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Dynamics of Intestinal Carriage of Extended-Spectrum Beta-lactamase–Producing *Enterobacteriaceae* in the Dutch General Population, 2014–2016

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Background. In the Netherlands, the prevalence of intestinal extended-spectrum beta-lactamase–producing *Enterobacteriaceae* (ESBL-E) carriage in community-dwelling subjects is ~5%. Little is known about the dynamics of ESBL-E carriage.

Methods. In a nationwide, population-based study (2014–2016) with 4177 community-dwelling subjects, fecal samples from 656 subjects were collected after 1 (time point [T] = 1) and 6 (T = 2) months. The growth of ESBL-E was quantified and a whole-genome sequence analysis was performed. Subjects were categorized as either an incidental, short-term, or long-term carrier or as a noncarrier. Risk factors were determined by random forest models and logistic regression. The transmissibility and duration of ESBL-E carriage was quantified using a transmission model, which also incorporated previous study data.

Results. Out of 656 participants, 96 were ESBL-E carriers at T = 0. Of these, 66 (10.1%) subjects were incidental carriers, 22 (3.3%) were short-term carriers, and 38 (5.8%) were long-term carriers; the remaining 530 (80.8%) were noncarriers. The risk factors for long-term carriage were travelling to Asia, swimming in a sea/ocean, and not changing the kitchen towel daily. The log-transformed colony forming units ratio at T = 0 was predictive for ESBL-E carriage at T = 1 (odds ratio [OR], 1.3; 95% confidence interval [CI], 1.2–1.6) and T = 2 (OR, 1.2; 95% CI, 1.1–1.4). Model simulations revealed a median decolonization rate of 2.83/year, an average duration of carriage of 0.35 years, and an acquisition rate of 0.34/year. The trend of the acquisition rate during the study period was close to 0.

Conclusions. The risk factors for long-term ESBL-E carriage were travel- and hygiene-related. The dynamics of ESBL-E carriage in the general Dutch population are characterized by balancing decolonization and acquisition rates.

Keywords. extended-spectrum beta-lactamase–producing *Enterobacteriaceae*; ESBL-E; general population; the Netherlands; longitudinal.

Extended-spectrum beta-lactamase–producing *Enterobacteriaceae* (ESBL-E) are widely present in healthcare settings and the community. Infections caused by these bacteria are a public health concern [1]. We recently quantified the prevalence of ESBL-E carriage in the general population of the Netherlands [2], but little is known about the dynamics of ESBL-E carriage. Knowledge about risk factors, acquisition, persistence, and loss of carriage is relevant for planning control measures and predicting future epidemiological trends.

Although the occurrence of intestinal ESBL-E carriage has been determined in multiple cross-sectional studies, few studies have addressed longitudinal carriage of ESBL-E, and most were performed in clinical settings [3–6], after an outbreak [7], or after travel-associated acquisition of ESBL-E [8]. In a rural, adult population in the Netherlands, 76 ESBL-E carriers and 249 noncarriers were subsequently tested for rectal ESBL-E carriage 5 times over an 8-month period; 32.9% of those harboring ESBL-producing *Escherichia coli* or *Klebsiella pneumoniae* in their first sample remained positive, whereas 12.9% acquired ESBL-E at 1 or more time points during the study. The investigators concluded that a single, positive test result might not provide an accurate prediction for prolonged carriage [9]. In a previous study in child-parent pairs in the Netherlands, bacterial loads of ESBL-E/Ambler class C cephalosporinases (AmpC) appeared higher when both the child and parent were carriers than when only 1 of them was a carrier [10], suggesting that bacterial load could also be predictive for ESBL-E carriage over time.

We performed a longitudinal study with follow-up sampling after 1 and 6 months in subjects carrying and not carrying ESBL-E, in order to better understand the dynamics

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of intestinal ESBL-E carriage in the general population. In addition, we determined the risk factors for incidental, short-term, and long-term ESBL-E carriage; explored the semi-quantitative ESBL-E loads in relation to the duration of carriage; and quantified the transmissibility and duration of ESBL-E carriage.

