Journal of Antimicrobial Chemotherapy Advance Access published June 7, 2014

J Antimicrob Chemother
doi:10.1093/jac/dku180

Journal of Antimicrobial Chemotherapy

Characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins

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Received 20 December 2013; returned 13 March 2014; revised 9 April 2014; accepted 30 April 2014

Objectives: To compare and characterize extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* from pigsties, pig farmers and their families on farms with previous high or no use of third- or fourth-generation cephalosporins.

Methods: Twenty farms with no third- or fourth-generation cephalosporin use and 19 herds with previous frequent use were included. The ESBL-producing isolates detected in humans and pigs were characterized by ESBL genotype, PFGE, susceptibility to non-β-lactam antibiotics and phylotype, and selected isolates were characterized by multilocus sequence typing (MLST). Furthermore, transferability of $bla_{CTX\text{-}M\text{-}1}$ from both human and pig isolates was studied and plasmid incompatibility groups were defined. The volunteers answered a questionnaire including epidemiological risk factors for carriage of ESBL-producing *E. coli*.

Results: ESBL-producing *E. coli* was detected in pigs on 79% of the farms with high consumption of cephalosporins compared with 20% of the pigs on farms with no consumption. ESBL-producing *E. coli* was detected in 19 of the 195 human participants and all but one had contact with pigs. The genes found in both humans and pigs at the same farms were $bla_{CTX\text{-}M\text{-}1}$ (eight farms), $bla_{CTX\text{-}M\text{-}14}$ (one farm) and $bla_{SHV\text{-}12}$ (one farm). At four farms ESBL-producing *E. coli* isolates with the same CTX-M enzyme, phylotype, PFGE type and MLST type were detected in both pigs and farmers. The majority of the plasmids with $bla_{CTX\text{-}M\text{-}1}$ were transferable by conjugation and belonged to incompatibility group IncI1, IncF, or IncN.

Conclusions: The present study shows an increased frequency of ESBL-producing *E. coli* on farms with high consumption of third- or fourth-generation cephalosporins and indicates transfer of either ESBL-producing *E. coli* or plasmids between pigs and farmers.

Keywords: swine, CTX-M, resistance, livestock, healthy humans, antibiotics, animal reservoirs, antibiotic usage

Introduction

Extended-spectrum β-lactamase (ESBL)-producing bacteria are emerging worldwide in both humans and animals. The use of third- and fourth-generation cephalosporins in food animal production leads to ESBL-producing *Escherichia coli* among food-producing animals and several studies have detected ESBL-producing *E. coli* in production animals.[1–5]

Studies from the Netherlands found clonally related ESBL-producing *E. coli* and similar plasmids in broilers, broiler meat and humans, suggesting broiler meat as a source of ESBL-producing *E. coli* causing infection in humans.[2] Dierikx *et al.*[6] detected ESBL genes on plasmids in *E. coli* from broilers at farm level that were identical to those in *E. coli* from the corresponding farmer. Moodley and Guardabassi[7] detected transfer of IncN plasmids with $bla_{CTX\text{-}M\text{-}1}$ between Danish pigs and pig farmers.

Andersen *et al.* investigated the effect of third- and fourth-generation cephalosporin use on the occurrence of extended-spectrum cephalosporinase-producing *E. coli* in Danish pig farms; they showed that previous regular use of third- or fourth-generation

cephalosporins was a significant risk factor for the occurrence of cephalosporinase-producing *E. coli* in pigs in Denmark (V. D. Andersen, V. F. Jensen, H. Vigre, M. Andreasen and Y. Agersø, unpublished results). In the present study cephalosporinase-producing *E. coli* from pigs found in the study by Andersen *et al.* were characterized by ESBL genotype, PFGE, susceptibility to non-β-lactam antibiotics and phylotype, and selected isolates were characterized by multilocus sequence typing (MLST).

The aim of this study was to compare and characterize ESBL-producing *E. coli* from pigsties, pig farmers and their families from farms with previous high or no use of third- or fourth-generation cephalosporins, to investigate whether ESBL-producing *E. coli* or plasmids with ESBL genes have spread between pigs and humans.

