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Potential Pathogenicity and Host Range of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Isolates from Healthy Poultry▽

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Thirty of 33 epidemiologically unrelated extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from healthy poultry lacked the virulence genes commonly associated with human-pathogenic strains. The main zoonotic risk is associated with the broad host range of avian *E. coli* belonging to sequence type complex 10 and of IncN and IncI1 plasmids carrying bla_{CTX-M} or bla_{SHV}.

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* is associated with a significant risk of increased mortality in human infections (1). ESBL-encoding genes, mainly belonging to the CTX-M type, have recently been reported to occur in various animal species, and the possible zoonotic risks derived from the emergence of ESBL producers in animals have been addressed by various authors (8). Further knowledge of the pathogenicity and host range of animal *E. coli* lineages carrying these antibiotic resistance determinants of high clinical relevance is needed to understand the zoonotic potential of specific animal reservoirs. In this study, the genetic background of selected ESBL-producing *E. coli* isolates from poultry was investigated by multilocus sequence typing (MLST) and plasmid typing, and their pathogenicity was inferred by PCR screening of virulence factors associated with extraintestinal pathogenic *E. coli* (ExPEC) and diarrheagenic *E. coli* (DEC).

Thirty CTX-M-producing and three SHV-producing *E. coli* strains previously isolated from the fecal flora of healthy chicken flocks in Italy in 2007 ($n = 15$) and in Denmark in 2009 ($n = 18$) and characterized in relation to their ESBL gene content, plasmid incompatibility group, and antimicrobial resistance profile were selected to represent each of the different clonal types identified by amplified fragment length polymorphism (AFLP) (5) or pulsed-field gel electrophoresis (PFGE) (6). DNA of plasmids harboring ESBL-encoding genes was extracted (18) from transformants or transconjugants that showed resistance to cefotaxime (MIC ≥ 2 μg/ml) and harbored a single plasmid as determined by plasmid profiling and was used to (i) estimate plasmid size by regression analysis of the relative electrophoretic mobility of plasmids of known size isolated from *E. coli* V517 and *E. coli* 39R and (ii) generate ClaI (FastDigest; Fermentas GmbH, St. Leon-Rot, Germany) restriction fragment length polymorphism (RFLP) patterns that were visualized on 0.8% agarose gels. The plasmids ranged in size from 19 to 149 kb (Table 1). Plasmids of the same size were generally indistinguishable by PCR-based replicon typing and RFLP (Table 1), thus showing that clonally related plasmids were disseminated among different *E. coli* types. Of note, a 43-kb IncN plasmid found in eight strains isolated from the same flock in Italy showed four distinct but closely related restriction profiles (a, a1, a2, and a3), each of which differed for the presence or absence of two bands of different sizes (data not shown). This IncN plasmid lineage was almost exclusively associated with $bla_{CTX-M-32}$, although it was also found to carry $bla_{CTX-M-1}$ in a single strain from the same flock, thus showing that the same plasmid lineage may contribute to the dissemination of different CTX-M variants. However, it is also possible that CTX-M-32 has evolved from CTX-M-1 on this IncN plasmid lineage as a result of a single amino acid substitution (Asp240Gly) or vice versa. IncI1 plasmids also harbored distinct ESBL-encoding genes, namely, bla_{SHV-12} and $bla_{CTX-M-1/61}$ (Table 1). By plasmid multilocus sequence typing (pMLST) (15), a single IncI1 plasmid was untypeable due to failure to amplify four of the five loci. This plasmid was also atypical being considerably smaller than the average IncI1 plasmids. Four of the five typeable IncI1 plasmids were classified as sequence type 3 (ST3), a variant previously described for *Salmonella enterica* strains from poultry and humans in France and in The Netherlands (10, 19) and in *E. coli* from humans in Spain and in The Netherlands (http://pubmlst.org/plasmid/) (19), while the remaining typeable IncI1 plasmid belonged to ST26, which has previously been reported to occur in The Netherlands and in the United Kingdom in *E. coli* and *S. enterica* strains of human origin (http://pubmlst.org/plasmid/). These results indicate that IncI1 ST3 and ST26 plasmids are widespread geographically and can be horizontally exchanged among different bacterial species and hosts.

