Faecal carriage of extended-spectrum β-lactamase-producing and AmpC β-lactamase-producing bacteria among Danish army recruits

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Abstract

During May and June 2008, 84 Danish army recruits were tested for faecal carriage of extended-spectrum β-lactamase (ESBL)-producing and AmpC β-lactamase-producing bacteria. Three ESBL-producing (CTX-M-14a) *Escherichia coli* isolates, two AmpC-producing (CMY-2) *E. coli* isolates and one AmpC-producing (CMY-34) *Citrobacter freundii* isolate were detected. Two of the CTX-M-14a *E. coli* isolates had similar pulsed-field gel electrophoresis and multilocus sequence typing profiles, indicating the same origin or transmission between the two army recruits. The $bla_{CTX\text{-}M\text{-}14a}$ genes were transferable to an *E. coli* recipient. These commensal bacteria therefore constitute a reservoir of resistance genes that can be transferred to other pathogenic bacteria in the intestine.

Keywords: Cephalosporins, CTX-M, *Enterobacteriaceae*, faecal carrier, gene transfer

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Extended-spectrum β-lactamases (ESBLs) are increasingly being reported in Europe. In human patients, ESBLs are mostly found in *Escherichia coli* isolates causing community-acquired infections; in particular, CTX-M enzymes are increasingly being detected [1]. As ESBL-producing bacteria have also been detected in food and animals, there might be a possibility of transfer of the ESBL-producing bacteria, or their resistance genes, from animals to humans, either by direct contact or via the food chain [2–8]. It is therefore of interest to look for faecal carriage of ESBL-producing or AmpC-producing bacteria in healthy humans in the community. However, only a few studies have investigated this [9–12].

The aim of the present study was to screen for ESBL-producing or AmpC-producing bacteria among Danish healthy humans not recruited through the healthcare system, and to characterize the resistant isolates in relation to virulence genes and transferability of the resistance genes.

Between 20 May and 17 June 2008, 84 of 120 army recruits (70.0%) from one military barracks in Denmark agreed to participate in the study. Each person mailed a faecal sample to the Statens Serum Institut (SSI) and filled in a questionnaire with information on gender, age, medications, gastrointestinal symptoms and diseases, travel activities, food consumption and animal contact. The Scientific Ethics Committee for the Copenhagen and Frederiksberg municipalities approved the protocol prior to the investigation (KF 01-006/02). Only eight of the 84 recruits were women, and the median age was 21 years (range: 18–31 years). The recruits had been resident in the barracks for 6 months for training, but went home each weekend.

Half a gram of faeces was suspended in 5 mL of saline (0.9%). One hundred microlitres of the diluted faecal sample was spread on two SSI Enteric agar plates (SSI Diagnostika, Hillerød, Denmark), one supplemented with 2 mg/L cefotaxime and the other with 2 mg/L ceftazidime. Both plates were incubated in ambient air for 18 h at 35°C. Presumptive third-generation cephalosporin-resistant *Enterobacteriaceae* were subcultured on 5% blood agar plates (SSI Diagnostika). Only one colony per sample was investigated further. The isolates were identified with API 20E (BioMérieux, Marcy-l'Etoile, France) and retested for susceptibility to cefotaxime and ceftazidime by Etest (AB Biodisk, Solna, Sweden).

ESBL and/or AmpC phenotypes were tested with the NeoSensitabs double-disk method according to the manufacturer's guidelines (Rosco Diagnostica A/S, Taastrup, Denmark). On the basis of the obtained phenotype, the presence of TEM, SHV, CTX-M, OXA, MOX, CIT, DHA, ACC, EBC and FOX β-lactamase genogroups was investigated by PCR [13] (F. Hansen, unpublished data). Positive amplicons were sequenced. *E. coli* isolates were typed by pulsed-field gel electrophoresis and multilocus sequence typing [14,15]. MICs were determined for chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulphamethoxazole, tetra-

cycline and trimethoprim (Trek Diagnostic, East Grinstead, UK). Data were interpreted with the EUCAST breakpoints.

The phylogenetic background (A, B1, B2, D) of the *E. coli* isolates was determined by triplex PCR with three DNA markers [16]. The results obtained allowed classification of the isolates into the four major *E. coli* phylogenetic lineages or as non-typeable isolates according to Gordon *et al.* [17].

The *E. coli* isolates were investigated for the extraintestinal pathogenic *E. coli* (ExPEC)-related virulence genes *kpsM*II, *papA*, *papC*, *iutA*, *sfaS*, *focG*, *afa* and *hlyD* (unpublished data).

In vitro matings were obtained according to Trobos *et al.* [15]. The recipient strain was a rifampin-resistant mutant of *E. coli* K-12 (MG1655). The selective plates used were Luria–Bertani agar (Sigma-Aldrich, Brøndby, Denmark) supplemented with: (i) 2 mg/L cefotaxime and 50 mg/L rifampin; (ii) 2 mg/L cefotaxime; or (iii) 50 mg/L rifampin.

