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Long-term Carriage of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* and *Klebsiella pneumoniae* in the General Population in The Netherlands

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Background. This longitudinal study aimed to investigate (risk factors for) persistence of carriage and molecular characteristics of extended-spectrum and plasmid-encoded AmpC β-lactamase–producing (ESBL/pAmpC) *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-E/K) in adults in the Dutch community.

Methods. Following a cross-sectional study (ESBL-E/K prevalence, 4.5%), a subset of ESBL-E/K–positive (n = 76) and –negative (n = 249) individuals volunteered to provide 5 monthly fecal samples and questionnaires. ESBL-E/K was cultured using selective enrichment/culture, and multilocus sequence types (MLSTs) were determined. ESBL/pAmpC-genes were analyzed using polymerase chain reaction (PCR) and sequencing. Plasmids were characterized and subtyped by plasmid MLST. Risk factors for persistent carriage were analyzed using logistic regression.

Results. Of the initially ESBL-E/K–positive participants, 25 of 76 (32.9%) remained positive in all subsequent samples; 51 of 76 persons (67.1%) tested ESBL-E/K negative at some time point during follow-up, of which 31 (40.8%) stayed negative throughout the longitudinal study. Carriers often carried the same ESBL gene and plasmid, but sometimes in different ESBL-E/K strains, indicative for horizontal transfer of plasmids. Of the 249 initially ESBL-E/K–negative participants, the majority (n = 218 [87.6%]) tested negative during 8 months of follow-up, whereas 31 of 249 (12.4%) participants acquired an ESBL-E/K. *Escherichia coli* phylogenetic group B2 and D and travel to ESBL high-prevalence countries were associated with prolonged carriage.

Conclusions. ESBL-E/K carriage persisted for >8 months in 32.9% of the initially ESBL-positive individuals, while 12.4% of initially negative individuals acquired ESBL-E/K during the study. A single positive test result provides no accurate prediction for prolonged carriage. Acquisition/loss of ESBL-E/K does not seem to be a random process, but differs between bacterial genotypes.

Keywords. ESBL; pAmpC; antibiotic resistance; carriage; ST131.

Infections due to extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae are increasing worldwide and are often preceded by asymptomatic carriage [1]. Previous cross-sectional studies in the Netherlands have reported a prevalence of fecal carriage of ESBL/plasmid-encoded AmpC (pAmpC)–producing Enterobacteriaceae of 5%–10% [2, 3].

Thus far, a limited number of longitudinal studies have been performed, mainly in patients. Alsterlund et al [4, 5] followed up on patients after an outbreak of ESBL-producing *Escherichia coli*: 12% still carried the bacteria after a median of 58 months, 43% had repeatedly negative cultures after shedding bacteria for a median of 7.5 months, and 38% had died while still shedding the bacteria for a median of 9 months. Zahar et al [6] found a median duration of carriage of 4.3 months in patients upon readmission after previous hospitalization. Titelman et al [7] reported that 43% of individuals with clinical infections with ESBL-producing Enterobacteriaceae still tested positive after 12 months, but often a different *E. coli* strain and/or ESBL gene was found. Löhr et al [8] found a median carriage time of 12.5 months in infants colonized with *Klebsiella pneumoniae* during an outbreak in a neonatal intensive care unit. A study investigating duration of fecal carriage of ESBL-producing *E. coli* or *K. pneumoniae* in patients with community-acquired urinary tract infection observed a clearance rate of 56% after 1 year [9]. Overdevest et al [10] found a half-life of 13 months for ESBL sequence type (ST) 131 carriage vs a 2- to 3-month half-life for other STs in a long-term care facility. The median duration of colonization with ESBL-producing Enterobacteriaceae after international travel was 30 days [11].

The aforementioned studies focused on patients and travelers, but little is known about the length of carriage in the population at large. Most studies investigate presence/absence of ESBL-producing bacteria, but data on the molecular characteristics of the strains are scarce and follow-up studies on ESBL-negative

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persons are lacking. The objectives of our longitudinal study were (1) to investigate duration of and risk factors for prolonged carriage of ESBL and pAmpC β-lactamase–producing *E. coli* and *K. pneumoniae* (ESBL-E/K) in predominantly healthy persons in the community; (2) to examine whether ESBL-E/K–positive individuals remained colonized with the same strain/ESBL gene/plasmid; and (3) to determine how many ESBL-E/K–negative persons acquire these resistant bacteria during follow-up.

MATERIALS AND METHODS

Study Population and Sample Collection

This study was part of the Livestock Farming and Neighbouring Residents' Health Study. First, a cross-sectional study was performed among 2432 adults with an ESBL-E/K prevalence of 4.5%, as described by Wielders et al [12]. The criteria for inclusion were age between 20 and 72 years old, living in the province North-Brabant or Limburg, and not living or working on a livestock farm. Per home address, 1 person was randomly selected. Extensive questionnaires were filled out by all participants [12].