MLST analysis (25) of the 33 *E. coli* strains allowed identification of 25 STs. Fourteen strains (42%) belonged to 13 novel STs (ST1626 to ST1638), 2 strains (6%) belonged to ST115, a member of the ST1006 complex, which to date has been recovered only from avian species (13, 21), and the remaining 17 strains (52%) belonged to 11 STs which have previously been described to occur in *E. coli* isolates from a broad variety of hosts worldwide. Namely, ST10, ST23, ST48, ST93, ST354,

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TABLE 1. Genetic and phenotypic traits of ESBL-producing *Escherichia coli* from healthy Italian and Danish poultry flocks

Strain	Flock	Plasmid characterization						Genetic background		
		bla gene(s)[a]	RFLP type[b]	Replicon type[b]	Plasmid ST	Plasmid size[b] (kb)	Cotransferred resistance[d]	Sequence type[e] (ST complex)	Virulence gene(s)[f]	Additional resistance[d]
52 IT	IT-4	CTX-M-32	a	N	–[c]	43	None	93 (10)	*iutA*	TET, SUL, TMP, CHL, NAL, CIP, STR
53 IT	IT-4	CTX-M-32	a	N	–	43	None	354 (354)	*iutA*	TET, SUL, TMP, CHL, NAL, CIP, STR, GEN
64 IT	IT-4	CTX-M-32	a	N	–	43	None	398 (46)	None	TET, SUL, CHL, NAL, CIP
30 IT	IT-4	CTX-M-32	a	N	–	43	None	1628 (206)	None	TET, NAL
18 IT	IT-4	CTX-M-32	a	N	–	43	None	1628 (206)	None	TET, NAL
49 IT	IT-4	CTX-M-32	a1	N	–	43	SUL	115 (1006)	*iutA*, *kii*	TET, NAL, CIP
31 IT	IT-4	CTX-M-1 (1/61)	a2	N	–	43	None	115 (1006)	*iutA*, *kii*	SUL, NAL, CIP, STR
51 IT	IT-4	CTX-M-32	a3	N	–	43	None	93 (10)	*iutA*	TET, SUL, TMP, CHL, NAL, CIP, STR
S1	DK-B	CTX-M-9 (14/17)	b	N	–	46	None	1630* (10)	None	None
S2	DK-B	CTX-M-9 group (14/17)	b	N	–	46	None	1631* (10)	None	None
S1	DK-C	CTX-M-1 (1/61)	c	I1	3	91	SUL, TET	746* (10)	None	None
CS1	DK-C	CTX-M-1 (1/61)	c	I1	3	91	SUL, TET	1637 (groups with ST1146)	None	None
CS2	DK-C	CTX-M-1 (1/61)	c	I1	3	91	SUL, TET	1638 (10)	None	TMP
35 IT	IT-6	CTX-M-1 (1/61)	d	N	–	37	None	10* (10)	None	TET
56 IT	IT-6	CTX-M-1 (1/61)	d	N	–	37	None	10* (10)	None	TET
3 IT	IT-1	SHV-12	e	FIB	–	119	SUL	1626* (10)	None	TET, TMP, FFC, CHL, NAL, CIP, STR
47 IT	IT-2	CTX-M-1 (1/61)	f	I1	26	112	SUL, TMP	1627 (groups with ST542)		TET, NAL
10 IT	IT-1	SHV-12	g	I1	Untypeable	19	None	752* (10)	*vtx2*	TET, SUL, TMP, NAL, STR
21 IT	IT-5	SHV-12	h	I1	3	104	SUL	1137* (10)	None	TET, TMP, FFC, CHL, STR
57 IT	IT-6	CTX-M-1 (1/61)	i	FIB	–	110	None	1629 (Singleton)	None	TET, SUL, CHL, NAL
S12	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	10* (10)	None	None
S14	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	10* (10)	None	None
F1	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	10* (10)	None	None
F3	DK-B	CTX-M-1 (1/61)	NI	Untypeable	–	149	SUL, TET, TMP	48* (10)	None	None
F5	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	48* (10)	None	None
F2	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	1632 (singleton)	None	None
F4	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	1633 (singleton)	None	None
F6	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	1634* (10)	None	None
F7	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	1635* (10)	None	None
CS2	DK-B	CTX-M-2 (2/20/44)	NI	Untypeable	–	149	SUL, TET, TMP	1636* (10)	None	None
S5	DK-B	CTX-M-1 (1/61)	–	–	–	–	None	23 (88)	*iutA*	STR, SUL, TET, TMP
CS1	DK-B	CTX-M-2 (2/20/44)	–	–	–	–	None	1303* (10)	None	SUL, TET, TMP
S13	DK-B	CTX-M-2 (2/20/44)	–	–	–	–	None	1564 (10)	*iutA*	SUL, TET, TMP

[a] Beta-lactamase (*bla*) genes were characterized by PCR and sequencing in the donors. The specific genes compatible with the obtained sequences are reported in parentheses. All *bla* genes were transferred by conjugation/transformation, as determined by PCR but not by sequencing.