METHODS

Study Design, Setting, and Data Collection

The prevalence of intestinal ESBL-E carriage in the Netherlands was investigated in a cross-sectional study, with monthly data collected from November 2014 to November 2016. From this, a random sample of ~2000 residents (including all ages), drawn monthly from registries covering the country's population (~17 million), was invited to complete a questionnaire and provide a fecal sample [11]. Participants were also invited to participate in a longitudinal sub-study with additional sample collections at 1 and 6 months after the first fecal sample. All phenotypically ESBL-E screen-positive participants and a random selection of non-carriers (2 for each suspected carrier), generated by R (R Foundation for Statistical Computing, Vienna, Austria), were invited. In case a phenotypically ESBL-E screen-positive sample was negative after molecular confirmation, the participant was classified as a noncarrier.

ESBL-E Detection

Details on the ESBL detection were described previously [11]. In brief, samples were cultured on MacConkey agar at 37°C with 1 mg/L cefotaxime and both with and without 2 mL of Luria-Bertani. Isolates were speciated by Matrix-Assisted Laser Desorption/Ionization-Time Of Flight mass spectrometry (Bruker, Bremen, Germany), and *Escherichia coli*, *Klebsiella pneumoniae*, and the *Enterobacter cloacae* complex were studied.

The number of colony forming units (CFU) of ESBL-E and the total *Enterobacteriaceae* per gram of feces in stool samples were determined with the "running-drop method" in any case of a growth on the MacConkey plate without enrichment. There were 10-fold dilutions made from fecal suspensions, of which a drop (10 µl per suspension) was allowed to run down the surface of the BioRad plates, both with and without 1 mg/L cefotaxime, which was then incubated overnight at 37°C. CFU were counted in the highest dilution where growth was still visible. The CFU ratio was calculated by dividing the CFU counts of the 2 plates.

Bacterial DNA was checked by specific polymerase chain reaction (PCR; active on CefoTaXime, first isolated in Munich (CTX-M) group 1 and 9 ESBLs) and, if negative, was tested for other ESBL-encoding genes by the Check MDR CT-101 microarray (Check-points, Wageningen, the Netherlands). PCR-based replicon typing was performed to identify the plasmid types [12].

A whole-genome sequence analysis (WGS) was performed. Assembled contigs that were larger than 500 bp and with at least 10x coverage were further analyzed by ResFinder, VirulenceFinder, PlasmidFinder, and multilocus sequence typing [13–16]. Epidemiologically related isolates were confirmed by WGS alignment (Kodon, Applied Maths, Belgium).

Data Analyses

Definitions of ESBL-E Carriage

All subjects were tested for intestinal ESBL-E carriage at 3 time points (T): T = 0 (baseline), T = 1 (1 month), and T = 2 (6 months). Those with ESBL-E carriage at a single time point were labeled as incidental carriers; those with ESBL-E carriage at T = 0 and T = 1 were labeled as short-term carriers; those with ESBL-E carriage at T = 0 and/or T = 1 and T = 2 were labeled as long-term carriers; and all others were labeled as noncarriers. We defined any positive sample at the next sampling moment as short- or long-term carriage, regardless of the molecular characteristics of the ESBL-E.

Risk Factors for ESBL-E Carriage

A random forest algorithm was used to identify the relative importance of potential risk factors for being incidental, short-term, and long-term carriers that were present at T = 0. In all models, the carriage profile of interest was compared to the noncarriers. We grew 1000 decision trees to form the random forests, and a random subset of variables was used at each split point, with 4 out of 30 variables used in each subset of all models. To assess the importance of the variables, we evaluated the accuracy decreases. A higher decrease of accuracy means a higher variable importance. The area under the curve (AUC) was calculated for every random forest model. An AUC value > 0.7 is generally considered useful [17]. Missing values (0–19%) were predicted by random forest methods as well [18]. Those variables most predictive for incidental, short-term, and long-term carriage were assessed in univariate and multivariate logistic regression analyses.

