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Cephalosporin-resistant *Escherichia coli* isolated from farm workers and pigs in northern Vietnam

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Abstract

OBJECTIVE Antimicrobial-resistant bacteria may be transmitted between farm workers and livestock. This study aimed to determine and compare the prevalence and the genetic determinants of cefotaxime-resistant and ESBL-producing *Escherichia coli* in faecal isolates from workers and pigs at 100 farms in northern Vietnam.

METHODS Farmers were interviewed about antimicrobial usage in livestock. *Escherichia coli* isolated on MacConkey agar containing 2 mg/l of cefotaxime (CTX) were tested for susceptibility to different cephalosporins by disc diffusion and screened for occurrence of ESBL-encoding genes by PCR.

RESULTS Antimicrobial usage was widespread and included classes regarded of critical or high importance in human medicine. Dosages were 0.5–2 times higher than recommended, and antimicrobials were often administered right until slaughter. Prevalence of CTX-resistant *E. coli* was 86% in farm workers and 89% in pigs. In 76% of farms, CTX-resistant *E. coli* were shared by pigs and farm workers. ESBL-producing *E. coli* were detected from pigs and workers at 66 and 69 farms, respectively. The ESBL phenotype was mainly mediated by CTX-M and to a lesser extent by TEM. Occurrence of $bla_{\text{CTX-M}}$ was similar in *E. coli* from pigs (66.7%) and humans (68.5%).

CONCLUSION The high occurrence of ESBL-producing *E. coli* in pig farmers and pigs could present a risk for spillover of these bacteria from pig farms into the community. Genomic studies are needed to elucidate reservoirs and transmission routes of ESBL-producing *E. coli* at livestock farms.

keywords cephalosporin resistance, ESBL, CTX-M, pigs, farmers, Vietnam

Introduction

Third- and fourth-generation cephalosporins are antimicrobials commonly used to treat infections by Gram-negative bacteria in humans and in selected geographical regions, also in livestock [1–3]. In *Escherichia coli*, resistance to third- and fourth-generation cephalosporins is generally mediated by extended-spectrum β-lactamases (ESBLs), which are encoded by plasmids easily transferable across bacterial species and reservoirs [4]. The possible exchange of ESBLs between humans and pigs has been debated in many countries for a long time [5, 6]. It has been suggested that antimicrobial-resistant bacteria may be transmitted from pigs to people by direct contact, for example occupational exposure of farm and slaughterhouse workers, consumption of contaminated food and indirect transmission through the environment [7].

In Vietnam, the population of pigs is expected to increase to 35 million in 2020 driven by fast-growing domestic demands for livestock meat products and plans to increase export [8]. Small-scale farms account for about 90% of pig production and pork supply in Vietnam [9]. Pig diseases, mainly respiratory and diarrhoeal diseases, are common and controlled by both prophylactic and therapeutic antimicrobial use despite the fact that most frequently occurring diseases, such as porcine reproductive and respiratory syndrome (PRRS) and foot-and-mouth disease, are of viral origin [10, 11].

Recent studies on antimicrobial use and resistance in Vietnamese livestock production document much higher amounts of antimicrobials used to produce pork than in other parts of the world [10, 12]. ESBL-producing Enterobacteriaceae are now found in livestock, food and people at similar or higher levels in Vietnam as in other parts of the world [13–17], although little is known

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about sources and routes of transmission of ESBL-producing bacteria found in humans.

In this study, we aimed to determine and compare the prevalence and the genetic determinants of cefotaxime-resistant and ESBL-producing *E. coli* in farm workers and pigs. To address this aim, we conducted a cross-sectional study analysing pig and human faecal samples from 100 small-scale pig farms located in Kien Xuong district (Thai Binh province) and Soc Son district close to Hanoi in northern Vietnam.