Following the cross-sectional study [12], a subset of the participants participated in the longitudinal study. All eligible participants from the cross-sectional study (sample moment T0) whose fecal sample tested phenotypically positive for ESBL-E/K were invited (n = 150). Two phenotypically ESBL-E/K–negative participants per phenotypically ESBL-E/K positive–participant were randomly selected (n = 300) and invited. Participants (n = 333; response, 74.0%) were asked to take 5 fecal swabs themselves using transport swabs with Amies medium and to fill in a short questionnaire with an interval of 1 month (sample moments 1–5 [T1–T5]). This questionnaire included questions on antimicrobial usage, contact with animals, travel, and hospitalization during the 4 weeks prior to sampling. When 2 consecutive samples were submitted within 15 or >45 days after each other, 1 of the samples was excluded from the analysis. Participants who provided samples and questionnaires for at least 4 of 6 sample moments were included in the analysis (Figure 1).

The medical ethical committee of the University Medical Center Utrecht, the Netherlands, approved the study (number 13/533). All participants provided written informed consent.

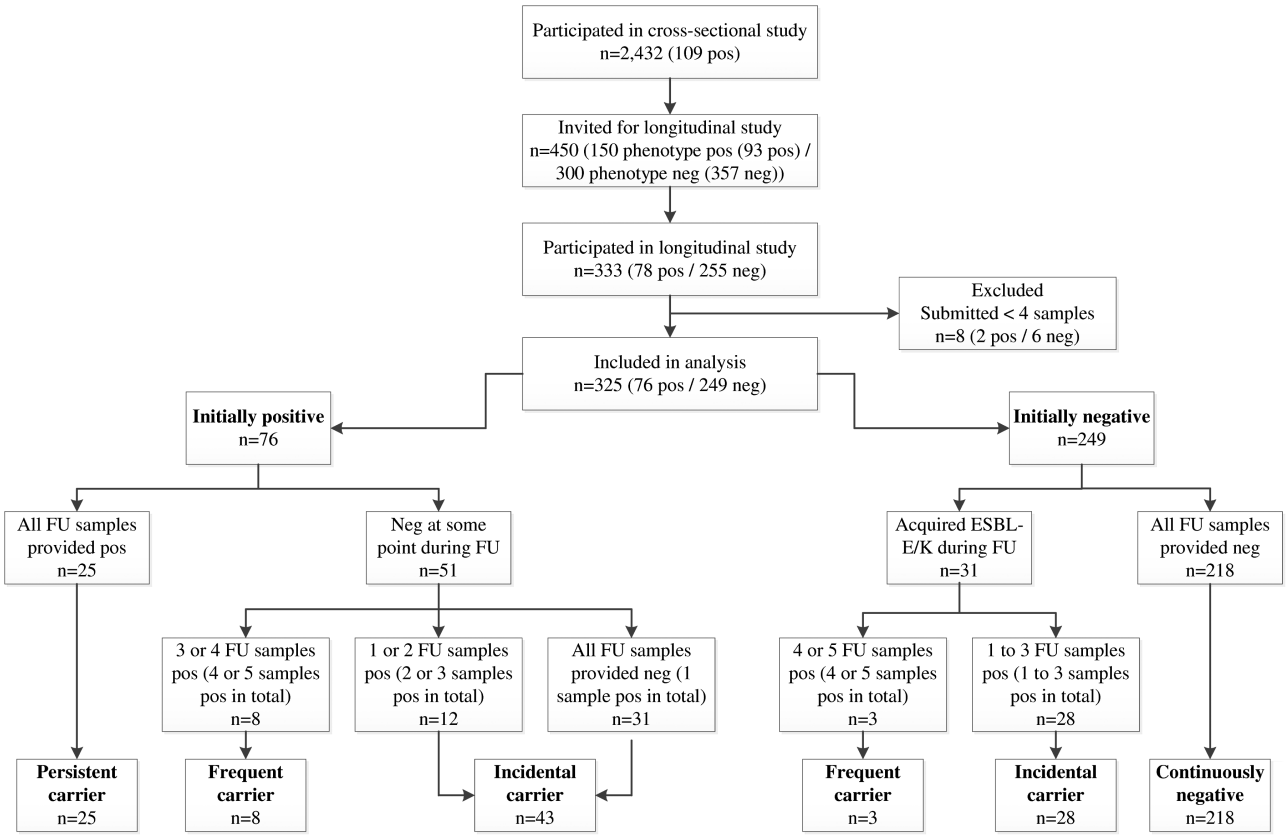

Figure 1. Flowchart of the results of the studied participants with a categorization into groups. Abbreviations: ESBL-E/K, extended-spectrum β-lactamase and plasmid-encoded AmpC β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*; FU, follow-up (the longitudinal study/sample moments T1–T5); neg, ESBL-E/K–negative genotype; pos, ESBL-E/K–positive genotype.