[b] Determination was done on plasmids obtained from the transconjugants/transformants. a1, a2, and a3 had two additional bands compared to a, but of different size. –, not characterized, as conjugation and transformation experiments failed repeatedly. NI, not interpretable.

[c] –, not characterized.

[d] Susceptibility to additional antimicrobials was determined for transconjugants/transformants and donors by the disk diffusion method. The antimicrobials tested were as follows: CHL, chloramphenicol; CIP, ciprofloxacin; FFC, florfenicol; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; SUL, sulfonamides; TET, tetracycline; and TMP, trimethoprim.

[e] Sequence types marked with asterisks belong to a cluster of closely related genotypes (Fig. 1).

[f] *iutA* and *kii* are extraintestinal virulence factors. The presence of ≥2 genes indicates an ExPEC strain. *vtx2*, verocytotoxin. Its presence indicates a VTEC strain.

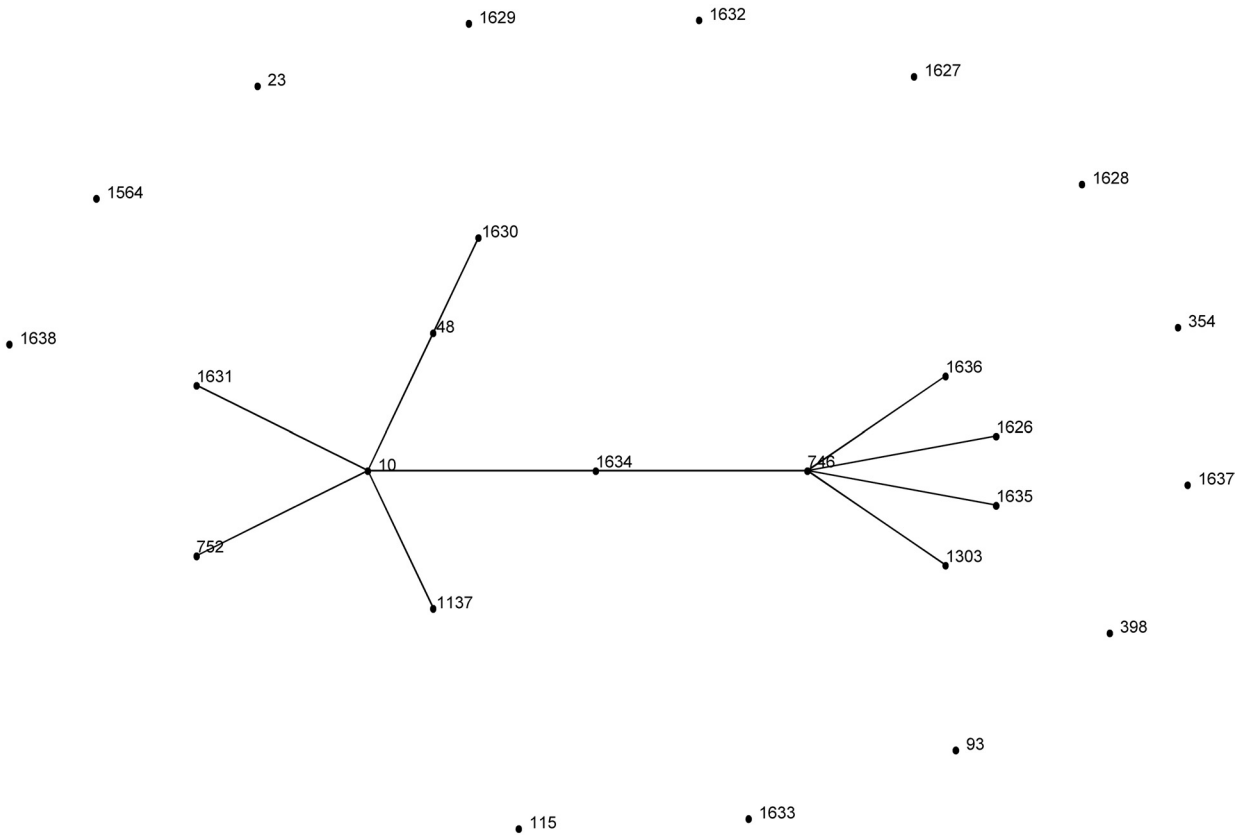

FIG. 1. Population snapshot of the *Escherichia coli* STs identified in this study. Single-locus variants are connected by a line. The linked cluster within the population snapshot represents closely related STs within the ST10 complex. ST93, ST1564, and ST1638 also belong to clonal complex 10 (Table 1) but are at least double-locus variants of other STs connected to the linked cluster but not identified in this study. This diagram does not show the genetic distance between unrelated STs.