A canonical correspondence analysis was used to explore associations of the incidental, short-term, and long-term ESBL-E carrier states with the ESBL types, sequence types (STs), and plasmid types. In addition, differences between incidental, short-term, and long-term carriage in ESBL types, STs, and plasmid types were tested with the χ^2 test or Fisher's exact test.

CFU of ESBL-E Over Time

The odds ratio (OR) for still being ESBL-E positive at T = 1 and T = 2, depending on the CFU load at T = 0, was calculated. In addition, the positive predictive value (PPV) and negative predictive value (NPV) were calculated as functions of the log-transformed CFU ratio.

Transmissibility of ESBL-E Carriage

We used a transmission model to estimate the transmissibility and duration of ESBL-E carriage for 2 independent data sets: the current, ESBL Attribution (ESBLAT) study and the results from another ESBL-E surveillance study, also performed in the Netherlands [9]. In this model, an individual either carries ESBL-E or is a noncarrier and, therefore, susceptible for the acquisition of colonization. A noncarrier may acquire ESBL-E at a time-dependent rate of $\lambda_0 + \lambda_1 t$, which is linearly increasing/decreasing with calendar time t. Here, λ_0 is the acquisition rate at the start of the study period (T = 0). A positive value of λ_1 corresponds to an acquisition rate that is increasing with time, while a negative value of λ_1 corresponds to a decreasing acquisition rate. Note that we required $\lambda_0 > 0$ and $\lambda_0 + \lambda_1 t$ to be positive during the study period. Carriers may lose ESBL-E at a constant rate (α). We assumed 100% sensitivity/specificity for the culture results. Our aim was to estimate the parameters α, λ_0, and λ_1, based on the data and the model. For this, we used a Bayesian framework with uninformative priors (Supplementary Information 1; Supplementary Figure S1).

Ethics

This study received approval from the Medical Research Ethics Committee of the University Medical Center, Utrecht (WAG/om/14/012490).

RESULTS

From the 4177 participants in the cross-sectional study, 786 were invited to participate in the longitudinal study (261 were screen-positive carriers and 525 were initially noncarriers). There were 725 (92.2%) who responded, of which 69 were excluded because of missing fecal samples, leaving 656 subjects for analyses, of which 96 (14.3%) were confirmed ESBL-E carriers at T = 0. The median times between sampling were 32 days (interquartile range, 27–38 days) between T = 0 and T = 1 and 152 days (interquartile range, 146–158 days) between T = 1 and T = 2.

There were 336 (51.4%) male participants. The minimum age of participants was 0 years, the maximum was 92 years, and the median was 56 years. There were 26 (4.0%) participants who were born outside the Netherlands, 263 (n = 651; 40.5%) who had children, 110 (n = 648; 17.0%) who had 1 or more dogs, and 127 (n = 648; 19.7%) who had 1 or more cats. In 34 (n = 527; 6.5%) households, there was at least 1 child who attended daycare. In the 12 months before T = 0, 53 (n = 648; 8.2%) persons had been hospitalized, and 134 (n = 627; 21.4%) had used antibiotics (Supplementary Table 1).

In all, 66 subjects (10.1%) were categorized as incidental carriers (77 isolates), 22 (3.3%) as short-term carriers (44 isolates), and 38 (5.8%) as long-term carriers (113 isolates); descriptive statistics, stratified by ESBL-E status, are provided in Table 1. Among short-term carriers, there were 22 isolate pairs (T = 0 and T = 1) available for WGS; 18 (81.8%) pairs were considered identical, and 2 plasmid transfer took place. Among long-term carriers, there were 4 pairs with 2 samples (T = 1 and T = 2) and 34 with 3 samples (T = 0, T = 1, and T = 3; of which 3 had an additional isolate at 1 of the 3 sampling moments) available for WGS. From these, 30 (78.9%) pairs were considered identical, with 4 determinations based on the evidence that a plasmid transfer took place. There were 6 isolate pairs that were not identical (neither by WGS or PCR-based replicon typing); from 2 of these, there was insufficient evidence that a plasmid transfer took place (Supplementary Table 2).