Methods

Study design

A cross-sectional study of ESBL-producing *E. coli* on Danish pig production farms stratified by previous consumption levels of third- or fourth-generation cephalosporin was conducted between November 2010 and May 2011. The study was started after the voluntary stoppage of third- and fourth-generation cephalosporin use for pigs in July 2010.[8] Therefore, the stratification was based on the consumption level 1 year before the study was started. Medium to large integrated pig herds were selected based on information from the Danish Central Husbandry Register (CHR) and two groups were selected based on the number of prescribed third- or fourth-generation cephalosporins within a year. These groups comprised 20 herds with no third- or fourth-generation cephalosporin use and 19 herds with frequent use (V. D. Andersen, V. F. Jensen, H. Vigre, M. Andreasen and Y. Agersø, unpublished results). Individuals working and living on farms were asked to participate after receiving full information from the investigators. Written informed consent was obtained from all participants who were ≥18 years of age, and proxy written informed consent for participants aged <18 years was obtained from parents or legal guardians. The volunteers signed an informed consent form and were asked to agree to faecal swabbing and to answer a standard questionnaire (available as Supplementary data at *JAC* Online), which was designed to collect demographic data and epidemiological risk factors for carriage of ESBL-producing bacteria, including gender, age, medications, livestock exposure, handling of antibiotics for pigs, travel activities, meat consumption and previous occupation. A total of 195 individuals from 19 farms with high-level third- or fourth-generation cephalosporin consumption and 20 farms with no consumption volunteered to participate. The questionnaire was originally in Danish, but has been translated into English for this publication. The protocol was approved by the Danish National Committee on Biomedical Research Ethics (no. H-4-2010-074) and the Danish Data Protection Agency (no. 2010-54-0976).

Description of sampling

On each farm 15 stool samples were collected from different pens in each of the following sections: sows/piglets, weaners and finishers. The 15 stool samples from each section were randomly pooled into three pooled samples, each containing five of the original stool samples. This approach resulted in three pooled samples per section per farm. (V. D. Andersen, V. F. Jensen, H. Vigre, M. Andreasen and Y. Agersø, unpublished results). The stool samples were sent to the Technical University of Denmark and analysed for cephalosporinase-producing *E. coli* within 24 h as described by Agersø *et al.*[9] In brief, 1 g of pooled sample was added to 10 mL of MacConkey broth supplemented with ceftriaxone (1 mg/L), and incubated overnight at 44°C. A 10 μL sample of the culture was streaked onto MacConkey agar plates with added ceftriaxone (1 mg/L) and incubated overnight at 44°C. From the agar plates, three presumptive colonies of cephalosporinase-producing organisms were selected and re-streaked.

Detection of ESBL-producing E. coli in human faecal samples

Farmers, employees and residents at the farms were asked to complete a questionnaire and send a rectal swab sample (Copan, Brescia, Italy) to Statens Serum Institut at the same time as the stool samples from the pigsties were sampled. The swabs were analysed within 24 h.

The human faeces from the swabs were spread on Discovery agar plates (Oxoid, Basingstoke, UK) with 1 mg/L ceftriaxone and incubated at 35°C for 18 h. Presumptive ESBL-producing *E. coli* were subcultured on 5% blood agar plates (SSI Diagnostika). Three colonies per sample were investigated further. The isolates were identified with API 20E (bioMérieux, Marcy-l'Étoile, France).

Molecular and phenotypic characterization of ESBL-producing E. coli

Three ESBL-producing *E. coli* isolates obtained from each human sample and one ESBL-producing *E. coli* isolate from each positive pooled sample obtained from the pigsties were further characterized.

The isolates were screened for bla_{CTX-M} by PCR. If negative, the isolates were further screened for bla_{SHV} and bla_{TEM} by PCR as previously described.[10–13] Positive control strains were *E. coli* O:149 77-30108-11 (bla_{CTX-M}), *E. coli* 76-33094-7 (bla_{TEM}) and *Salmonella* Keurmassar DAK-2 (bla_{SHV}). Positive amplicons were sequenced.