ST398, ST746, ST752, ST1137, ST1303, and ST1564 have been described to occur among commensal and pathogenic *E. coli* isolates from humans as well as various avian and mammalian species in Europe, Africa, America, and Asia (http://mlst.ucc.ie/mlst/dbs/Ecoli) (3, 9, 12, 13, 19, 22, 23, 24). As defined by eBURST version 3 (http://eburst.mlst.net/) using the stringent group definition (6/7 shared alleles), 14 (78%) strains from Denmark and 7 (47%) strains from Italy, representing 15 STs, belonged to the ST10 complex (Table 1). Twelve of the 15 STs formed a linked cluster within the ST10 complex (Fig. 1). Of note, this ST complex was the most prevalent also in two previous studies, one examining ESBL producers isolated from hospitalized patients in Spain and one examining isolates from broilers, retail chicken meat, and patients in The Netherlands (19, 23). *E. coli* isolates belonging to the ST10 complex are not associated with a particular type of ESBL, as shown in both this study (Table 1) and the study by Oteo et al. (23). Members of this ST complex appear to have a broad host range and therefore may facilitate zoonotic transfer of ESBL-encoding plasmids from poultry to humans.

According to the scheme proposed by Johnson et al. (16), two (6%) strains were classified as ExPEC since they harbored *iutA* and *kii*. The presence of *iutA*, which is considered a genetic marker for identification of avian pathogenic *E. coli* (APEC) (17), was demonstrated for five additional strains (Table 1). A single strain was classified as a verocytotoxin-producing *E. coli* (VTEC) strain containing *vtx2* by a commercial PCR kit for detection of DEC (DEC primer mix; Statens Serum Institut, Denmark). Detection of a VTEC strain from poultry was surprising since this zoonotic pathotype is traditionally associated with cattle and other ruminants (7). However, the occurrence of VTEC in poultry has been sporadically reported in recent years (2, 11, 14). The *vtx2*-harboring strain described in the present study was assigned to ST752, which is represented in the MLST database by a clinical enteropathogenic *E. coli* (EPEC) strain isolated in Germany in 2007.

The present study represents a first effort to assess the pathogenicity and host range of avian ESBL-producing *E. coli*. Altogether, 9% of the avian ESBL-producing *E. coli* isolates displayed pathotypes of zoonotic interest, 21% were potentially pathogenic to poultry, and 44% belonged to STs previously isolated from humans. These findings suggest that a moderate proportion of avian ESBL-producing *E. coli* isolates are pathogenic to humans or poultry, but many of them, especially strains belonging to the ST10 complex, may be transmitted between the two host populations. The potential pathogenicity of ESBL producers in poultry production is particularly worrying due to the multidrug resistance phenotype of these bacteria (Table 1) and the limited therapeutic options available for treatment of chickens (20). The main zoonotic risk is likely

to derive from horizontal transfer of broad-host-range IncN or IncI1 plasmids carrying $bla_{CTX\text{-}M}$ or bla_{SHV} from avian *E. coli* to zoonotic human pathogenic *Enterobacteriaceae* such as *Salmonella*. Zoonotic transfer of these plasmids may also be facilitated by the apparent broad host range of strains belonging to sequence type complex 10, which according to the results of this study is relatively common among ESBL-producing *E. coli* isolates from the intestinal microbiota of healthy poultry.