Method

Selection of pig farms and farmer interviews

One hundred small-scale pig farms, each farm raising between 15 and 30 pigs, were visited in Kien Xuong and Soc Son districts from May to July, 2015. Most (80%) farms produced their own piglets and raised them until slaughter (approximately 80 kg). Pigs sampled had a weight between 20 and 50 kg. The two study sites are located within a 100 km distance from the capital Hanoi and are main suppliers of pork to the city population. According to the 2015 annual report of the local Department of Animal Health (DAH) in Vietnam, farmers in Kien Xuong and Soc Son districts produced 200 000 and 121 580 pigs, respectively. Pig farms in the two districts were similar in size, type of production and general management. District veterinarians employed by the DAH provided a list of pig farms and representative farms were selected in five communes in each of the two locations. Most small-scale farms in Vietnam, including those selected for this study, feed pigs a commercial diet mixed with local traditional feed items, for example powder of corn and cassava as well as rice bran. Vegetables, for example morning glory, water hyacinth and banana tree leaves, are also fed to the pigs. Antimicrobials were sold to farmers in so-called veterinary chemical and drug shops, by visiting veterinarians employed by pharmaceutical companies as well as local government and private veterinarians.

A survey of antimicrobial usage at the selected farms was conducted by questionnaire-based interviews of the pig farm owners who all reportedly had daily contact with pigs. The questionnaire covered the following subject areas: antimicrobial use practices, including type of antimicrobial, dosage, duration of antimicrobial use; withdrawal time before slaughter; main type of pig diseases and therapeutic antimicrobials typically used; and factors determining farmer's choice of antimicrobials to be purchased, including prize, experience and advice from local veterinarians.

Collection of faecal samples from pigs and farm workers

Individual rectal swab samples were collected from five randomly selected pigs in each pen at a farm using Fecal-swab (COPAN, Brescia, Italia). Farms typically had between three to five pens, and swabs were collected from three pens. All swabs representing one farm were placed into a sterile plastic bag (composite sample) containing 50 ml of 0.1% peptone water with 0.85% NaCl (BO0471; Oxoid, Cambridge, England). At each farm, a human faecal sample of approximately 20 g was collected by the farm owner during defecation using sterile gloves. The sample was placed immediately into a labelled sterile plastic bag provided by the survey team. The farm owner was informed orally about the survey and told that he/she could withdraw at any time. Both pig and human faecal samples were stored in an insulated box with cooling elements and transported to the laboratory where analysis was started on the day of collection.

Isolation of cefotaxime-resistant *E. coli*

One millilitre of composite faecal sample was mixed with 0.1% peptone diluents containing 0.85% NaCl (Oxoid) in 1:9 ratio in a sterile plastic bag and homogenised in a Bag Mixer model VW400 (Seward, England) for 30 s. Serial 10-fold dilutions were prepared to obtain individual colonies after plating onto the surface of MacConkey agar (Merck, Germany) plates supplemented with 2 mg/l of cefotaxime (CTX; Oxoid, Hampshire, England) and incubated at 37 °C for 24 h [18]. One gram of human faecal sample was mixed with 9 ml peptone diluent. Dilutions were prepared and cultured onto MacConkey agar as described above to allow for the selection of individual *E. coli* colonies.

Up to five presumptive *E. coli* colonies were selected from each composite pig and human faecal sample. Isolates were confirmed as *E. coli* by biochemical testing (API 20E, BioMerieux, France) with *E. coli* ATCC 25922 used as reference strain. Isolates were streaked onto blood agar plates to ensure purity and stored at −80 °C in Eppendorf tubes containing brain heart infusion broth (CM1135, Oxoid) with 10% glycerol.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by disc diffusion according to standard procedures [19]. All selected isolates confirmed as *E. coli* were tested for susceptibility to the following cephalosporins: ceftazidime (CAZ, 3rd generation, 30 μg), cefpodoxime (CPD, 3rd, 10 μg), ceftriaxone (CRO, 3rd, 30 μg), cefuroxime

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(CXM, 2nd, 30 μg), cefepime (FEP, 4th, 30 μg), cefoperazone (CFP, 3rd, 75 μg) and cefoxitin (FOX, 2nd, 30 μg) (Oxoid, Cambridge, England). Antimicrobial susceptibility test results were interpreted according to CLSI criteria [19]. Isolates classified as intermediate resistant were categorised as susceptible to avoid overestimation of resistance (http://www.crl-ar.eu/203-reports.htm#eqas). Up to two CTX-resistant *E. coli* isolates per faecal sample were randomly selected to detect presumptive ESBL-producers by the modified double-disc synergy test (amoxicillin/clavulanic acid, 20/10 μg and cefpodoxime, 10 μg) [19, 20].

