ml buffered peptone water and subjected to stomaching. One hundred milliliters of homogenate with addition of 1 mg/liter cefotaxime (Sigma-Aldrich) was incubated overnight at 37°C. After enrichment, ESBL/pAmpC-producing *E. coli* was isolated using CHROMagarESBL and CHROMagarOrientation with 1 mg/liter cefotaxime (EMM Life Science AB, Hägersten, Sweden). Identifi-

cation of *E. coli* was performed using API20E (bioMérieux Sweden, Askim, Sweden), and ESBL/pAmpC phenotypes were verified using EtestESBL and EtestAmpC (bioMérieux Sweden). One *E. coli* isolate per sample was collected.

Antimicrobial susceptibility of the isolates was assessed using VetMIC GN-mo plates, applying epidemiological cutoff values by EUCAST, and tested by EtestImipenem. ESBL/pAmpC genes were detected by PCR (11–13). The gene variants were determined by sequencing using BigDye v1.1 and the following primers: bla_{CTX-M-1}, 5'-CAGAATAAGGAATCCCATGGTT-3' and 5'-GGCGATAAACAACAAACGGAAT-3'; bla_{CMY-2}, 5'-AAATCGTTATGCT(G/C)CGCTCT-3' (14) and 5'-CATGGGATTTTCCTTGCTGT-3'; and bla_{TEM} (5'-TTCTTGAAGACGAAAGGGC-3' and 5'-ACGCTCAGTGAACGAAAC-3') (15). Isolates were genotyped by multilocus sequence typing (MLST) according to <http://mlst.ucc.ie/>.

Transferability of cephalosporin resistance from the broiler isolates was tested by conjugation to *E. coli* HMS174.

Transformation was performed on isolates unable to transfer ESBL/pAmpC phenotypes by conjugation and transconjugants PCR positive for multiple plasmid replicon types. Plasmid DNA was transformed using ElectroMax DH10B (Gibco Invitrogen). Transconjugants and transformants were subjected to PCR-based plasmid replicon typing (16) and PCRs for ESBL/pAmpC genes (11–13).

Additional isolates from previous studies on Swedish broilers (S. Börjesson, C. Jernberg, A. Brolund, P. Edquist, M. Finn, A. Landén, B. Olsson Liljequist, K. Tegmark-Wisell, B. Bengtsson, and S. Englund, submitted for publication) and imported chicken meat (17) were included in the present study based on identical plasmid replicon type and MLSTs and antibiotic resistance patterns. These isolates were compared with isolates from Swedish meat by PFGE (18) and analyzing the patterns obtained in BioNumerics v6.6 (Applied Maths, Ghent, Belgium).

In total, 44 of the 100 Swedish chicken fillets analyzed carried *E.*

Received 16 December 2012 Accepted 22 January 2013

Published ahead of print 25 January 2013

Address correspondence to Stefan Börjesson, Stefan.Borjesson@sva.se.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AEM.03893-12

TABLE 1 Results of strain and plasmid typing of *E. coli* isolates carrying *bla*_{CTX-M-1} and *bla*_{CMY-2} obtained from Swedish meat during 10 weeks from September to November 2010

ESBL/ pAmpC gene	Non-ESBL β-lactamase gene	<i>E. coli</i> MLST result	Antibiotic resistance ^a	ESBL/ pAmpC location(s)	No. of isolates
<i>bla</i> _{CMY-2}		ST10	Ci Nal	IncK	1
		ST10	Ci Nal Tc	IncK	1
		ST10	Tc	IncK	2
		ST38		IncK	10
		ST38		IncI1	1
		ST69		IncK	1
		ST69		IncI1	1
		ST69	Km Sm Su Tc	IncK	2
		ST115		IncK	1
		ST115	Su	IncK	3
		ST135		IncK	1
		ST212		IncK	1
		ST373		IncK	3
		ST648	Su Tc	IncK	1
		ST770	Ci Nal Su	IncK	1
		ST1594		IncK, IncF, IncFIB	1
		ST2184		IncK	1
		ST2207		IncK	1
		ST2370		IncI1	1
		ST2167	Su	IncK	4
<i>bla</i> _{TEM-1}		ST38	Su	IncK	1
<i>bla</i> _{TEM-1}		ST69	Km Sm Su Tc	IncK	1
<i>bla</i> _{CTX-M-1}		ST117	Su Tc	IncI1	1
		ST1640	Su Tc	IncI1	2
		ST2183	Su Tc	IncI1	1

^a Resistant to all β-lactams tested. Ci, ciprofloxacin; Nal, nalidixic acid; Km, kanamycin; Sm, streptomycin; Su, sulfamethoxazole; and Tc, tetracycline.

coli strains producing ESBL or pAmpC. Four of these isolates carried *bla*_{CTX-M-1}, 38 *bla*_{CMY-2}, and 2 *bla*_{CMY-2} plus *bla*_{TEM-1}. None of the isolates showed decreased susceptibility to imipenem.