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REFERENCES

1. **Bhavnani, S. M., P. G. Ambrose, W. A. Craig, M. N. Dudley, and R. N. Jones.** 2006. Outcomes evaluation of patients with ESBL- and non-ESBL-producing *Escherichia coli* and *Klebsiella species* as defined by CLSI reference methods: report from the SENTRY Antimicrobial Surveillance Program. Diagn. Microbiol. Infect. Dis. **54:**231–236.
2. **Bohaychuk, V. M., S. L. Checkley, G. E. Gensler, and P. R. Barrios.** 2009. Microbiological baseline study of poultry slaughtered in provincially inspected abattoirs in Alberta, Canada. Can. Vet. J. **50:**173–178.
3. **Bonnedahl, J., et al.** 2009. Dissemination of *Escherichia coli* with CTX-M type ESBL between humans and yellow-legged gulls in the south of France. PLoS One **4**(6):e5958.
4. **Bortolaia, V., M. Bisgaard, and A. M. Bojesen.** 2010. Distribution and possible transmission of ampicillin- and nalidixic acid-resistant *Escherichia coli* within the broiler industry. Vet. Microbiol. **142:**379–386.
5. **Bortolaia, V., L. Guardabassi, M. Bisgaard, J. Larsen, and A. M. Bojesen.** 2010. Escherichia coli producing CTX-M-1, -2, -9 group β-lactamases in organic chicken egg production. Antimicrob. Agents Chemother. **54:**3527–3528.
6. **Bortolaia, V., et al.** 2010. High diversity of extended-spectrum β-lactamases in *Escherichia coli* from Italian broiler flocks. Antimicrob. Agents Chemother. **54:**1623–1626.
7. **Caprioli, A., S. Morabito, H. Brugere, and E. Oswald.** 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet. Res. **36:**289–311.
8. **Carattoli, A.** 2008. Animal reservoirs for extended-spectrum beta-lactamase producers. Clin. Microbiol. Infect. **14**(Suppl. 1):117–123.
9. **Cerquetti, M., et al.** 2009. First report of plasmid-mediated quinolone resistance determinant qnrS1 in an *Escherichia coli* strain of animal origin in Italy. Antimicrob. Agents Chemother. **53:**3112–3114.
10. **Cloeckaert, A., et al.** 2010. IncI1 plasmid carrying extended-spectrum-beta-lactamase gene blaCTX-M-1 in Salmonella enterica isolates from poultry and humans in France, 2003 to 2008. Antimicrob. Agents Chemother. **54:** 4484–4486.
11. **Cobeljic, M., et al.** 2005. The prevalence of Shiga toxin-producing *Escherichia coli* in domestic animals and food in Serbia. Epidemiol. Infect. **133:** 359–366.
12. **Coque, T. M., et al.** 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg. Infect. Dis. **14:**195–200.
13. **Ewers, C., E. M. Antão, I. I. Diehl, H. C. Philipp, and L. H. Wieler.** 2009. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. Appl. Environ. Microbiol. **75:**184–192.
14. **Farooq, S., I. Hussain, M. A. Mir, M. A. Bhat, and S. A. Wani.** 2009. Isolation of atypical enteropathogenic *Escherichia coli* and Shiga toxin 1 and 2f-producing *Escherichia coli* from avian species in India. Lett. Appl. Microbiol. **48:**692–697.
15. **García-Fernández, A., et al.** 2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J. Antimicrob. Chemother. **61:**1229–1233.
16. **Johnson, J. R., A. Gajewski, A. J. Lesse, and T. A. Russo.** 2003. Extraintestinal pathogenic *Escherichia coli* as a cause of invasive nonurinary infections. J. Clin. Microbiol. **41:**5798–5802.
17. **Johnson, T. J., et al.** 2008. Comparison of extraintestinal pathogenic *Escherichia coli* strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. Appl. Environ. Microbiol. **74:** 7043–7050.
18. **Kado, C. I., and S. T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
19. **Leverstein-van Hall, M. A., et al.** 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin. Microbiol. Infect. doi:10.1111/j.1469-0691.2011.03497.x.
20. **Loehren, U., A. Ricci, and T. S. Cummings.** 2009. Guidelines for antimicrobial use in poultry, p. 126–142. *In* L. Guardabassi, L. B. Jensen, and H. Kruse (ed.), Guide to antimicrobial use in animals. Blackwell Publishing, Oxford, United Kingdom.
21. **Mora, A., et al.** 2009. Extraintestinal pathogenic *Escherichia coli* O1:K1: H7/NM from human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution. BMC Microbiol. **9:**132.
22. **Nicklasson, M., et al.** 2010. Enterotoxigenic *Escherichia coli* multilocus sequence types in Guatemala and Mexico. Emerg. Infect. Dis. **16:**143–146.
23. **Oteo, J., et al.** 2009. Extended-spectrum beta-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. Int. J. Antimicrob. Agents **34:**173–176.
24. **Valverde, A., et al.** 2009. Spread of *bla*(CTX-M-14) is driven mainly by IncK plasmids disseminated among *Escherichia coli* phylogroups A, B1, and D in Spain. Antimicrob. Agents Chemother. **53:**5204–5212.
25. **Wirth, T., et al.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. **60:**1136–1151.