PCR for bla_{TEM}-, $bla_{CTX\text{-}M}$- and bla_{SHV} genes

PCR was performed to detect common ESBL-encoding genes, that is, bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ using previously reported primers and protocols [21, 22]. In each PCR experiment, *E. coli* strains previously confirmed to carry bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ by PCR and sequencing (Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi, Vietnam) were used as positive controls. Buffered phosphate solution was used as a negative control.

Ethical approval

Ethical approval for the collection of human faecal specimens was obtained from the Hanoi School of Public Health, Ministry of Health before field work was initiated (Decision on ethical approval for research involving human subject participation. No. 251/2015/YTCC-HD3).

Results and discussion

Antimicrobial use practices at the pig farms

70% of the pig farm owners interviewed (n = 100) had graduated from primary school. In 65 farms, pigs were fed commercial feed supplemented with traditional feed items like corn- and cassava powder and rice bran mixed with vegetables, for example morning glory, water hyacinth and banana tree leaves. Pig feed containing antimicrobials such as colistin sulphate, ampicillin or ampicolin (a mix of ampicillin and colistin sulphate) was seen stored at 12 farms (data not shown). Farmers stated that the use of commercial feed (representing three different brands) containing growth promoters was cost-effective as it enabled the pigs to reach a weight of about 80 kg in four months vs. 70 kg reached in 6–7 months without use of growth promoters.

During our visits, we noted the antimicrobials and their route of administration (Table 1) and gathered information on antimicrobial use practices. 73% of farmers often contacted veterinarians when their pigs were diseased and asked for advice on which antimicrobials to purchase. At 24 farms (24%), household owners went directly to shops operated by veterinarians to buy antibiotics. The main reasons for treatment were diarrhoea and respiratory diseases (details not collected). Despite advice from the veterinarians, almost all farmers (94%) decided on antimicrobial dosages based on their own experience. Such dosages were typically from 0.5 to 2 times higher than recommended on product labels and antimicrobial treatment continued until slaughter with little adherence to withdrawal periods. Six farmers did not provide information about withdrawal periods. All interviewed farmers (100%) stated that they tried to save money by purchasing cheap antimicrobial products. Only 27 farmers had attended free training courses on antimicrobial use offered by both DAH and private medical companies. Most farms had an allocated space where farmers discarded empty bottles, containers and other items related to used antimicrobials. Our findings of substantial antimicrobial use in pig production are similar to those of Nguyen *et al.* [10]. The use of higher antimicrobial dosages than recommended may be due to poor quality of the antimicrobials; a problem that has been documented for antimicrobials used in aquaculture in Vietnam [23]. Antimicrobial treatment of pigs until slaughter inevitably leads to accumulation of residues in pork products as documented by Hanh *et al.* (2015), who found sulphadimidine residues in 50% and chloramphenicol residues in 17% of pork samples sold at wet markets in two Vietnamese provinces [24].

A recent study conducted from 2013 to 2014 estimated that 563.6 mg of antimicrobials was used to produce 1 kg (live weight) of pigs in Tien Giang province, Vietnam [10]. The farms in that study were larger than our farms, and therefore likely to have different antimicrobial usage practices. Nevertheless, the amounts of antimicrobials used are very high, corresponding to more than three times the global average annual consumption of antimicrobials per kilogram of pig produced [25]. Similar data were provided by Carrique-Mas *et al.* (2015) who reported that a total of 470 mg of antimicrobial compound were used to raise one chicken in Vietnam, vs. 80 mg in Europe [26]. Overall, these estimates of antimicrobial consumption in Vietnamese livestock indicate inadequate knowledge on the adverse consequences of inappropriate use of antimicrobials among farmers, veterinarians and other stakeholders involved.

