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Transmission of IncN Plasmids Carrying *bla*$_{CTX-M-1}$ between Commensal *Escherichia coli* in Pigs and Farm Workers▽†

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CTX-M-1-producing *Escherichia coli* were isolated from 56 pigs, three farm personnel, two manure samples, and two air samples from two Danish pig farms where an association between prophylactic ceftiofur use and the occurrence of cephalosporin resistance was previously demonstrated. Human, animal, and environmental strains displayed high genetic diversity but harbored indistinguishable or closely related IncN plasmids carrying *bla*$_{CTX-M-1}$, indicating that IncN plasmids mediating cephalosporin resistance were transmitted between pigs and farm workers across multiple *E. coli* lineages.

Extended-spectrum cephalosporins have been classified by the World Health Organization as critically important antibiotics in human medicine (19). Ceftiofur is an extended-spectrum cephalosporin licensed for the treatment of respiratory infections in pigs and cattle. Various authors have hypothesized that the veterinary use of extended-spectrum cephalosporins may select for extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* in animals, resulting in an increased risk of the zoonotic transmission of ESBL-carrying bacteria and plasmids (2, 10, 11, 17, 18).

A statistical association between the prophylactic use of ceftiofur and the occurrence of cefotaxime-resistant *E. coli* in healthy pigs was recently demonstrated at two Danish pig farms, and most of the isolates were shown to be CTX-M-1 producers (11). In this study, we revisited the farms 1 year after the previous study to investigate the distribution, persistence, and transmission of *bla*$_{CTX-M-1}$ between pigs, farm workers, and the farm environment. Fecal samples were randomly obtained from one sow-piglet pair or weaner in every fifth pen. Limited to one farm, composite manure samples were collected from pens containing slaughter pigs. Environmental surface swabs were collected from door handles, wash areas, and food carts. Rectal swabs were obtained from four consenting farm workers and one family member. All samples were cultured on MacConkey agar (Oxoid) plates containing cefotaxime (2 μg/ml). Air samples were obtained by exposing cefotaxime plates to air for 1 h. The identification of colonies with typical *E. coli* morphology was confirmed by the citrate, indole, methyl red, and Voges-Proskauer tests. The presence of *bla*$_{CTX-M-1}$ was determined by PCR using CTX-M universal primers (9) and by DNA sequencing using CTX-M-1-specific primers (F primer, 5′-CCATGGTTAAAAAATCACTGCG-3′, and R primer, 5′-GTRAARTARGTSACCAGAAY SAGCGG-3′). The 803-bp fragment (approximately 92% of the entire gene) displayed 100% nucleotide and predicted amino acid identity to *bla*$_{CTX-M-1}$.

E. coli producing CTX-M-1 was detected at high frequencies in animal, human, and environmental samples from both farms (Table 1). Most pigs (56/70) harbored CTX-M-1-positive *E. coli*, including 20 of the 30 sow-piglet pairs tested. CTX-M-1-producers were also isolated from three of the four samples from the farm workers and from the manure and air samples but not from the surface swabs or from the sample from the family member, who did not have daily exposure to the pigs. Four *E. coli* phylogenetic groups (A, B1, B2, and D) were detected by multiplex PCR (60%, 22%, 6%, and 12%, respectively) (4) (Table 1). XbaI pulsed-field gel electrophoresis (PFGE) (3) was used to investigate the genetic diversity among the 55 typeable CTX-M-1-producing isolates (see Fig. S1 in the supplemental material). Nineteen isolates from the previous study (11) were included to assess the possible persistence of CTX-M-1-producing *E. coli* clones. PFGE cluster analysis (unweighted-pair group method using average linkages, Dice similarity coefficient, optimization, and position tolerance of 2%) was done by Gelcompar II (Applied Maths, Belgium), resulting in 15 PFGE types displaying less than 80% similarity and 63 subtypes showing minor band differences within PFGE types. Indistinguishable PFGE patterns were observed only in isolates from farm 2 on four occasions, including two piglet isolates from 2006 and one human isolate from 2007.