The isolates were phylotyped by multiplex PCR and classified as phylogroup A, phylogroup B1, phylogroup B2, phylogroup D or non-typeable as previously described by Clermont *et al.*[14]

PFGE was performed with XbaI as a restriction enzyme according to Tenover *et al.*[15] The genetic profiles were compared using BioNumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). PFGE types were defined with a cut-off value of 90% homology for relatedness.

MICs were determined for 12 non-β-lactam antimicrobial agents by the use of Sensititre panels (Trek Diagnostic Systems Ltd, East Grinstead, UK). Results for chloramphenicol, ciprofloxacin, gentamicin and trimethoprim were interpreted according to EUCAST clinical breakpoints (http://www.eucast.org), whereas results for apramycin, florfenicol, neomycin, and spectinomycin were interpreted according to the EUCAST MIC epidemiological cut-off value, and results for nalidixic acid, tetracycline, streptomycin and sulfamethoxazole were interpreted according to CLSI standards.[16] *E. coli* ATCC 25922 was used for quality control.

MLST

For each representative PFGE cluster type, one isolate was chosen for MLST. In cases where the same PFGE cluster type was detected in both pigs and humans, both isolates were typed by MLST. MLST was performed using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) (http://mlst.warwick.ac.uk/mlst/).

Conjugative transfer and characterization of plasmid replicons

All ESBL-producing *E. coli* with $bla_{CTX-M-1}$ belonging to different PFGE cluster types were used as donors in filter mating experiments. In cases where the same PFGE cluster type was detected in both pigs and humans, both isolates were used as donor; in total 51 isolates were used in these experiments. The donor and recipient (*E. coli* 1005RN, rif^R, nal^R) were grown in brain heart infusion broth overnight at 37°C. Donor and recipient broths were mixed 1:1 and 500 μL of the mixture was added to a 0.45 μm filter

(EMD Millipore Corporation, Billerica, MA, USA) placed on a 5% blood agar plate and the plates were incubated overnight at 37°C. The filter was transferred to 10 mL of 0.9% NaCl and vortex mixed. Transconjugants were selected on brain heart infusion agar supplemented with 1 mg/L ceftriaxone and 100 mg/L rifampicin. The presence of $bla_{CTX\text{-}M\text{-}1}$ in the transconjugants was tested and confirmed by PCR.[12]

If no transconjugants were obtained or if more than one replicon was found in the transconjugant, plasmid purification was performed. Afterwards, plasmids were introduced to electrocompetent plasmid-free *E. coli* Invitrogen ElectrMax™ DH10B™ cells by means of transformation by electroporation.[17]

PCR-based replicon typing (PBRT) was used to characterize plasmids from the ESBL-producing *E. coli* with $bla_{CTX\text{-}M\text{-}1}$ chosen for mating experiments and the isolates were tested for the following incompatibility groups by single PCRs: IncI1, IncN, IncA/C, IncHI2, IncK and IncF.[18]

Statistical analysis

Statistical significance tests of differences between proportions of samples positive for ESBL-producing *E. coli* were performed using the χ^2 test or Fisher's exact test (two-tailed) when the number of positive samples was low (less than five) (GraphPad Prism software, version 5, GraphPad, La Jolla, CA, USA).

Results

Detection of ESBL-producing E. coli in pigs

At 15 of the 19 (79%) farms with a high consumption of cephalosporins at least one of the tested stool samples from pigs tested positive for ESBL-producing *E. coli*. This occurrence was significantly ($P=0.0004$) higher compared with farms with no consumption of third- or fourth-generation cephalosporins, where 4 out of 20 (20%) farms had pigs with ESBL-producing *E. coli*. In all farms with pig samples positive for ESBL-producing *E. coli*, positive samples were found among the stool samples from sows/piglets. Some of these farms also had ESBL-producing *E. coli* in samples from weaning pigs or finisher pigs (data not shown). In total, 13 out of 177 stool samples from pig farms with no consumption of cephalosporins and 50 out of 162 stool samples from farms with high consumption of cephalosporins were positive for ESBL-producing *E. coli*. The detected genes encoding ESBL production in the isolates from pigs were $bla_{CTX\text{-}M\text{-}1}$ (14 farms), $bla_{CTX\text{-}M\text{-}14}$ (2 farms), $bla_{SHV\text{-}12}$ (1 farm) and $bla_{CTX\text{-}M\text{-}97}$ (2 farms) [Figure 1, Table 1 and Table S1 (available as Supplementary data at *JAC* Online)].