Most of the antimicrobials used at the examined farms (Table 1) are considered of critical or high importance for human medicine by WHO [27]. Adding colistin to

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Table 1 Antimicrobials reportedly used to prevent and treat pig diseases in the examined farms, Vietnam

Antimicrobial class	Type of antimicrobial	Route of administration	Farm ID
Aminoglycosides	Gentamicin	Injection	3, 6, 78
	Neomycin	Drinking water	13, 20, 29, 43
	Streptomycin	Injection	4
β-lactams	Ampicillin	Injection	11, 13, 15
	Amoxicillin	Drinking water	4, 18, 32, 75, 78
	Penicillin	Drinking water	32
	Cefixime	Injection	87
Phenicols	Thiamphenicol	Injection	90
Lincosamides	Lincomycin	Injection	1, 11, 29, 77, 87, 88
Macrolides	Tylosin	Feed or drinking water	1, 11, 12, 78, 79
Pleuromutilins	Tiamulin	Injection	12, 17, 18, 19, 20
Polypeptides	Colistin	Feed or drinking water	11, 13, 19, 43
Quinolones	Enrofloxacin	Injection	6, 44, 78, 86
	Norfloxacin	Drinking water	78
Tetracyclines	Doxycycline	Drinking water	11
	Oxytetracycline	Feed	12, 13, 16, 77, 80, 90

feed or water is worrisome as such selective pressure will lead to resistance in pigs as documented by Nguyen *et al.* (2016a) [10]. A recent farm-based study in Vietnam of the colistin resistance gene *mcr-1* in *E. coli* showed that detection of *mcr-1* in humans was associated with exposure to *mcr-1*-positive chickens [28].

Occurrence of cefotaxime-resistant *E. coli* in pigs and farmers

CTX-resistant *E. coli* were detected in 89 (89%) composite faecal samples from pigs and in 86 (86%) faecal samples from farm owners. As *E. coli* were isolated without any selective enrichment step, it appears that CTX-resistant *E. coli* were very highly prevalent at the sample, host species and farm level. The very high prevalence of pig farms positive for CTX-resistant *E. coli* is similar to that reported in pig farms using 3rd-generation cephalosporins in Europe [29]. Although we could not retrieve exact figures of cephalosporin and other antimicrobial use, it was clear from the interviews that farmers were administering antimicrobials without adequate knowledge on dosages and treatment regimens (see the subsection above).

The prevalence of ESBL-resistant *E. coli* in farm owners is higher than previously reported in clinical settings which is surprising as prevalence of cephalosporin-resistant bacteria in clinical settings is expected to be higher than in the general population [30]. Global trends of increasing carriage of CTX-resistant *E. coli* in the community over time [31] can at least partially explain such difference. A study from 2013–2014 in a Vietnamese healthy rural population reported 88% of people being positive for CTX-resistant *E. coli* in at least one of three sampling times over a 12-month study period [13] which is a percentage similar to what we observed and which the authors proposed was due to improper use of antimicrobials among people [32]. However, in the same study, when considering each sampling time separately, the prevalence of healthy people carrying CTX-resistant *E. coli* fluctuated between 46% and 53% [13], which is considerably lower than the prevalence detected in our study.

As there are no specific reasons to believe that irrational overuse of antimicrobials happens more frequently among pig farmers than in the general population, it appears that pig farming might have an impact on the very high prevalence of CTX-resistant *E. coli* detected in Vietnamese farmers. In Europe, higher prevalence of CTX-resistant *E. coli* has been reported in workers at pig farms with CTX-resistant *E. coli*-positive pigs than in workers at farms with CTX-resistant *E. coli*-negative pigs [29, 33]. It is, however, unknown if this is due to continuous acquisition of resistant bacteria from animals or to other livestock farming-related factors (e.g. handling of antimicrobials and other compounds which may coselect for antimicrobial resistance such as feed additives, disinfectants, etc.) promoting long-term colonisation of farmers. The fact that CTX-resistant *E. coli* were simultaneously detected both in pigs and in pig farmers in 76% of the farms in our study strongly reinforces previous findings that contact with pigs has an influence on carriage of CTX-resistant *E. coli* in farmers through mechanisms yet to be elucidated. We also observed 13

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farms (seven in Kien Xuong and six in Soc Son) in which CTX-resistant *E. coli* were isolated only from pigs and 11 farms (eight in Kien Xuong and three in Soc Son) in which CTX-resistant *E. coli* were isolated only from humans. It is, however, unclear if this might be linked to different biosecurity measures in the farms, to host-related factors (e.g. resistance to colonisation by CTX-resistant *E. coli* from a different host species) or simply to presence of CTX-resistant *E. coli* at undetectable levels in the samples testing negative.