The four *bla*_{CTX-M-1} isolates belonged to three different sequence types (STs) and were resistant to sulfamethoxazole and tetracycline (Table 1). *bla*_{CTX-M-1} was identified on plasmids belonging to replicon type incI. The *bla*_{CTX-M} incI1 plasmid also carried sulfamethoxazole and tetracycline resistance phenotypes.

Twelve of the *bla*_{CMY-2} isolates belonged to MLST ST38, and the remaining isolates were associated with 13 different STs (Table 1). Twenty-three (out of 40) *bla*_{CMY-2} isolates were sensitive to all additional antibiotics tested, and six were defined as multiresistant (i.e., resistant to more than three antimicrobial classes) (Table 1). Thirty-six of the isolates carried *bla*_{CMY-2} on an incK plasmid, and three isolates carried it on an incI plasmid. In the remaining isolate, both the corresponding transconjugant and transformant were positive for incK, IncF, and incFIB (Table 1). None of the plasmids carrying *bla*_{CMY-2} transferred any additional resistance phenotypes, and neither of the two isolates carrying *bla*_{TEM-1} transferred *bla*_{TEM-1} together with *bla*_{CMY-2}.

A total of 18 isolates from Swedish meat, four isolates from Swedish broiler cecum, four isolates from Danish meat, and one isolate from Finnish meat were compared using PFGE (Fig. 1). Isolates belonging to the same STs were found to be related or closely related (Fig. 1). Three isolates from Swedish broiler cecum, Swedish meat, and Danish meat were found to share identical PFGE patterns.

The occurrence of ESBL/pAmpC-producing *E. coli* on Swedish meat is most likely due to fecal contamination, because isolates with identical or closely related PFGE patterns were found in both broiler cecum and meat. A previous study has also shown that

FIG 1 Dendrogram showing the genotypic relatedness of *Escherichia coli* carrying *bla*_{CMY-2} on an incK plasmid isolated from Swedish broilers, Swedish meat, and meat imported from Denmark and Finland based on pulsed-field gel electrophoresis with XbaI. One isolate from Swedish meat proved to be untypeable with PFGE. For comparison, cluster analyses using the Dice coefficient and unweighted-pair group method using average linkages (UPGMA) were performed, with position tolerance and optimization both set at 1%. The scale bar represents the percentage of similarity, the vertical dotted line indicates ≥90% similarity, and the vertical intact line indicates ≥80% similarity. Resistance to antimicrobials was defined according to EUCAST epidemiological cutoffs. Km, kanamycin; Sm, streptomycin; Su, sulfamethoxazole; Tc, tetracycline.

contamination of meat by fecal bacteria is a common event during slaughter (19).

The Swedish situation is similar to that described in Denmark, where 44% of the chicken meat was reported to be contaminated (20). However, the prevalence is still lower than, for example, that in The Netherlands, where up to 94% of the meat may contain ESBL-producing *E. coli* (7, 8). The high percentage of chicken meat contaminated is worrying, because studies have suggested that isolates, the plasmid, and/or the genes can be transferred from broilers to humans via the meat (6–8, 21, 22). *In situ* experiments have demonstrated that ESBL-producing *E. coli* strains of poultry origin can establish themselves and that the genes can be transferred in the human commensal bowel microbiota even without antimicrobial pressure (23). It is therefore possible that such a high occurrence on chicken meat increases the risk of contribution to the human ESBL/pAmpC load.

The majority of the *E. coli* isolates producing ESBL or pAmpC found in the present study carried *bla*_{CMY-2} on an *incK* plasmid, reflecting the situation in Swedish broilers (Börjesson et al., submitted). The *bla*_{CMY-2} gene has generally been identified on other replicon types in humans and animals, but *bla*_{CMY-2} on *incK* is increasing in poultry, and recent studies have identified this combination in human clinical settings (1, 24–28; Börjesson et al., submitted). In addition, the combination of *incI1* and *bla*_{CTX-M-1} identified in this study has frequently been identified in both human clinical and broiler settings (29, 30).