Microbiological Examination

A detailed description of the microbiological methods can be found in Wielders et al [12]. Fecal samples were cultured using selective enrichment and selective agar supplemented with 1 mg/L cefotaxime. The mean time from taking the sample to incubation was 2.5 days. Five colonies per person per positive sample were chosen on morphological appearance and investigated.

Phenotypically confirmed ESBL-E/K samples were screened for the presence of bla_{CTX-M} and/or bla_{CMY} and bla_{DHA} β-lactamase genes by polymerase chain reaction (PCR). If negative, these isolates were additionally screened for bla_{OXA}, bla_{SHV}, and bla_{TEM}. The complete ESBL and/or pAmpC gene sequence was determined as described previously [13].

Phylogenetic groups were determined for *E. coli* [14]. One isolate per phylogenetic group of each sample was genotyped by multilocus sequence typing (MLST) [15]. *Klebsiella pneumoniae* isolates were typed by MLST using protocol 2 with universal sequencing primers (http://bigsdb.pasteur.fr/klebsiella/primers_used.html) [16]. Plasmids were characterized by transformation and PCR-based replicon typing [17]. Subtyping was done by plasmid MLST (pMLST) [18–20].

An individual was considered positive for ESBL-E/K if presence of an ESBL/pAmpC gene was confirmed.

Statistical Analyses

Participants were categorized into 4 groups depending on their number of ESBL-E/K–positive test results: continuously negative, incidental, frequent, or persistent carriers (Figure 1). Univariate logistic regression analysis was performed to study potential risk factors for prolonged (ie, persistent and frequent carriage of ESBL-E/K combined) compared to no carriage (continuously negative) using the extensive cross-sectional questionnaire (T0). Fisher exact test (when expected count <5) and χ^2 tests were performed, and odds ratios (ORs) with 95% confidence intervals (CIs) were obtained. The Benjamini-Hochberg procedure with a 10% false discovery rate was used to correct for the number of univariate tests performed [21]. Data from the short questionnaire (T1–T5) were analyzed on the individual level and recoded to antibiotic use, hospitalization, travel, and contact with animals at any moment during 4 weeks before sampling. Molecular typing data (phylogenetic group, CTX-M group, ESBL/pAmpC gene, MLST) of prolonged carriers were compared to incidental carriers by univariate and multiple logistic regression analysis. A P value <.10 (χ^2 test or Fisher exact test) was used as threshold to be included in the multiple logistic regression analysis and a P value <.05 was used to determine significance. Furthermore, Kaplan-Meier survival analysis was used (T1–T5) to estimate the rate of ESBL-E/K clearance similar to the method described by Jørgensen et al [9]. ESBL-E/K clearance was defined as 2 consecutive negative samples and the event end point was set to occur at the first negative sample if the following sample was also negative. Data were analyzed using SAS version 9.4 software (SAS Institute, Cary, North Carolina).

RESULTS

The median time between T0 and T1 was 125 days (range, 71–234 days; interquartile range [IQR], 106–141 days), where T0 is the sample from the cross-sectional study and T1 is the first sample from the longitudinal study. The median total study duration (time between T0 and the last submitted sample) was 243 days (range, 189–345 days; IQR, 221–257 days).

In total, 1923 samples were taken by participants submitting ≥4 samples and 27 samples were excluded because they were submitted within 15 days or >45 days of the consecutive sample. This resulted in 1896 included samples from 325 participants. *E. coli* was found in 282 samples originating from 100 participants; *K. pneumoniae* was found in 15 samples originating from 10 participants. In total, 107 participants tested positive at least once during the study (Figure 1). ESBL-E/K was found in all consecutive samples of 25 participants (6/6 [n = 22]; 5/5 [n = 2]; 4/4 [n = 1]) further defined as persistent carriers. In addition, 11 participants had 4 or 5 positive samples and 1 or 2 negative samples (5/6 [n = 8]; 4/6 [n = 3]), further defined as frequent carriers (Table 1). The number of ESBL-E/K–positive and –negative participants at the different time points is summarized in Table 2.

Of the 76 initially ESBL-E/K–positive participants, 25 (32.9%) tested positive for ESBL-E/K in all samples provided, of which 24 carried the same ESBL gene and 17 also carried the same E/K ST for all positive samples. The carriage rate was 39 of 76 (51.3%) at sample T1 (median, 4.0 months since sample moment T0), 34 of 69 (49.3%) at T2 (median, 5.2 months), 33 of 75 (44.0%) at T3 (median, 6.1 months), 31 of 72 (43.1%) at T4 (median, 7.1 months), and 32 of 75 (42.7%) at T5 (median, 8.0 months).