Prevalence of resistance to additional cephalosporins in cefotaxime-resistant *E. coli*

Up to five *E. coli* isolates from each sample were randomly selected from CTX-containing MacConkey agar plates yielding a total of 254 isolates from pigs and 263 isolates from humans tested for susceptibility to seven additional cephalosporins.

Overall, CTX-resistant *E. coli* showed 15 patterns of resistance to other cephalosporins (Table 2). Twelve profiles were observed in pig isolates and 11 in human isolates (Table 2), thus indicating that the overall diversity of *E. coli* clones and/or mechanisms of cephalosporin resistance was slightly higher in pig than in human isolates. This might have been influenced by the sampling strategy, with pig samples representing pooled faecal samples from different individuals and human samples deriving from one individual only. In each of 89 farms, the isolates from pigs showed at least two cephalosporin resistance profiles. Similarly, two or more cephalosporin resistance profiles were detected in isolates from humans at 86 farms (data not shown). This suggests that different CTX-resistant *E. coli* clones and/or mechanisms mediating cephalosporin resistance coexist both in the general pig and pig farmers *E. coli* population and at the farm level.

Nearly all CTX-resistant *E. coli* isolates were resistant to cefpodoxime (CPD) alone or in association with resistance to other cephalosporins (Table 2) which was expected, as this third-generation cephalosporin has a very high sensitivity compared to that of cefotaxime for detection of ESBLs [34]. Surprisingly, no isolate displayed cefepime resistance, which is generally considered indicative of CTX-M type ESBLs. However, recent European Food Safety Authority (EFSA) guidelines do not recommend use of cefepime for inferring determinants of cephalosporin resistance from phenotypic data (http://www.crl-ar.eu/data/images/ws_April-2016/f11_efsa_criteria.pdf). Coresistance to CXM, CRO, CPD and CFP, CRO, CXM, CPD were the most common patterns of resistance to different generation of cephalosporins among isolates from both pigs and humans (Table 2) and at farm level (Table 3). CXM, CRO, CPD resistance occurred in 32% of isolates from pigs from 51 farms and in 27% of human isolates from 41 farms. At 20 farms, this profile was detected both in pig and in human isolates (Table 3). The CFP, CRO, CXM, CPD resistance profile occurred in 15% of isolates from pigs from 33 farms and in 25.9% of human isolates from 47 farms. In 22 farms, this profile was detected both in pig and in human isolates (Table 3). The predominance of a few indistinguishable cephalosporin resistance profiles in isolates from different host species and from unrelated farms suggests the presence of related resistance determinants. Additional patterns of resistance to different generations of cephalosporins occurred less frequently and overall at similar levels in isolates from pigs and humans (Table 2). Such profiles were also scattered across farms in the majority of cases, but only three occurred in isolates from pigs and humans at the same farm (Table 3). In total, six phenotypic profiles were shared by epidemiologically related pig and human isolates (Table 3), with at least one shared profile found in 63 farms (data not shown). An exchange of isolates and/or resistance genes between the pig and the human population might partly explain our observations, and it is clear that selective pressure for cephalosporin resistance is high in both host species. Within each shared phenotypic profile, the highest proportion of farms in which the profile was shared by pig and human isolates out of the total number of farms in which the profile was observed was moderate (38%) (Table 3). This suggests that, although *E. coli* with identical cephalosporin resistance profiles can be detected in pigs and humans, transmission across hosts is likely affected by a complex of ecological factors beyond the resistance determinants involved. Studies to understand which contexts promote or limit transfer of identical resistance determinants across host species in pig farms in Vietnam are needed.