Antimicrobial susceptibility was performed on all CTX-M producers according to CLSI standards (5). Due to the absence of standard breakpoints for *E. coli*, resistance (R) to ceftiofur (R = 6 mm for all isolates), florfenicol (R = 6 mm; susceptibility/intermediate resistance [S/I] ≥ 15 mm) and spectinomycin (R ≤ 10 mm; S/I ≥ 12 mm) was defined based on cutoff values determined by the distribution of inhibition zone diameters. Such values were in agreement with the clinical breakpoints for animal respiratory pathogens (6). Most (80%) isolates were resistant to two or more non-β-lactam antimicrobial classes. The human isolates showed variable resistance profiles, but all were resistant to at least four antimicrobial classes, including aminoglycosides, phenicols, tetracyclines, and sulfamethoxazole-trimethoprim. Ten strains from each farm displaying diverse PFGE subtypes or strain origins (pig, human, or environmental) and three strains from 2006 (20) (Table 2)

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TABLE 1. Occurrence and diversity of $bla_{CTX\text{-}M\text{-}1}$-positive *E. coli*

Farm	Sample type (no. of isolates)	No. of $bla_{CTX\text{-}M\text{-}1}$-positive PCR isolates	No. of PFGE subtypes[a]	*E. coli* phylotype(s) (no. of isolates)
1	Sow (20)	15	11, NT (4)	A (7), B1 (4), B2 (2), D (2)
	Piglets (20)	16	14, NT (2)	A (11), B1 (4), D (1)
	Surface swabs (7)	0		
	Air samples (7)	1	1	D (1)
	Humans (1)	0		
2	Sow (10)	10	10	A (7), B1 (2), D (1)
	Piglets (10)	9	6, NT (1)	A (4), B1 (5)
	Weaners (10)	6	5, NT (1)	A (3), B1 (2), B2 (1)
	Manure (4)	2	2	A (1), B1 (1)
	Surface swabs (8)	0		
	Air samples (9)	1	1	A (1)
	Humans (4)	3	3	A (2), D(1)
Subtotal	Pigs (70)	56		A (32), B1 (17), B2 (3), D (4)
	Humans (5)	3		A (2), D (1)
	Environment (35)	4		A (2), B1 (1), D (1)

[a] See Fig. S1 in the supplemental material for complete PFGE dendrograms. NT indicates nontypeable isolates, and the number of nontypeable isolates is indicated in parentheses.

were selected for plasmid characterization. Plasmids mediating cefotaxime resistance were transformed into electrocompetent Genehog *E. coli* (Invitrogen) using a Bio-Rad gene pulser and analyzed by replicon PCR (1) and restriction fragment length polymorphism (RFLP) using HincII and EcoRV. All plasmids belonged to IncN, and most (19/23) of them were either indistinguishable or closely related (RFLP type A) (Table 2). Four variants of plasmid RFLP type A were found in *E. coli* isolates from farm 1 (A1 to A3) and farm 2 (A4). Such variants were characterized by equal size (approximately 45 kb), identical EcoRV patterns, and up to two or three band differences following digestion with HincII (data not shown). The remaining four transformants contained IncN plasmids with different RFLP patterns (B to E). On farm 2, the plasmid detected in all the farm workers, the air sample, the manure, and most of the pigs was identical to that identified in the isolate from 2006 (RFLP pattern A4) and related to those found on farm 1 in 2006 (RFLP pattern A3) and 2007 (RFLP patterns A1 and

TABLE 2. Characterization of IncN plasmids harboring $bla_{CTX\text{-}M\text{-}1}$ by RFLP analysis and replicon typing

Strain	Origin	Farm	*E. coli* phylotype	PFGE subtype	EcoRV plasmid RFLP	HincII plasmid RFLP	Cotransferred resistance[a]	Coresistance genes
HA6	Slaughter[b]	1	A	F1	I	A3		
6232P	Piglet	1	A	H2	I	A1		
6232S	Sow	1	A	H3	III	C	SXT, FFC, TET, CHL	*sul1*, *sul2*, *tetA*, *floR*, *catA1*
1184S	Sow	1	A	I1	I	A1		
1285P	Piglet	1	A	J1	I	A2		
1233S	Sow	1	B1	A1	II	B	SUL, FFC, CHL	*sul1*, *sul2*, *floR*
1285S	Sow	1	B1	A2	I	A2		
1184P	Piglet	1	B1	B1	I	A2		
1170P	Piglet	1	B1	C1	I	A1		
951P	Piglet	1	D	B2	I	A1		
Env1	Air	1	D	B4	I	A1		
KV7	Piglet[b]	1	D	G1	IV	D		
1484S	Sow	1	D	I2	I	A2		
Hu2	Human	2	A	L4	I	A4		
FP7	Piglet[b]	2	A	L4	I	A4		
Env2	Air	2	A	L5	I	A4		
W3	Weaner	2	A	L9	V	E	CHL, SPX, SXT	*sul1*, *sul3*, *cmlA*, *aadA*
S2	Sow	2	A	L16	I	A4		
Hu1	Human	2	A	M1	I	A4		
Pen2	Slaughter	2	A	M2	I	A4		
P2	Piglet	2	B1	L5	I	A4		
Pen4	Slaughter	2	B1	L10	I	A4		
Hu3	Human	2	D	M6	I	A4		