Of the 249 initially ESBL-E/K–negative participants, the majority (n = 218 [87.6%]) tested continuously negative. Overall, 31 of 249 (12.4%) participants acquired an ESBL-E/K during the course of the longitudinal study and 3 (1.2%) became frequent carriers. The carriage rate for initially ESBL negatives was 7 of 249 (2.8%) at T1 (median, 4.1 months since sample moment T0), 8 of 226 (3.5%) at T2 (median, 5.3 months), 15 of 247 (6.1%) at T3 (median, 6.1 months), 10 of 243 (4.1%) at T4 (median, 7.1 months), and 10 of 239 (4.2%) at T5 (median, 8.1 months).

The Kaplan-Meier analysis showed that 29 of the 45 (64.4%) individuals positive for ESBL-E/K at T1 were still positive at T5 (1 person was excluded because of 1 missing sample followed by a negative and a positive sample). When this is translated to the findings of the cross-sectional study, this means that after 4 months, 70 of the 109 ESBL-E/K positive individuals would still be carriers (2.9% of the total cross-sectional study population).

Table 1. Description of Persistent, Frequent, Incidental, and Continuously Negative Carriers (N = 325)

Type of Carrier	No. of Positive/Negative ESBL-E/K Samples	No. of Participants	No. of Initial ESBL-E/K–Positive/Negative Participants at Sample Moment T0	Percentage of the Total No. of Participants
Persistent carriers[a] (n = 25)	6/0	22	22/0	6.8
	5/0	2	2/0	0.6
	4/0	1	1/0	0.3
Frequent carriers[b] (n = 11)	5/1	8	6/2	2.5
	4/2	3	2/1	0.9
Incidental carriers[c] (n = 71)	3/3	8	5/3	2.5
	3/2	1	1/0	0.3
	2/4	8	6/2	2.5
	1/5	45	24/21	13.8
	1/4	8	6/2	2.5
	1/3	1	1/0	0.3
Continuously negative (n = 218)	0/6	184	0/184	56.6
	0/5	29	0/29	8.9
	0/4	5	0/5	1.5

Abbreviation: ESBL-E/K, extended-spectrum β-lactamase and plasmid-encoded AmpC β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*.

[a]Persistent carriers: all samples provided were ESBL-E/K positive.

[b]Frequent carriers: 4 or 5 positive samples and 1 or 2 negative samples.

[c]Incidental carriers: 1, 2, or 3 positive samples.

ESBL/pAmpC Genes

The predominant genes found were $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}1}$, $bla_{CMY\text{-}2}$, $bla_{SHV\text{-}12}$, and $bla_{CTX\text{-}M\text{-}27}$ found in samples originating from 44 (41.1%), 20 (18.7%), 13 (12.1%), 11 (10.3%), 9 (8.4%), and 8 (7.5%) participants, respectively. Other genes characterized were $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}3}$, $bla_{CTX\text{-}M\text{-}8}$, $bla_{CTX\text{-}M\text{-}65}$, $bla_{SHV\text{-}2}$, $bla_{TEM\text{-}52}$, and $bla_{DHA\text{-}1}$, found in only 1 or 2 participants.

E. coli and *K. pneumoniae* Sequence Types

In total, 85 different *E. coli* STs were found. The predominant *E. coli* STs in this study were ST131, ST10, ST38, and ST69. ST131 was found in 72 samples originating from 19 participants; ST10 in 37 samples from 17 participants, ST38 in 26 samples from 8 participants, and ST69 in 14 samples from 8 participants. ST58 and ST88 were both found in 5 participants. For *K. pneumoniae*, STs identified were ST45, ST221, ST429, ST902, ST1715, ST2459, ST2462, and ST2670.

Plasmids

Plasmids were analyzed from all persistent and frequent carriers except for 1 frequent carrier.

In general, the first and last isolate with the same *E. coli* or *K. pneumoniae* ST were selected. IncF plasmids were most commonly found (in total in 21/35 [60.0%] persons) and were often associated with $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CTX\text{-}M\text{-}27}$. *E. coli* ST131 carrying different genes on incF plasmids predominated in persistent carriers (Table 3). In some occasions, the ESBL gene was not transferable by transformation or not typeable by PBRT (Table 3). All results including pMLST of persistent, frequent, and incidental carriers are displayed in Supplementary Table 1.

Within-Host Diversity

Testing multiple colonies per positive sample revealed that 23 of 107 (21.5%) participants carried >1 *E. coli* and/or *K. pneumoniae* ST in 46 of 295 (15.6%) of the total number of positive samples. In the majority of the samples with multiple isolates, 2 different isolates were found, of which 1 sample included 1 *E. coli* and 1 *K. pneumoniae* strain; in 5 samples, 3 different strains were identified, of which 1 sample included 2 *E. coli* strains and 1 *K. pneumoniae* strain. The maximum amount of different STs within 1 person during the study (T0–T5) was 6. This person carried 3 different ESBL genes, 5 different plasmid types, and 6

Table 2. Number of Extended-Spectrum β-Lactamase and Plasmid-Encoded AmpC β-Lactamase–Producing *Escherichia coli* and *Klebsiella pneumoniae*–Positive and –Negative Individuals Throughout the Study