Phenotypic and genotypic detection of ESBL-producing *E. coli* in faecal samples from farm workers and pigs

Phenotypic detection of ESBLs was performed in up to two cefotaxime-resistant isolates per sample leading to a total of 149 and 163 *E. coli* tested from porcine and human samples, respectively. Phenotypic tests suggested ESBL production in 101 (68.5%) isolates from pigs representing 66 farms and in 117 (71.8%) isolates from farm workers from 69 farms. At 44 farms, *E. coli* with ESBL phenotype were isolated both from pigs and from humans. An ESBL phenotype was observed in isolates across almost all resistance profiles (Table 2). In case of FOX coresistance, a likely explanation is the presence of

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Table 2 Resistance to various cephalosporins in cefotaxime-resistant *Escherichia coli* from pigs and pig farmers in Vietnam, 2015

	Phenotypic and genotypic traits	Cephalosporin resistance profile*																
		CXM; CPD	CRO; CPD	CXM; CRO; CPD	CPD	CXM; FOX; CRO; CPD	CFP; CRO; CXM; CPD	CFP; CRO; CPD	CFP; CRO; CXM; FOX; CPD	CFP; CRO; CXM; FOX	CXM; FOX; CAZ; CPD	FOX; CPD	CRO	CFP; CRO; FOX; CPD	CFP; CXM; CPD	CRO; FOX; CPD	None	Total, %
Pig faeces	Cephalosporin resistance phenotype (*n*/N†; %)	24/254 (10)	10/254 (4)	80/254 (32)	72/254 (28)	1/254 (0.4)	38/254 (15)	1/254 (0.4)	14/254 (6)	1/254 (0.4)	–	10/254 (4)	1/254 (0.4)	1/254 (0.4)	–	–	1/254 (0.4)	254
	Positive at the double-disk test (*n*/N†)	11/12	1/6	45/48	3/33	–	31/31	0/1	8/8	–	–	0/7	0/1	1/1	–	–	1/1	101/149 (68.5)
	Positive for bla_{CTX-M} *n*(/E⁺‡/N†)	5 (5/5)	0 (1/1)	11 (12/12)	0 (2/4)	–	10 (11/11)	–	2 (3/3)	–	–	0 (0/7)	–	1 (1/1)	–	–	1 (1/1)	30/45 (66.7)
	Positive for bla_{TEM} *n*(E⁺‡/N†)	5 (5/5)	0 (1/1)	11 (12/12)	2 (2/4)	–	5 (11/11)	–	2 (3/3)	–	–	7 (0/7)	–	0 (1/1)	–	–	0 (1/1)	32/45 (71.1)
	Positive for bla_{TEM} and bla_{CTX-M} *n*(E⁺‡/N†)	5 (5/5)	0 (1/1)	10 (12/12)	0 (2/4)	–	4 (11/11)	–	2 (3/3)	–	–	0/0 (0/7)	–	1 (1/1)	–	–	1 (1/1)	21/45 (46.7)
	Negative for bla_{CTX-M} and bla_{TEM} *n*(E⁻‡/N)	0 (5/5)	1 (1/1)	0 (12/12)	2 (2/4)		0 (11/11)	–	1 (3/3)	–	–	0/0 (0/7)	–	0 (1/1)	–	–	0 (1/1)	5/45 (11.1)
Human Excreta	Cephalosporin resistance phenotype (*n*/N†; %)	28/263 (10.6)	3/263 (1.1)	71/263 (27.0)	49/263 (18.6)	3/263 (1.1)	68/263 (25.9)	–	26/263 (10.0)	–	1/263 (0.4)	7/263 (2.7)	–	–	1/263 (0.4)	2/263 (0.8)	4/263 (1.5)	263
	Positive at the double-disk test (*n*/N†)	12/16	1/1	39/39	7/27	2/2	45/47	–	12/18	–	0/2	0/6	–	–	–	0/2	0/4	117/163 (71.8)
	Positive for bla_{CTX-M} *n*(E⁺‡/N†)	3 (3/3)	–	10 (13/13)	1 (2/9)	–	25 (27/27)	–	10 (8/11)	–	1 (0/1)	0 (0/6)	–	–	–	0 (0/2)	0 (0/1)	50/73 (68.5)
	Positive for bla_{TEM} *n*(E⁺‡/N†)	2 (3/3)	–	7 (13/13)	1 (2/9)	–	7 (27/27)	–	5 (8/11)	–	1 (0/1)	6 (0/6)	–	–	–	2 (0/2)	0 (0/1)	31/73 (42.5)
	Positive for bla_{TEM} and bla_{CTX-M} *n*(E⁺‡/N†)	2 (3/3)	–	5 (13/13)	0 (2/9)	–	6 (27/27)	–	4 (8/11)	–	1 (0/1)	0 (0/6)	–	–	–	0 (0/2)	0 (0/1)	18/73 (24.7)
	Negative for bla_{CTX-M} and bla_{TEM} *n*(E⁻‡/N†)	1 (3/3)	–	1 (13/13)	7 (2/9)	–	1 (27/27)	–	0 (8/11)	–	0 (0/1)	0 (0/6)	–	–	–	0 (0/2)	1 (0/1)	11/73 (15.1)