Detection of ESBL-producing E. coli in humans

A total of 195 persons participated in the study. The number of participants at each farm varied from 2 to 10. Of the 195 persons, 136 had direct contact with pigs (data not shown).

ESBL-producing *E. coli* was detected in 19 of the 195 participants (Table 2 and Table S1). Unfortunately, not all questionnaires were filled completely, most likely because the questionnaire was in Danish and nine persons with ESBL-producing *E. coli* originated from other countries and might not have possessed Danish literacy. The 19 persons were from 13 of the 39 investigated farms (Figure 1, Table 2 and Table S1). From each of the 13 farms, one or two persons were carrying ESBL-producing *E. coli* (Table 2 and Table S1). All but one of the humans with ESBL-producing *E. coli* had contact with pigs, 14 were from farms with previous high use of third- or fourth-generation cephalosporins and 5 were from farms with no previous use (Table 2). Persons with direct contact with pigs (15/72; 21%) more often had ESBL-producing *E. coli* in their stools if ESBL-producing *E. coli* was detected in the pigsties, compared with persons with contact with pigs (3/64; 5%) if no ESBL-producing *E. coli* was detected in the pigsties ($P=0.0056$) (data not shown).

Phylotyping

One of the tested isolates from pigs belonged to phylogroup B2 and three isolates belonged to phylogroup D, all other pig isolates belonged to phylogroup A ($n=26$) or B1 ($n=17$) or were non-typeable ($n=16$). Six isolates from two humans belonged to phylogroup B2 and three isolates from another human belonged to phylogroup D, whereas all other isolates from humans belonged to phylogroup A ($n=25$) or B1 ($n=12$) or were non-typeable ($n=11$) (Table S1).

Antimicrobial susceptibility testing

All but seven ESBL-producing *E. coli* isolates from pigs were resistant to more than two of the tested non-β-lactam antibiotics. Three of the human isolates were susceptible to all tested non-β-lactam antibiotics, one isolate was only co-resistant to sulfamethoxazole, and one isolate was only co-resistant to trimethoprim. All other ESBL-producing *E. coli* isolates from humans were resistant to two or more of the tested antibiotics (Table S1).

PFGE

In total, 33 PFGE cluster types were identified whilst 32 isolates had a unique PFGE type (Table 1 and Table S1).

MLST

Several different sequence types (STs) were detected in both humans and pigs, including 11 new STs (Table 1 and Table S1). Isolates belonging to ST10 were detected at eight farms, and these included isolates from both humans and pigs (Table S1). Isolates from two humans belonged to B2/ST131 (Table 1 and Table S1).

$bla_{CTX\text{-}M\text{-}1}$ plasmid transfer

Plasmid transfer by conjugation was possible for 44 of the 51 tested *E. coli* isolates with $bla_{CTX\text{-}M\text{-}1}$ (Table S1). The investigated plasmids belonged to IncI1, IncF and IncN (Table 1 and Table S1). At seven farms the same Inc plasmid type was detected in CTX-M-1-producing *E. coli* transconjugants from both humans and pigs (Table 1 and Table S1). The isolates from two of the seven farms (farms 29 and 84) belonged to the same PFGE type/MLST type. The CTX-M-1-producing *E. coli* transconjugants from humans and pigs from the remaining five farms (1, 17, 31, 38 and 46) where the same Inc plasmid type was detected in both humans and pig isolates did not belong to the same clone type according to PFGE and MLST (Table 1 and Table S1).