		Sample Moment					
Participants	Cross-sectional Study [12]	0[a]	1	2	3	4	5
No. of positive	109	76	46	42	48	41	42
No. of negative	2323	249	279	253	274	274	272
Total No.	2432	325	325	295	322	315	314
% positive	4.5%	23.4%	14.2%	14.2%	14.9%	13.0%	13.4%

[a]Included from the cross-sectional study [12]

Table 3. Bacterial Strain (Sequence Type), Extended-Spectrum β-Lactamase (ESBL)/Plasmid-Encoded AmpC (pAmpC) Gene, and Plasmid Type Found in 36 Persistent and Frequent Carriers in Their First and Last ESBL-Positive Sample

First Positive Sample			Last Positive Sample		
Gene	ST	Plasmid	Gene	ST	Plasmid
CTX-M-15	131	**incFII/FIA**	CTX-M-15	131	**incFII/FIA**
	131	**incFII/FIA**		131	**incFII/FIA**
	131	**incFII**		2451	**incFII**
	405	**incFII/FIA/FIB**		405	**incFII/FIA/FIB**
	902[a]	**incFIIk**		902[a]	**incFIIk**
	95	incFII		95	TF
	131	incFII		131	TF
	131	TF		131	TF
	501	TF		501	TF
	93, 5017	NT, **incK**		10	**incK**
	2076, New 2	incK		227, 2076	incFIA/FIB/incK, incFIA/incK
	44, 394	**incI1**		44, 322	incFII/FIA/FIB,**incI1**
	1122	**incI1**		675	**incI1**
	701	**incX1**		701	**incX1**
	131	TF		398	incFIB
	New 1	TF		New1	Not determined
	58	NT		405	incFII/FIB
CTX-M-14	10, 69	**incFII**	CTX-M-14	10	**incFII**
	131	**incFII/FIA/FIB**		131	**incFII/FIA/FIB**
	648	**incFII**		648	**incFII**
	38	TF		38	TF
	38	TF		38	TF
	131	TF		131	TF
	38	incFII		38	TF
	69	NT		10	NT
	131	Not determined		69	incFII
	10	incFII		44, 1001	Not determined
CTX-M-27	131	IncFII/FIA/FIB	CTX-M-27	131	IncFII/FIA/FIB
	131	IncFII/FIA/FIB		131	IncFII/FIA/FIB
	1193	incFIA/FIB		1193	incFIA/FIB
	131	TF		131	Not determined
CTX-M-1	1250	**incN**	CTX-M-1	69, 1277	**incN**
CMY-2	1656	**incI1/FIB**	CMY-2	10	**incI1/FIB**
	429, 3727	**incK**	CMY-2	3727	**incK**
TEM-52	224	**incX1**	TEM-52	224	**incX1**
SHV-12	354	NT	SHV-12	155	NT

Bold text indicates that the gene was found on similar plasmid at different time points. This table summarizes results from the first and last sample per person in time that was extended-spectrum **β**-lactamase positive. Supplementary Table 1 displays all data from all different data points that were analyzed.

Abbreviations: NT, not typable with PBRT; PBRT, PCR-based replicon typing; ST, sequence type; TF, transformation failed.

[a] *Klebsiella* species.

E. coli STs and had traveled to Vietnam several times and once to Thailand during the study period. Diversity in resistance genes found was less pronounced: >1 ESBL/pAmpC gene was found in 12 persons either in the same sample or in subsequent samples. The maximum number of different ESBL/pAmpC genes found in 1 sample was 2, and 3 in the same person in different samples.

Risk Factors for Prolonged Carriage

Characteristics of the ESBL-E/K persistent, frequent, and incidental carriers and continuously negative individuals at T0 are shown in Table 4. Prolonged carriers (persistent and frequent combined, n = 36) were compared to individuals testing negative only using the questionnaire data at T0. Statistically significant determinants for prolonged carriage were antibiotic use during the last 6 months (OR, 4.68; 95% CI, 1.73–12.67), proton pump inhibitor use (OR, 2.71; 95% CI, 1.21–6.08), and living within 1000 m of at least 1 pig farm (OR, 2.87; 95% CI, 1.07–7.71). Antibiotic use during the last 3 months was also found to be statistically significant, but numbers were relatively small. None of the univariate-identified risk factors remained statistically

Table 4. Descriptive Characteristics of the Extended-Spectrum β-Lactamase and Plasmid-Encoded AmpC β-Lactamase–Producing *Escherichia coli* and *Klebsiella pneumoniae* Persistent, Frequent, and Incidental Carriers and Continuously Negative Individuals at Sample Moment T0