*CXM, cefuroxime; FOX, cefoxitin; CAZ, ceftazidime; CPD, cefpodoxime; CRO, ceftriaxone; CFP, cefoperazone; FEP, cefepime.
†*n*, number of isolates with the specific phenotypic or genotypic trait; *N*, total number of isolates tested.
‡E⁺, ESBL phenotype; E⁻, non-ESBL phenotype.

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Table 3 Host and farm-level distribution of cephalosporin-resistant *Escherichia coli* subtypes

Source	Antimicrobials*	Profile ID	No. of farms/total no. of farms in which the profile was detected (%)
Pigs only	CXM;CPD	1	11/32 (34%)
	CRO;CPD	2	9/14 (64%)
	CXM;CRO;CPD	3	31/72 (43%)
	CPD	4	21/51 (41%)
	CXM;FOX;CRO;CPD	5	1/4 (25%)
	CFP;CRO;CXM;CPD	6	11/58 (19%)
	CFP;CRO;CPD	7	1/1 (100%)
	CFP;CRO;CXM;FOX;CPD	8	9/29 (31%)
	CFP;CRO;CXM;FOX	9	1/1 (100%)
	FOX;CPD	12	1/2 (50%)
	CRO	13	1/1 (100%)
	CFP;CRO;FOX;CPD	14	1/1 (100%)
	None	10	1/1 (100%)
Humans only	CXM;CPD	1	14/32 (44%)
	CRO;CPD	2	4/14 (29%)
	CXM;CRO;CPD	3	21/72 (29%)
	CPD	4	19/51 (37%)
	CXM;FOX;CRO;CPD	5	3/4 (75%)
	CFP;CRO;CXM;CPD	6	25/58 (43%)
	CFP;CRO;CXM;FOX;CPD	8	18/29 (62%)
	CXM; FOX;CAZ;CPD	11	1/1 (100%)
	FOX;CPD	12	1/2 (50%)
	CFP;CXM;CPD	15	1/1 (100%)
	CRO;FOX; CPD	2	2/2 (100%)
	None	10	4/5 (80%)
Pigs and humans at the same farm	CXM;CPD	1	7/32 (22%)
	CRO;CPD	2	1/14 (7%)
	CRO;CXM;CPD	3	20/72 (28%)
	CPD	4	11/51 (22%)
	CFP;CRO;CXM;CPD	6	22/58 (38%)
	CFP;CRO;CXM;FOX;CPD	8	2/29 (7%)

CXM, cefuroxime; CPD, cefpodoxime; CRO, ceftriaxone; FOX, cefoxitin; CAZ, ceftazidime; FEP, cefepime; CFP, cefoperazone.
*Only cephalosporins other than cefotaxime are reported since all isolates were resistant to cefotaxime.

AmpC-type beta-lactamases that mask the ESBL phenotype [35, 36]. In case of resistance to different third-generation cephalosporins (CFP, CRO, CPD along with CTX resistance), occurrence of beta-lactam resistance mechanisms other than enzymatic drug inactivation cannot be excluded.

