Faecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae is common 12 months after infection and is related to strain factors

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Abstract

We aimed to determine the duration of faecal carriage of extended-spectrum β-lactamase (ESBL) -producing Enterobacteriaceae (EPE) in patients with clinical infection caused by an EPE, to study host strains during carriage, and to identify factors associated with prolonged carriage. Patients (n = 61) were followed with faecal samples and questionnaires about antimicrobial treatment and risk factors for EPE, 1, 3, 6 and 12 months after EPE infection. The EPE isolates were subjected to ESBL genotyping, epidemiological typing with pulsed-field gel electrophoresis and PCR-based replicon typing. *Escherichia coli* isolates were analysed with PCR for phylogrouping, detection of *pabB* (ST131) and virulence content. Patient-related and strain-related variables were compared for carriers and non-carriers at 12 months. Carriage of EPE was observed in 51 of 61 (84%) patients after 1 month, 36 of 61 (66%) after 3 months, 31 of 61 (55%) after 6 months and 26 of 61 (43%) after 12 months. Of the 26 carriers at 12 months, five had previous negative samples. In 17 of 61 patients, ESBL was found in a new bacterial species and/or strain during carriage. Among *E. coli*, 14 of 49 belonged to the international clone ST131. Phylogroup B2 and CTX-M-gr.-9 were associated with being carriers at 12 months (OR 4.3, 95% CI 1.1–16.3 and OR 6.4, 95% CI 1.3–30.9, respectively). In conclusion, EPE carriage is common 12 months after infection and persisting carriage may be associated with *E. coli* phylogroup B2 and CTX-M-gr.-9. The host strain frequently changes throughout carriage and negative samples do not imply eliminated carriage.

Keywords: CTX-M-15, extended-spectrum β-lactamase, phylogroup B2, ST131, stool colonization
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Introduction

Extended-spectrum β-lactamase (ESBL) -producing *Escherichia coli* and *Klebsiella pneumoniae* have become important causes of nosocomial and community-acquired infections, and high numbers of ESBL carriers among healthy individuals have been reported from several countries [1]. The rapid increase of CTX-M-15 among ESBL-producing *E. coli* is largely linked to the internationally spread *E. coli* clone ST131-O25b [2]. Comorbidities, use of antimicrobial agents and hospital contact are well-known risk factors for community-onset infections with ESBL-producing Enterobacteriaceae (EPE) [1], but transmission of EPE within households outweighs nosocomial dissemination in the non-outbreak setting [3].

Although the prevalence and risk factors for EPE carriage have been studied, little is known about the time course of stool colonization. Previous reports show that EPE carriage often persists for 3 months and may be prolonged by antibiotic treatment [4]. In other studies, 51.5% were EPE carriers after 6 months (22nd European Congress of Clinical Microbiology and Infectious Diseases, poster 1673), 20% after 12 months,

but <5% after 2 years [5]. Persisting carriage of *K. pneumoniae* carbapenemase-producing *K. pneumoniae* was recently associated with catheter use, low functional status and long-term care facility stay [6].

Non-ESBL *E. coli* strains differ widely in their capacity to colonize the colon, and this capacity is linked to virulence factors associated with uropathogenicity [7]. Genes encoding P fimbriae, type 1 fimbriae and other virulence factors are enriched in resident strains, and there seems to be an additive effect of these factors promoting persistence [7–9]. Strains belonging to phylogroups B2 and D have an enhanced ability to persist in the intestinal microbiota [7]. How pathogen-related factors impact the duration of carriage of EPE is unknown.

ESBL genes are often located on conjugative plasmids belonging to the IncF group [10,11], 11, N and K, with high potential of recombination [12]. It is therefore likely that ESBL enzymes are transferred from transient strains to resident strains in the normal intestinal microbiota. However, knowledge about transfer of ESBL enzymes between different strains and species during the course of ESBL carriage is limited.

We present prospective data on the duration of faecal carriage of EPE after first-time EPE infection, and identify patient and strain factors associated with prolonged carriage. We also report on changes in host strains throughout the carriage, and present data on plasmid replicon content for cases for which the ESBL production was detected in a new strain or species during the course of carriage.

Materials and Methods

Study design, outcome measures and definitions

The study was designed as a prospective cohort study. Sixty-one patients were subjected to follow up with faecal samples and questionnaires about antimicrobial treatment and risk factors for EPE infection at 1, 3, 6 and 12 months after diagnosis. The study outcome was faecal carriage of EPE. Prolonged carriage was defined as the finding of EPE in faeces at 12 months.

Study population

The Karolinska University Hospital laboratory covers 80% of the Stockholm County population. In 2009 the proportions of ESBL-producing *E. coli* and *K. pneumoniae* in Stockholm were 3.3% and 2.8% [13]. The incidence of EPE infections was 40/100 000 inhabitants [14].

All patients' clinical isolates of EPE detected at Karolinska University Hospital between February and December 2009 were identified (n = 508). Patients that were likely to be able to understand instructions in Swedish and that would be able to perform self-collection and submission of faecal samples were eligible for inclusion. Since EPE was notifiable and was still rare in Sweden at the time of the study, the regional research ethics committee requested that the patients' physicians were contacted to obtain approval to contact the patients. Reasons for exclusion are shown in the Supporting information (Fig. S1). Seventy-one patients were included. Nine were lost to follow-up and one patient died. Characteristics of the patients included and not included in analyses are presented in Table 1.

Collection of samples and clinical data

At the specified time-points, 1, 3, 6 and 12 months after the patients' diagnosis with EPE infection, self-collected faecal samples were obtained. At 1 month the patients filled in a questionnaire about antimicrobial treatment received and previously described risk factors for EPE infection (antimicrobial treatment, hospital stay, urinary catheter, travel abroad and hospital admissions abroad within 6 months before the EPE infection, and abnormalities of the urinary tract). At months 3, 6 and 12 the patients filled in a questionnaire about new acquired infections and recently received antimicrobial treatment.

TABLE 1. Characteristics of 508 patients with extended spectrum β-lactamase-producing *Enterobacteriaceae* detected at the clinical microbiology laboratory at Karolinska University Hospital between February and December 2009[a]

	All patients (n = 508)	Patients included in analyses (n = 61)	Patients not included in analyses (n = 447)	p value
Female	337 (66)	38 (61)	299 (67)	0.48
Age (mean)	57.8	58.3	57.7	0.87
Level of care				
Hospital inpatients	308 (60)	35 (57)	273 (61)	0.002
Hospital outpatients	50 (10)	14 (23)	36 (8)	
Long-term care facilities	25 (5)	0	25 (6)	
Primary care	125 (25)	12 (20)	113 (25)	
Culture material				
Urine	437 (86)	51 (84)	386 (86)	0.39
Blood	24 (5)	5 (8)	19 (4)	
Other	47 (9)	5 (8)	42 (9)	

[a]Data presented as number and column percentage in parenthesis if not otherwise stated.