Comparison of ESBL-producing isolates from pigs and humans

On 10 farms the same ESBL gene was detected in both pig stools and human faeces (Figure 1, Table 1, Table 2 and Table S1). The

Table 1. Comparison of ESBL-producing *E. coli* isolates from humans and pigs[a]

	Humans[c]				Pigs[c]			
Farm no.[b]	ESBL type	MLST	PFGE[d]	Inc plasmid type[e]	ESBL type	MLST	PFGE[d]	Inc plasmid type[e]
1	CTX-M-1	10	A	ND	CTX-M-1	10	R	*IncN*
		58	CC	IncI1		10	UT	*IncN*
		2936	R	ND		4048	UT	*IncN*
		4047	Y	*IncN*				
14	**CTX-M-14**	**10**	**P**		**CTX-M-14**	**10**	**P**	
		10	U			10	EE	
						86	UT	
						4051	UT	
17	CTX-M-1	10	J	*IncI1*	CTX-M-1	1406	UT	IncF
						4052	UT	*IncI1*
						4053	UT	*IncI1*
29	**CTX-M-1**	**10**	**I**	*IncF*	**CTX-M-1**	**10**	**I**	*IncF*
		542	AA	*IncN*		10	UT	*IncF*
		46	UT	*IncN*		189	FF	*IncN*
		L	10	*IncF*				
31	CTX-M-1	1486	B	*IncN*	CTX-M-1	10	UT	*IncN*
						206	UT	*IncN*
32	SHV-12	4054	E		SHV-12	10	UT	
		4055	BB			58	UT	
						641	W	
						3322	UT	
38	CTX-M-1	2739	T	*IncF*	CTX-M-1	2739	GG	*IncF*
						453	UT	*IncF*
						542	UT	*IncF*
46	CTX-M-1	34	H	*IncI1*	CTX-M-1	910	UT	*IncI1*
	CTX-M-27	131	C			10	UT	*IncI1*
						1684	UT	*IncI1*
65	**CTX-M-1**	**4056**	**V**	ND	**CTX-M-1**	**4056**	**V**	ND
						4056	HH	
78[f]	CTX-M-1	131	Z	IncI1				
84	**CTX-M-1**	**744**	**S**	*IncI1*	**CTX-M-1**	**744**	**S**	*IncI1*
		93	UT			UT	1564	
			34	N				
88[f]	CTX-M-97	1436	UT					
		453	G					
102[f]	CTX-M-24	38	J					
		4057	X					

ND, not detected.
[a]Farms where only pigs tested positive for ESBL-producing *E. coli* are not included in this table.
[b]Farms with a number above 65 did not use third- and fourth-generation cephalosporins within 6 months before the faecal sample was taken.
[c]Bold formatting indicates observation of the same ESBL genotype, PFGE type and MLST type in both humans and pigs.
[d]UT, unique type; only one isolate with this type was detected.
[e]Inc plasmid types detected in transconjugants from CTX-M-1-producing *E. coli*. Italic formatting indicates observation of the same Inc plasmid type in both humans and pigs at the same farm.
[f]No ESBL-producing *E. coli* was detected among the pigs on these farms.

genes found in both humans and pig stool samples were $bla_{\text{CTX-M-1}}$ (eight farms), $bla_{\text{CTX-M-14}}$ (one farm) and $bla_{\text{SHV-12}}$ (one farm) (Figure 1, Table 1, Table 2 and Table S1). In one case, ST34 CTX-M-1-producing *E. coli* was found in the stool samples from pigs and in the faecal sample from the farmer (46-P3), whereas his wife (46-P2) was carrying an ST131 CTX-M-27-producing *E. coli*. She worked as an office assistant, ate meat and had been in contact with a cat, but no other animals (Table 2). In three farms, ESBL-producing *E. coli* was detected in humans but not in pig stool samples (Figure 1, Table 1 and Table S1). The genes encoding ESBL in these isolates were $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-24}}$ and $bla_{\text{CTX-M-97}}$, respectively (Figure 1, Table 1 and Table S1). All three farms did not use third- or fourth-generation cephalosporins.