Determinant[a]	Total No.	Missing Info	ESBL-E/K Persistent Carrier (n = 25)	ESBL-E/K Frequent Carrier (n = 11)	ESBL-E/K Incidental Carrier (n = 71)	ESBL-E/K Continuously Negative (n = 218)
Demographics						
Sex						
Female	176		14 (56.0)	6 (54.6)	39 (54.9)	117 (53.7)
Male	149		11 (44.0)	5 (45.5)	32 (44.4)	101 (46.5)
Age, y						
20–29	2		0 (0.0)	0 (0.0)	1 (1.4)	1 (0.5)
30–39	16		1 (4.0)	0 (0.0)	3 (4.2)	12 (5.5)
40–49	44		3 (12.0)	0 (0.0)	9 (12.5)	32 (14.8)
50–59	90		6 (24.0)	3 (27.3)	19 (26.4)	62 (28.6)
≥60	173		15 (60.0)	8 (72.7)	39 (54.9)	111 (50.9)
Country of birth						
The Netherlands	316		25 (100.0)	11 (100.0)	69 (97.2)	211 (96.8)
Other	9		0 (0.0)	0 (0.0)	2 (2.8)	7 (3.2)
Place of residence during youth not in the study area	93		10 (40.0)	2 (18.2)	21 (29.6)	60 (27.5)
Educational level						
Low	88		8 (32.0)	3 (27.3)	17 (23.9)	60 (27.5)
Medium	145		9 (36.0)	5 (45.5)	34 (47.9)	97 (44.5)
High	92		8 (32.0)	3 (27.3)	9 (28.2)	61 (28.0)
Health						
Smoking						
Never smoker	129		9 (36.0)	5 (45.5)	25 (35.2)	90 (41.3)
Current or ex-smoker	196		16 (64.0)	6 (54.6)	46 (64.8)	128 (58.7)
Comorbidity[b]	43	75	6 (31.6)	2 (22.2)	10 (17.5)	25 (15.2)
Antibiotic use during last 6 months	26	75	4 (21.1)	4 (44.4)	5 (8.8)	13 (7.9)
Proton pump inhibitor use	55	3	7 (28.0)	4 (36.4)	14 (19.7)	30 (14.0)
Hospitalized during last 12 months[c]	40	1	4 (16.0)	1 (9.1)	13 (18.3)	22 (10.1)
Travel						
Travel during last 12 months		1				
No travel, travel to Western/Northern Europe, North America, Australia, or New Zealand	174		11 (44.0)	7 (63.6)	39 (54.9)	117 (53.9)
Travel to Southern/Eastern Europe	106		11 (44.0)	2 (18.2)	18 (25.4)	75 (34.6)
Travel to Africa, Asia (West/South/Southeast/East/Central, including Turkey), or Latin America	44		3 (12.0)	2 (18.2)	14 (19.7)	25 (11.5)
Exposure at work/study/home						
During work/study contact with patients	44	21	3 (13.6)	1 (9.1)	12 (18.5)	28 (13.6)
During work/study contact with residents of nursing homes	47	21	3 (13.6)	3 (27.3)	10 (15.4)	31 (15.1)
During work/study contact with children	36	21	1 (4.6)	4 (36.4)	7 (10.8)	24 (11.7)
During work/study contact with animals	24	21	2 (9.1)	3 (27.3)	6 (9.2)	13 (6.3)
Lived on a farm during childhood	97	2	6 (24.0)	5 (45.5)	19 (26.8)	67 (31.0)
Kept pets during the last 5 years[d]	151		11 (44.0)	7 (63.6)	36 (50.7)	97 (44.5)
Kept a cat	48		1 (4.0)	2 (18.2)	13 (18.3)	32 (14.7)
Kept a dog	74		7 (28.0)	3 (27.3)	24 (33.8)	69 (31.7)
Kept farm animals for a hobby during the last 5 years[e]	46	4	3 (12.0)	1 (9.1)	12 (17.4)	30 (13.9)
Visit to a farm last 12 months	193		11 (44.0)	8 (72.7)	48 (67.6)	126 (57.8)
Contact with animals during farm visit	103	5	6 (24.0)	5 (45.5)	28 (40.0)	64 (29.9)
Environmental exposure (livestock farms)						
Living within 1000 m of 1 or more farms (based on environmental license)						
All farm types combined	316		25 (100.0)	11 (100.0)	68 (95.8)	212 (97.3)
Cattle farm(s)	313		24 (96.0)	11 (100.0)	68 (95.8)	210 (96.3)
Goat farm(s)	9		0 (0.0)	1 (9.1)	3 (4.2)	5 (2.3)

Table 4. Continued

Determinant[a]	Total No.	Missing Info	ESBL-E/K Persistent Carrier (n = 25)	ESBL-E/K Frequent Carrier (n = 11)	ESBL-E/K Incidental Carrier (n = 71)	ESBL-E/K Continuously Negative (n = 218)
Horse farm(s)	216		18 (72.0)	5 (45.5)	47 (66.2)	146 (67.0)
Mink farm(s)	37		4 (16.0)	4 (36.4)	6 (8.5)	23 (10.6)
Pig farm(s)	236		22 (88.0)	9 (81.8)	45 (63.4)	149 (68.4)
Poultry farm(s)	150		13 (52.0)	5 (45.5)	35 (49.3)	97 (44.5)
Sheep farm(s)	62		2 (8.0)	3 (27.3)	13 (18.3)	44 (20.2)

Data are presented as No. (%).