It was found that *bla*_{CMY-2} *E. coli* isolates from chicken meat imported from Denmark and Finland were identical or closely related to isolates from Sweden (Fig. 1). It has been suggested that the occurrence of ESBL- and pAmpC-producing *E. coli* strains in Swedish broilers is due to vertical transmission from imported breeding stock (10). Therefore, the likely connection between the Swedish, Danish, and Finnish isolates is via vertical transmission through the broiler production pyramid. Swedish and Danish broilers share the same parent birds, as Swedish parent birds are exported to Denmark (31). In addition, Sweden and Finland import grandparents from the same companies (Thomas Carlson, Aviagen-Swechick, personal communication, 2012). A large proportion of the Swedish meat isolates tested were also resistant to ciprofloxacin, nalidixic acid, sulfamethoxazole, and tetracycline, the same resistance pattern seen in isolates from broiler cecum. This is noteworthy because these types of antibiotics are not used in Swedish production (9, 32). This may indicate that these resistant *E. coli* isolates were not selected through antibiotic pressure, but introduced into the flocks.

A large proportion of the samples from Swedish chicken meat were contaminated with ESBL- or pAmpC-producing *E. coli*, primarily carrying the *bla*_{CMY-2} gene. A selection of isolates from meat were identical or closely related to those identified in slaughtered Swedish broilers, indicating that the occurrence on meat is due to fecal contamination at slaughter. The results offer support for the theory that occurrence of ESBL- and pAmpC-producing *E. coli* in the Swedish broiler population is due to introduction and spread from imported breeding stock.

ACKNOWLEDGMENT

This work was supported by The Swedish Civil Contingencies Agency.

REFERENCES

- Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D. 2010. Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. *Vet. Microbiol.* 145:273–278.
- Pitout JD, Laupland KB. 2008. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. *Lancet Infect. Dis.* 8:159–166.
- WHO. 2007. The World Health Report 2007. A safe future: global public health security in the 21st century. World Health Organization, Geneva, Switzerland.
- Wieler LH, Ewers C, Guenther S, Walther B, Lubke-Becker A. 2011. Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing *Enterobacteriaceae* in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. *Int. J. Med. Microbiol.* 301:635–641.
- Su LH, Chu C, Cloeckaert A, Chiu CH. 2008. An epidemic of plasmids? Dissemination of extended-spectrum cephalosporinases among *Salmonella* and other *Enterobacteriaceae*. *FEMS Immunol. Med. Microbiol.* 52: 155–168.
- European Food Safety Authority. 2011. Scientific opinion on the public health risks of bacterial strains producing extended-spectrum β -lactamases and/or AmpC β -lactamases in food and food-producing animals. *EFSA J.* 9:2322. doi:10.2903/j.efsa.2011.2322.
- Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin. Microbiol. Infect.* 17:873–880.
- Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X, Kluytmans J. 2011. Extended-spectrum beta-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. *Emerg. Infect. Dis.* 17:1216–1222.
- Bengtsson B, Börjesson S, Englund S, Ericsson-Unnerstad H, Greko C, Grönlund-Andersson U, Landén A, Pringle M. 2012. Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) 2011. National Veterinary Institute (SVA), Uppsala, Sweden.
- Bengtsson B, Ericsson-Unnerstad H, Greko C, Grönlund-Andersson U, Landén A. 2011. Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) 2010. National Veterinary Institute (SVA), Uppsala, Sweden.
- Fang H, Ataker F, Hedin G, Dornbusch K. 2008. Molecular epidemiology of extended-spectrum beta-lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *J. Clin. Microbiol.* 46:707–712.
- Perez-Perez FJ, Hanson ND. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* 40:2153–2162.
- Woodford N, Fagan EJ, Ellington MJ. 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. *J. Antimicrob. Chemother.* 57:154–155.
- Sundsfjord A, Simonsen GS, Haldorsen BC, Haaheim H, Hjelmvoll SO, Littauer P, Dahl KH. 2004. Genetic methods for detection of antimicrobial resistance. *APMIS* 112:815–837.
- Costa D, Poeta P, Saenz Y, Vinue L, Rojo-Bezares B, Jouini A, Zarazaga M, Rodrigues J, Torres C. 2006. Detection of *Escherichia coli* harbouring extended-spectrum beta-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild animals in Portugal. *J. Antimicrob. Chemother.* 58:1311–1312.
- Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* 63:219–228.
- Egervärn M, Englund S, Börjesson S, Lindblad M. 2011. Occurrence of ESBL-producing *E. coli* and *Salmonella* in meat obtained from the Swedish market. National Food Agency and National Veterinary Institute (SVA), Uppsala, Sweden. (In Swedish.)
- Maslow JN, Mulligan ME, and Arbeit RD. 1993. Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. *Clin. Infect. Dis.* 17:153–164.
- Herman L, Heyndrickx M, Grijspeerd K, Vandekerchove D, Rollier I,