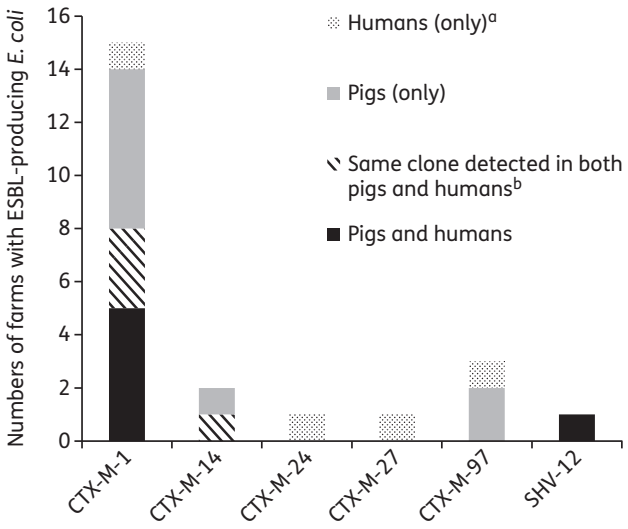

Figure 1. Occurrence of ESBL-producing *E. coli* at the farms. [a]The CTX-M-27-producing *E. coli* was detected in faeces from a woman with no contact with pigs; at the same farm, CTX-M-1-producing *E. coli* was detected in a farmer and pigs. [b]ESBL-producing *E. coli* isolates with the same ESBL type, phylotype, MLST type and PFGE type detected from both pigs and humans.

ESBL-producing *E. coli* isolates with the same ESBL type, phylotype, MLST type and PFGE type from both pigs and humans at the farm level were detected at four farms (Table 1 and Table S1). The involved persons had all been in contact with pigs.

Discussion

The study by Andersen *et al*. showed that previous use of third- or fourth-generation cephalosporins selects for cephalosporinase-producing *E. coli* in pigs (V. D. Andersen, V. F. Jensen, H. Vigre, M. Andreasen and Y. Agersø, unpublished results).

In our study, ESBL-producing *E. coli* isolates with the same CTX-M enzyme, phylotype, PFGE type and MLST type were detected in both pigs and in humans with contact with pigs, indicating transfer of ESBL-producing *E. coli* between pigs and humans.

In July 2010, a voluntary ban on cephalosporins was started in Danish pig production. This led to a significant reduction in the occurrence of extended-spectrum cephalosporinase-producing *E. coli* in slaughter pigs.[8] So it seems likely that the pig reservoir of ESBL-producing *E. coli* can be reduced both in Denmark and elsewhere if pigs are not treated with cephalosporins, and by this means the possible transfer of ESBL-producing *E. coli* from pigs to farmers can also be reduced.

In our study ESBL-producing *E. coli* was detected in 5% of the humans with contact with pigs that tested negative for ESBL-producing *E. coli*. This corresponded to the findings on ESBL-producing *E. coli* from Danish army recruits.[19] In both cases another community source, such as food or contact with ESBL carriers in the community, seems likely.

Rodríguez-Baño *et al*.[20] showed that the prevalence of faecal carriage of ESBL-producing *E. coli* was higher in relatives of patients with a urinary tract infection with ESBL-producing *E. coli* than in unrelated persons. This could be explained by person-to-person transmission or acquisition from a common source, probably related to food.[20] Transfer of ESBL-producing *E. coli* from farmers working with pigs to family members not working with pigs was not detected in our study. Only in one case did a family member test positive for ESBL-producing *E. coli*, but since the farmer was carrying an ST34 CTX-M-1-producing *E. coli*, whereas his wife was carrying an ST131 CTX-M-27-producing *E. coli*, person-to-person transmission does not seem likely.