A total of 45 isolates from pigs (including 30 phenotypically positive and 15 isolates negative for ESBL production) and 73 isolates from humans (including 50 isolates phenotypically positive and 23 negative for ESBL production) were randomly selected for PCR targeting the most common genes mediating beta-lactam resistance in *E. coli*. Of pig isolates subjected to PCR, 66.7% were positive for $bla_{\text{CTX-M}}$ only, 71.1% for bla_{TEM} only, and 46.7% for both $bla_{\text{CTX-M}}$ and bla_{TEM} (Table 2). Among the samples from humans subjected to PCR, 68.5% were positive for $bla_{\text{CTX-M}}$ only, 42.5% for bla_{TEM} only and 24.7% for both $bla_{\text{CTX-M}}$ and bla_{TEM} (Table 2). Occurrence of $bla_{\text{CTX-M}}$ was similar in isolates from pigs and humans, whereas occurrence of bla_{TEM} was higher in pigs than in humans. Consequent to this, it was more common to observe these genes together in isolates from pigs than in those from humans (Table 2). The reasons for this are unknown, and it would be relevant to determine whether $bla_{\text{CTX-M}}$ and bla_{TEM} are genetically linked (e.g. harboured on the same plasmid) and therefore co-selected, in pig isolates. $bla_{\text{CTX-M}}$ was detected only among isolates showing an ESBL phenotype, whereas bla_{TEM} was detected both among ESBL-positive and ESBL-negative isolates (Table 2). The latter finding could be due to the presence of non-ESBL TEM variants such as TEM-1. Overall, it appeared that the ESBL phenotype

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was mainly mediated by CTX-M enzymes and to a lesser extent by TEM enzymes, thus being in line with findings in pig farm environments in Europe and Asia [18, 28, 33, 37]. Only three isolates from pigs and humans displayed an ESBL phenotype and were negative for the genes screened by PCR (Table 2), which suggests that additional ESBL types may occur sporadically. $bla_{\text{CTX-M}}$ was detected in isolates exhibiting different cephalosporin resistance profiles (Table 2), thus suggesting presence of different CTX-M variants and/or additional mechanisms contributing to cephalosporin resistance (e.g. porin loss). Our findings show that the epidemiology of cephalosporin resistance can be highly complex also in a population from a restricted geographical area sampled during a limited time frame.

Exchange of ESBL-producing *E. coli* between pig and pig farmers might have taken place at least in a subset of the studied farms as the same resistance genes were identified in eight of 11 cases in which isolates from both sources sharing the same resistance profiles were tested by PCR. Further genomic investigations including plasmid analysis would be necessary to assess this hypothesis, which is supported by studies in the Netherlands and in Denmark where highly related CTX-M-1-encoding plasmids were detected in *E. coli* from pig and pig farmers [29, 38]. The high occurrence of ESBL-producing *E. coli* in pigs and pig farmers presents a risk for spillover of these bacteria in Vietnamese communities both via social interactions of the farmers and via food. Human-human interactions are considered important routes of transmission of ESBLs [39]. Pork contamination by ESBL-producing bacteria is very high in Vietnam, with a recent study showing that 40.6% of pork samples sold in Nha Trang were ESBL-positive [14]. This is not surprising considering that *E. coli* originating from the pigs' own faeces or cross-contamination between pigs slaughtered on the same day is an important source of carcass contamination [39], and we have shown in this study that pig faeces carry ESBL-producing *E. coli* at high prevalence. The human health consequences of ESBLs in Vietnamese pork are unknown but efforts should be made to reduce their numbers in line with a precautionary principle.

Together with previous studies, our findings document a strong and immediate need to strengthen control measures to ensure prudent antimicrobial use practices in Vietnamese pig production. This includes coordinated efforts across different ministries and authorities. Not only should the antimicrobial use practices be changed and access to quality, independent veterinary services improved, but biosecurity measures at pig farms, including use of disinfectants and vaccines, should be implemented. Our study strongly indicates the urgent need to establish integrated national programs of surveillance of antimicrobial use and occurrence of ESBL-producing *E. coli* (and other antimicrobial-resistant bacteria with zoonotic potential) among people, livestock and foods in Vietnam.

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