Abbreviation: ESBL-E/K, extended-spectrum **β**-lactamase and plasmid-encoded AmpC **β**-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*.

[a]All determinants were assessed at sample moment T0 unless indicated otherwise (eg, antibiotic use during last 6 months indicates antibiotic use during 6 months prior to sample moment T0).

[b]Comorbidity includes cerebrovascular disease, chronic cardiovascular disease, liver disease, chronic lung disease, chronic renal disease, autoimmune disease, neurological comorbidity, diabetes, and malignancy.

[c]Hospitalized in the Netherlands and/or abroad.

[d]Bird, cat, dog, fish, guinea pig, hamster, mouse, rabbit, rat, or turtle.

[e]Chicken, cow, donkey, duck, goat, goose, horse, pig, pony, sheep, or turkey.

significant after performing the Benjamini-Hochberg procedure. Of the questionnaire data collected at sample moments T1–T5 (n = 1543 questionnaires), only travel at any time in the 4 weeks prior to sampling was found to be significantly associated with prolonged carriage—in particular, travel to Africa, Asia (West/South/Southeast/East/Central, including Turkey),

Table 5. Univariate and Multiple Logistic Regression Analysis[a] of Molecular Typing Characteristics of Extended-Spectrum β-Lactamase and Plasmid-Encoded AmpC β-Lactamase–Producing *Escherichia coli* and *Klebsiella pneumoniae* Persistent and Frequent (Prolonged) Carriage Compared to Incidental Carriage

Determinant	Total No.	ESBL-E/K Persistent and Frequent (Prolonged) Carrier (n = 36)	ESBL-E/K Incidental Carrier (n = 71)	Odds Ratio (95% CI)	Adjusted Odds Ratio[a] (95% CI)
Phylogenetic group					
A	42	14 (38.9)	28 (39.4)	0.98 (0.43–2.22)	
B1	27	11 (30.6)	16 (22.5)	1.51 (0.61–3.73)	
B2	29	19 (52.8)	10 (14.1)	6.82 (2.68–17.38)	11.24 (1.97–64.07)
D	39	19 (52.8)	20 (28.2)	2.85 (1.24–6.56)	10.37 (2.74–39.30)
B2 and D combined (presence of *chuA*)	63	33 (91.7)	30 (42.3)	15.03 (4.21–53.65)	
ESBL/pAmpC genes and CTX-M group					
CTX-M-1 group[b]	58	19 (52.8)	39 (54.9)	0.92 (0.41–2.05)	
$bla_{CTX-M-1}$	13	1 (2.8)	12 (16.9)	0.14 (0.02–1.13)	
$bla_{CTX-M-15}$	44	18 (50.0)	26 (36.6)	1.73 (0.77–3.90)	
CTX-M-9 group	28	15 (41.7)	13 (18.3)	3.19 (1.30–7.80)	1.85 (0.63–5.48)
$bla_{CTX-M-14}$	20	11 (30.6)	9 (12.7)	3.03 (1.12–8.21)	
$bla_{CTX-M-27}$	8	6 (16.7)	2 (2.8)	6.90 (1.32–36.17)	
Other genes					
bla_{CMY-2}	11	3 (8.3)	8 (11.3)	0.72 (0.18–2.88)	
bla_{SHV-12}[c]	9	1 (2.8)	8 (11.3)	0.23 (0.03–1.87)	
Multilocus sequence typing					
ST10	17	6 (16.7)	11 (15.5)	1.09 (0.37–3.23)	
ST38	8	5 (13.9)	3 (4.2)	3.66 (0.82–16.27)	
ST69	8	4 (11.1)	4 (5.6)	2.09 (0.49–8.91)	
ST131	19	14 (38.9)	5 (7.0)	8.40 (2.72–25.99)	2.48 (0.46–13.39)
Klebsiella pneumoniae	10	1 (4.0)	9 (12.7)	0.20 (0.02–1.62)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CI, confidence interval; ESBL-E/K, extended-spectrum **β**-lactamase and plasmid-encoded AmpC **β**-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*; ESBL/pAmpC, extended-spectrum **β**-lactamase and plasmid-encoded AmpC; ST, sequence type.

[a]Only the CTX-M groups were considered to be included in the multiple logistic regression analysis, not the separate ESBL/pAmpC genes.

[b]One isolate with 2 CTX-M-1 group genes that could not be distinguished is included in the number of CTX-M-1 group isolates, but is not included in the number of specific genes.

[c]One isolate with 2 SHV group genes that could not be distinguished is not included in the numbers.

or Latin America compared with no travel, travel to Western/Northern Europe, North America, Australia, or New Zealand (OR, 3.54; 95% CI, 1.12–11.21).