In the present study CTX-M-1-producing *E. coli* was the most prevalent ESBL enzyme detected. This corresponds to findings from the Danish integrated surveillance programme (DANMAP), where CTX-M-1 was the most common ESBL enzyme among ESBL-producing *E. coli* in Danish pigs both at farm level and at slaughter.[8] CTX-M-1 was also the most common ESBL enzyme detected in pigs from Spain and Portugal.[21,22] Transfer of $bla_{\text{CTX-M-1}}$ by conjugation was possible for 44 of the 51 tested isolates. The same Inc plasmid types were detected in isolates from both humans and pigs at seven farms. This could indicate horizontal plasmid transfer between humans and pigs besides clonal spread. To investigate this further, plasmids have to be typed either by plasmid MLST or RFLP. Highly similar IncN plasmids carrying $bla_{\text{CTX-M-1}}$ have previously been detected in Danish pigs and pig farmers.[7]

SHV-12-producing *E. coli* and CTX-M-14-producing *E. coli* were also detected in our study from both pigs and humans. Both enzymes have been detected in pigs from Denmark, Korea, Spain and Portugal.[21–23] Furthermore, CTX-M-14-producing *E. coli* has been detected in Danish army recruits, indicating a community source for this enzyme.[19]

CTX-M-97-producing *E. coli* was detected in both humans and pigs. To our knowledge, CTX-M-97-producing *E. coli* has been detected only in a patient previously and not in pigs (submitted by J. Hacker to http://mlst.warwick.ac.uk/mlst/).

CTX-M-24-producing *E. coli* was detected in a farmer on a farm where ESBL-producing *E. coli* was not detected in pig stool samples. The origin of the CTX-M-24-producing *E. coli* is unknown. The farmer had, besides pigs, been in contact with a dog and a cat. CTX-M-24-producing *E. coli* has previously been detected in dogs.[24]

The office assistant with no contact with pigs was carrying B2-ST131 CTX-M-27-producing *E. coli*. The origin of the isolate was unknown, but B2-ST131 CTX-M-27-producing *E. coli* has been detected from Danish patients.[25,26] CTX-M-27 has, to our knowledge, not been detected in *E. coli* from Danish animals, but in *E. coli* from pigs and poultry in China.[27]

Most isolates from both pigs and humans in our study were resistant to two or more of the tested antibiotics besides β-lactams, which defines them as multidrug resistant. The resistance to non-β-lactams included antibiotics that are used for treatment of *E. coli* infections in humans (e.g. sulphonamides, ciprofloxacin and gentamicin).

UTIs are most often associated with phylogroup B2 and, to some extent, phylogroup D.[28] In our study B2 and D were not very common; only isolates from two humans and one of the pigs belonged to B2 and one isolate from one pig and one human belonged to D.

CTX-M-15 is the predominant enzyme among ESBL-producing *E. coli* in both urine and bloodstream infections in Denmark, while the enzymes detected in the present study (CTX-M-1, CTX-M-14 and CTX-M-27) are less common.[29] CTX-M-15-producing *E. coli*