- De Zutter L. 2003. Routes for *Campylobacter* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiol. Infect.* 131:1169–1180.
20. Agerso Y, Hald T, Helwich B, Brock Hog B, Jensen LB, Frokjaer Jensen V, Korsgaard H, Larsen LS, Seyfarth AM, Struve T. 2012. DANMAP 2011—use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Technical University of Denmark (Statens Serum Institut), Lyngby, Denmark.
 21. Doi Y, Paterson DL, Egea P, Pascual A, Lopez-Cerero L, Navarro MD, Adams-Haduch JM, Qureshi ZA, Sidjabat HE, Rodriguez-Bano J. 2010. Extended-spectrum and CMY-type beta-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, U. S. A. and Seville, Spain. *Clin. Microbiol. Infect.* 16:33–38.
 22. Dutil L, Irwin R, Finley R, Ng LK, Avery B, Boerlin P, Bourgault AM, Cole L, Daignault D, Desruisseau A, Demczuk W, Hoang L, Horsman GB, Ismail J, Jamieson F, Maki A, Pacagnella A, Pillai DR. 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerg. Infect. Dis.* 16:48–54.
 23. Smet A, Rasschaert G, Martel A, Persoons D, Dewulf J, Butaye P, Catry B, Haesebrouck F, Herman L, Heyndrickx M. 2011. In situ ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration. *J. Appl. Microbiol.* 110:541–549.
 24. Baudry PJ, Mataseje L, Zhanel GG, Hoban DJ, Mulvey MR. 2009. Characterization of plasmids encoding CMY-2 AmpC beta-lactamases from *Escherichia coli* in Canadian intensive care units. *Diagn. Microbiol. Infect. Dis.* 65:379–383.
 25. Carattoli A, Miriagou V, Bertini A, Loli A, Colino C, Villa L, Whichard JM, Rossolini GM. 2006. Replicon typing of plasmids encoding resistance to newer beta-lactams. *Emerg. Infect. Dis.* 12:1145–1148.
 26. Dierikx C, van der Goot J, Fabri T, van Essen-Zandbergen A, Smith H, Mevius D. 2013. Extended-spectrum-beta-lactamase- and AmpC-beta-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. *J. Antimicrob. Chemother.* 68:60–67.
 27. Martin LC, Weir EK, Poppe C, Reid-Smith RJ, Boerlin P. 2012. Characterization of bla_{CMY-2} plasmids in *Salmonella* and *Escherichia coli* isolates from food animals in Canada. *Appl. Environ. Microbiol.* 78:1285–1287.
 28. Naseer U, Haldorsen B, Simonsen GS, Sundsfjord A. 2010. Sporadic occurrence of CMY-2-producing multidrug-resistant *Escherichia coli* of ST-complexes 38 and 448, and ST131 in Norway. *Clin. Microbiol. Infect.* 16:171–178.
 29. Carattoli A. 2009. Resistance plasmid families in Enterobacteriaceae. *Antimicrob. Agents Chemother.* 53:2227–2238.
 30. Naseer U, Sundsfjord A. 2011. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. *Microb. Drug Resist.* 17:83–97.
 31. Secher S. 2012. Aviagen SweChick ökar nu till över tre miljoner föräldradjur. *Fjäderfä* 8:30–35. (In Swedish.)
 32. Grave K, Greko C, Kvaale MK, Torren-Edo J, Mackay D, Muller A, Moulin G. 2012. Sales of veterinary antibacterial agents in nine European countries during 2005–2009: trends and patterns. *J. Antimicrob. Chemother.* 67:3001–3008.