[a] CHL, chloramphenicol; FFC, florfenicol; SPX, spectinomycin; SUL, sulfamethoxazole; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline.
[b] Isolate data from Jørgensen et al. (11).

A2). The cotransfer of resistance to non-β-lactam antimicrobials was only observed for three plasmids, and the cotransferred resistance genes were identified by PCR (8, 12, 13, 15) (Table 2).

CTX-M-1-producing *E. coli* was widespread on the two farms. PFGE and plasmid analysis indicated that the spread of CTX-M-1-producing *E. coli* between pigs and farm workers was predominantly due to the horizontal dissemination of IncN plasmids among distinct *E. coli* lineages. This study illustrates that plasmids carrying ESBL genes of clinical interest can be easily transferred between animals and humans by direct contact. The high genetic diversity indicated that the spread of $bla_{CTX\text{-}M\text{-}1}$ was not a result of clonal dissemination. Interestingly, even sows and piglets harbored distinct strains despite being housed together. The same PFGE pattern was occasionally observed in *E. coli* of porcine and human origin, suggesting that some strains may be able to exist in the intestinal tracts of both pigs and humans, thereby allowing plasmid transfer. The three farm workers harboring CTX-M-1-producing *E. coli* had no contact with hospitals and did not receive antimicrobials in the 6 months prior to sampling. Therefore, it is reasonable to assume that the farm workers acquired IncN plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ from pigs, where the presence of such plasmids was selected by antibiotic exposure.

The association between $bla_{CTX\text{-}M\text{-}1}$ and IncN plasmids has previously been observed among porcine clinical *E. coli* isolates in Denmark (H. Hasman, unpublished data) and among human clinical isolates in Spain (16) and Italy (14). Less frequently, CTX-M-1 has been associated with other incompatibility groups, such as IncI1 in poultry in France (7) and IncL/M in human patients in Spain (16). The small band differences observed among the three most prevalent plasmids at farm 1 (RFLP patterns A1 to A3) and farm 2 (RFLP pattern A4) indicate that such plasmid variants may have originated from a common ancestor. The dissemination of this plasmid lineage in Danish pig farming could have been enhanced by the use of veterinary cephalosporins, as suggested by the fact that $bla_{CTX\text{-}M\text{-}1}$ was the only detectable resistance gene. As indicated by the results of the previous cohort study (11), the high prevalences of CTX-M-1-producing *E. coli* observed among healthy pigs and farm workers in this study are likely to reflect the continuous selective pressure exerted through the prophylactic use of ceftiofur. The effects of veterinary cephalosporins on the selection of CTX-M-1-producing *E. coli* in the intestinal tracts of pigs have recently been illustrated under experimental conditions (2).

The spread of IncN plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ in pig farming may have significant implications for both human and animal health. Beside the zoonotic risk of transmission, there is also a concrete risk that IncN plasmids carrying $bla_{CTX\text{-}M\text{-}1}$, which are typically conjugative and broad-host-range plasmids, are transferred from commensal *E. coli* to swine respiratory pathogens, thereby resulting in a veterinary therapeutic problem. On the basis of these considerations, ceftiofur and other veterinary cephalosporins should be used prudently in animal farming and prophylactic use should be avoided. More research is needed to assess the occupational health risks associated with the occurrence of CTX-M-1-producing *E. coli* in farm workers as well as to investigate the possible zoonotic transmission of IncN plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ through the food chain.

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