Table 2. Description of the 19 persons with ESBL-producing *E. coli*

	Background data										
Farm no.[a]	person no.	profession	gender	age (years)	place of birth	intake of antimicrobial agents[b]	meat eaten[c]	contact with animals[d]	handling of antibiotics for pigs[e]	travel within 3 months	*bla* genes detected
1	01-P1	farmer	male	44	Denmark	no	yes	pigs, cat	yes	no	$bla_{CTX\text{-}M\text{-}1}$
	01-P4	farmer	male	44	Denmark	no	yes	pigs, cat, dog	yes	no	$bla_{CTX\text{-}M\text{-}1}$
14	14-P2	farmer	male	40	Denmark	no	yes	pigs	yes	Egypt	$bla_{CTX\text{-}M\text{-}14}$
	14-P8	farm assistant	female	25	Romania	NI	NI	pigs	NI	NI	$bla_{CTX\text{-}M\text{-}14}$
17	17-P1	farm assistant	male	31	Romania	no	yes	pigs, dog	yes	no	$bla_{CTX\text{-}M\text{-}1}$
29	29-P1	farm assistant	male	29	Romania	yes	yes	pigs	yes	Germany	$bla_{CTX\text{-}M\text{-}1}$
	29-P5	farm assistant	male	23	Romania	yes	yes	pigs, cat, other animals	yes	no	$bla_{CTX\text{-}M\text{-}1}$
	29-P6	farm assistant	female	25	Romania	yes	yes	pigs, cat	yes	no	$bla_{CTX\text{-}M\text{-}1}$
31	31-P3	farmer	male	51	Denmark	no	yes	pigs, cat, dog	yes	no	$bla_{CTX\text{-}M\text{-}1}$
32	32-P5	farmer trainee	female	20	Denmark	no	yes[f]	pigs, cattle, horses	yes	no	$bla_{SHV\text{-}12}$
	32-P8	farm assistant	male	25	Ukraine	yes	NI	pigs, other information missing	NI	NI	$bla_{SHV\text{-}12}$
38	38-P5	farm assistant	male	25	Denmark	no	yes	pigs, cat, dog	yes	no	$bla_{CTX\text{-}M\text{-}1}$
46	46-P2	office assistant[g]	female	48	Denmark	no	yes	cat	no	no	$bla_{CTX\text{-}M\text{-}27}$
	46-P3	farmer	male	46	Denmark	no	yes	pigs, cat	yes	no	$bla_{CTX\text{-}M\text{-}1}$
65	65-P3	farm assistant[h]	female	33	The Netherlands	yes	yes	pigs, cat, dog, horse	yes	no	$bla_{CTX\text{-}M\text{-}1}$
78	78-P6	farm assistant	female	NI	Romania	NI	NI	pigs, other information missing	NI	NI	$bla_{CTX\text{-}M\text{-}1}$
84	84-P1	student	female	24	Romania	no	NI	pigs, other information missing	NI	NI	$bla_{CTX\text{-}M\text{-}1}$
88	88-P5	farmer	male	22	Denmark	no	yes	pigs, dog	yes	no	$bla_{CTX\text{-}M\text{-}97}$
102	102-P2	farmer	male	51	Denmark	no	yes	pigs, dog, cat	yes	no	$bla_{CTX\text{-}M\text{-}24}$

NI, no information.
[a]Farms with a number above 65 did not use third- and fourth-generation cephalosporins within 6 months before the faecal sample was taken.
[b]Within 6 months before the faecal sample was taken.
[c]Pork, poultry meat and beef eaten regularly.
[d]Within 1 week before the faecal sample was taken.
[e]Within 1 month before the faecal sample was taken.
[f]Only beef.
[g]Farmer's wife, living on the farm.
[h]Hospitalized more than 1 year ago.

was not detected in our study, but this enzyme has previously been detected in Danish pigs.[30]

As in many other countries, the majority of the CTX-M-15-producing *E. coli* causing UTIs and bloodstream infections in humans belong to B2-ST131.[25,31] None of the pig isolates in our study belonged to B2-ST131, but *in vitro* transfer of $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ of porcine origin to B2-ST131 has been shown.[30] In our study most $bla_{CTX\text{-}M\text{-}1}$ genes were transferable by conjugation *in vitro*.

Our study shows that ESBL-producing *E. coli* are transferred between pigs and humans. Furthermore, pigs can be a reservoir for ESBL genes, which may be transferred to other *E. coli* that might be more pathogenic.

It seems likely that the CTX-M-1-, CTX-M-14- and CTX-M-27-producing *E. coli* detected in patients could be of animal origin, but further studies are needed to investigate and quantify a possible zoonotic link between ESBL-producing *E. coli* and human infections.

Acknowledgements

We thank all the farmers, farm assistants and the farmer's families for participation in this study. We thank Karin Sixhøj Pedersen for excellent technical assistance. We thank Frederik Skov for excellent handling of the data from the questionnaires.

Funding

This work was supported by the Danish Ministry of Science, Technology and Innovation and the Danish Ministry of Health and Prevention as part of The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) and by the Danish Ministry of Food, Agriculture and Fisheries (grant no.: 3304-FVFP-09-F-002-1).

Transparency declarations

None to declare.

Supplementary data

The questionnaire and Table S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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