Prolonged carriage was statistically significantly associated with the detection of phylogenetic group B2 and D, CTX-M-9 group ESBL genes, and ST131 (Table 5). After adjustment with multiple logistic regressions analysis, the phylogenetic groups B2 and D showed the highest statistically significant ORs, whereas the CTX-M-9 group and ST131 were no longer significant. Furthermore, *K. pneumoniae* seemed to be more common among incidental carriers than among prolonged carriers in univariate analysis.

DISCUSSION

A cross-sectional study followed by a prospective cohort study was performed to investigate the duration of and risk factors for prolonged fecal carriage of ESBL-E/K in individuals in the population at large. Of the initially ESBL-E/K–positive persons, 32.9% stayed positive in all subsequent samples after a median carriage of 242 days. This carriage rate was similar to that of patients after a clinical infection in Sweden [7], but generally, the carriage rate among patients was slightly higher than in the present study in predominantly healthy persons [9]. In a study in travelers, median duration of colonization after travel was 30 days and only 14.3% and 11.3% remained colonized at 6 and 12 months after return, respectively [11], which is shorter than in the present study. A rapid clearance of *E. coli* resistant to third-generation cephalosporins in travelers after return was also found by Kennedy and Collignon [22].

Most initially ESBL-E/K–negative persons (87.6%) remained negative during the study, while 12.4% acquired an ESBL-E/K at least once during the study. Due to the active selection of ESBL-E/K positives, the carriage rate was 23.4% at the start of the longitudinal study, declined to 14.2% at T1, and remained stable at around 13%–15% between T2 and T5 (Table 2). This was higher than the prevalence of 4.5% found in the cross-sectional study [12]. It suggests that acquisition and loss of ESBL-E/K is not a random process. Host and bacterial factors play a role in colonization; *E. coli* strains differ in their capacity to colonize the colon [23]. Prolonged carriers more often carried *E. coli* isolates belonging to phylogenetic group B2 and D with ST131 as the major contributor to B2. This confirms the findings of Jørgensen et al [9] that these phylogenetic groups are associated with long-term carriage.

Interestingly, nearly all persistent carriers (24/25 [96.0%]) remained positive with the same gene often carried on the same plasmid, but this gene was not always found in the same ESBL-E/K strain. Two or more strains were found in 15.6% of all samples and in 87.0% of these, the same ESBL gene was found in different STs. This is indicative of horizontal gene transfer within the intestinal tract from 1 bacterial strain to the other through conjugation rather than the acquisition of a different ESBL-producing strain by the same person. In the latter case, one would expect a higher diversity in ESBL genes and plasmids within an individual. In addition, more uncommon ESBL genes such as $bla_{\text{CTX-M-2}}$ and $bla_{\text{TEM-52}}$ were also found in different STs in the same sample, and the chance of persons acquiring diverse *E. coli* strains with uncommon genes on different occasions seems unlikely. Titelman et al [7] found multiple ESBL-producing strains in fecal samples from Swedish patients. Thus, to describe the molecular epidemiology of ESBL, testing only 1 isolate leads to an underestimation of the diversity of the genotypes and resistance genes.

In prolonged carriers, CTX-M-9 group ESBL genes ($bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-27}}$) were more often found than in incidental carriers. In contrast, $bla_{\text{CTX-M-1}}$ persisted in only 1 person, although this gene was common in incidental carriers and in the cross-sectional study it was the third common ESBL gene. Only 1 persistent carrier carried *K. pneumoniae*. In 6 of the 12 persons who carried $bla_{\text{CTXM-14}}$ or $bla_{\text{CTX-M-27}}$, the gene was present on the same plasmid in the same *E. coli* ST in the first and last sample, indicative of persistence of successful clones rather than horizontal gene transfer.

At T0, prolonged carriers were more likely to have used antibiotics in the previous 6 months, proton pump inhibitors, and to live within 1000 m of at least 1 pig farm, although these were not statistically significant after performing the Benjamini-Hochberg procedure. During follow-up (T1–T5), only travel to Africa, Asia, and Latin America was associated with prolonged carriage; however, due to low numbers we were unable to perform multiple logistic regression analysis. Comparable results were found when analyzing persistent carriers vs continuously negative individuals, persons who tested positive at least once vs continuously negative, persons who acquired ESBL-E/K during T2–T5 vs persons who did not acquire ESBL-E/K, and prolonged carriers without comorbidity vs continuously negative individuals without comorbidity (data not shown).

In conclusion, ESBL-E/K carriage persisted for >8 months in one-third of the initially ESBL-positive individuals and was acquired by 12.4% of the ESBL-E/K negatives at least once during the longitudinal study. Certain ESBL-E strain/gene combinations were more often found in prolonged carriers, indicating that acquisition/loss of ESBL-E/K is not a random process. A single positive test result provides no accurate prediction for prolonged carriage, and repeated testing may be needed in clinical practice and population-based studies.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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