From faecal samples of six male army recruits, five *E. coli* isolates and one *Citrobacter freundii* isolate were obtained (one isolate per person) (Table 1). Three of the five *E. coli* isolates had an ESBL phenotype and were positive for CTX-M-14a. The two other *E. coli* isolates had an AmpC phenotype and were positive for CMY-2. The *C. freundii* isolate had an AmpC phenotype and was positive for CMY-34 (Table 1). Two of the ESBL-producing *E. coli* isolates (3807X08 and 3827X08) had identical pulsed-field gel electrophoresis, multilocus sequence typing and resistance profiles. Both isolates were obtained from faecal samples sent within 1 week; it is therefore likely that these isolates had the same origin or that there had been transmission between these two army recruits. Only one of the recruits had been in contact with animals. CTX-M-14 has also been detected in faecal samples from healthy humans in Spain, indicating a community reservoir of this enzyme in other countries [10–12].

Two isolates (3786Z08 and 3823Z08) contained the *iutA* gene, whereas the other *E. coli* isolates were negative for all virulence genes tested. None of the five *E. coli* isolates was an ExPEC isolate according to the molecular definition of ExPEC isolates [18]. Urinary tract infections are most often associated with phylogroup B2 and, to some extent, with phylogroup D. One of the tested *E. coli* isolates belonged to phylogroup D, whereas none of them belonged to phylogroup B2 (Table 1). Rates of transfer of phenotypic resistance to the *E. coli* and *C. freundii* donors are presented in Table 1. The $bla_{CTX\text{-}M\text{-}14a}$ genes had a high transfer rate.

The six army recruits had not been hospitalized within 1 month before the faecal samples were taken, and nor had they travelled abroad within 3 months prior to sampling. All had been eating meat (pork, poultry meat and beef) on a regular basis (Table 1). Two of the six army recruits had

TABLE 1. Description of six army recruits with extended-spectrum β-lactamase (ESBL)-producing or AmpC-producing bacteria

	Background data												
ID	Gender	Age (years)	Intake of antimicrobial agents[a]	Meat eaten[b]	Contact with animals[c]	Species	Phenotype	Genes encoding the β-lactamases	MLST type	PFGE type	Resistence to non-β-lactams	Phylotype	ExPEC-related genes	Transfer rate[d]
3786Z08	Male	22	Penicillin	Yes	Dog	*Escherichia coli*	AmpC	bla CMY-2	ST1822	1	Chloramphenicol, spectinomycin, streptomycin, sulphamethoxazole	D	*iutA*	$<1 \times 10^{-8}$
3823Z08	Male	21	Dicloxacillin	Yes	Dog	*E. coli*	AmpC	bla CMY-2	ST1800	2	Ciprofloxacin, nalidixic acid, spectinomycin, streptomycin, sulphamethoxazole, trimethoprim	B1	*iutA*	$<1 \times 10^{-8}$
3826Z08	Male	20	No	Yes	Dog, cat, wild birds, hunting animals	*Citrobacter freundii*	AmpC	bla CMY-34		–	None		–	$<1 \times 10^{-8}$
3792X08	Male	21	No	Yes	Dog	*E. coli*	ESBL	bla CTX-M-14a	ST641	3	None	B1	–	7.6×10^{-4}
3807X08	Male	19	No	Yes	None	*E. coli*	ESBL	bla CTX-M-14a	ST1631	4	None	NT	–	4.7×10^{-4}
3827X08	Male	19	No	Yes	Dog, cat, reptiles, parrot	*E. coli*	ESBL	bla CTX-M-14a	ST1631	4	None	NT	–	4.9×10^{-4}

ExPEC, extraintestinal pathogenic *E. coli*; MLST, multilocus sequence typing; NT, non-typeable; PFGE, pulsed-field gel electrophoresis.
[a]Within 6 months before the faecal sample was taken.
[b]Pork, poultry meat and beef on a regular basis.
[c]Within 1 week before the faecal sample was taken.
[d]Transconjugants/donor.

been treated with a penicillin compound (penicillin and dicloxacillin, respectively) within 6 months before sampling. Such treatment might have selected for the resistant *E. coli* isolates. Five recruits had been in contact with companion animals or hunting animals, but not with food animals, within the last week before sampling.

It was not possible to screen the meat that the recruits had been eating or the animals that they had been in contact with for ESBL/AmpC-producing bacteria, but a common source seems likely.

The investigated *E. coli* isolates were not virulent, and the ESBL-producing isolates were susceptible to all of the non-β-lactam antibiotics tested, but, because of their excellent ability to transfer resistance genes, they may act as donors of ESBL genes for other more virulent and resistant *E. coli* isolates.

In summary, even though the ESBL prevalence in hospitals is low in Denmark as compared with southern European countries, six of the 84 studied Danish army recruits (7%) harboured ESBL-producing or AmpC-producing bacteria in their gut, which indicates a human faecal reservoir in the community.

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Transparency Declaration

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