Of the 96 ESBL-E carriers at T = 0, 57 (59.4%) were ESBL-E carriers at T = 1, of which 35 (36.5%) were ESBL-E carriers at T = 2 (Figure 1). Of the 560 noncarriers at T = 0, 14 (2.5%) and 16 (2.9%) acquired ESBL-E at T = 1 and T = 2, respectively (Figure 1).

Risk Factors for ESBL-E Carriage

We created models to identify the risk factors for incidental, short-term, and long-term ESBL-E carriage, which yielded AUCs of 0.51, 0.50, and 0.70, respectively (Supplementary Information 2; Supplementary Figures S2–4). A protective factor associated with ESBL-E incidental carriage in multivariate logistic regression analyses was having a cat in the household (OR, 0.4; 95% confidence interval [CI], .2–.8). A risk factor associated with ESBL-E short-term carriage was being hospitalized in the past 12 months (OR, 5.9; 95% CI, 1.7–18.1); the risk factors for ESBL-E long-term carriage were travelling to Asia (OR, 4.8; 95% CI, 1.5–15.8), swimming in a sea/ocean (OR, 2.8; 95% CI, 1.3–6.1), and not changing the kitchen towel daily (OR, 3.1; 95% CI, 1.2–10.9; Supplementary Table 3).

CFU as Predictor for Persistence of ESBL-E Carriage

In participants that were ESBL-E carriers at T = 0 and still carriers at T = 1 and/or T = 2, the log-transformed CFU ratio at T = 0 was significantly higher, as compared to the noncarriers, at T = 1 and T = 2 (Figure 2). For every log-value increase at T = 0, the OR for ESBL-E carriage at T = 1 increased by 1.3 (95% CI, 1.2–1.6) and the OR for ESBL-E carriage at T = 2 increased by 1.2 (95% CI, 1.1–1.4). The log-transformed CFU ratio at T = 1 did not predict ESBL-E carriage at T = 2 (OR, 1.1; 95% CI, .9–1.3). The log-transformed CFU counts did not improve ORs, compared to the log-transformed CFU ratio. The best PPV for persistence was derived with a log-transformed CFU ratio of -4 at T = 0 (PPV 0.90 and NPV 0.55).

Molecular Characteristics Associated to Different Extended-spectrum Beta-Lactamase–Producing Enterobacteriaceae *States Over Time*

In all 3 carrier-states, the most prevalent ESBL type was $bla_{\text{CTX-M-15}}$ (n = 28 [42.4%], n = 14 [63.6%], and n = 16 [42.1%] in incidental, short-term, and long-term carriers, respectively), and

Table 1. Descriptive Statistics, Stratified by Extended-spectrum Beta-Lactamase–Producing *Enterobacteriaceae* Status

		Non-Carriers	Incidental Carriers	Short-term Carriers	Long-term Carriers
No. of individuals		530	66	22	38
No. of isolates		0	77	44	113
Based on the individual level					
Sex	Male	273 (51.5)	35 (53.0)	9 (40.9)	20 (52.6)
	Female	257 (48.5)	31 (47.0)	13 (59.1)	18 (47.4)
Country of birth	The Netherlands	510 (96.2)	63 (95.5)	22 (100.0)	35 (92.1)
	Other	20 (3.8)	3 (4.5)	0 (0)	3 (7.9)
Degree of urbanization, addresses per km^2	≥2500	36 (6.8)	4 (6.1)	1 (4.5)	1 (2.6)
	1500–2500	84 (15.8)	14 (21.2)	1 (4.5)	4 (10.5)
	1000–1500	101 (19.1)	12 (18.2)	8 (36.4)	10 (26.3)
	500–1000	130 (24.5)	17 (25.8)	5 (22.7)	15 (39.5)
	<500	179 (33.8)	19 (28.8)	7 (31.8)	8 (21.1)
SES	High SES	131 (24.9)	16 (24.2)	5 (22.7)	12 (32.4)
	Intermediate SES	292 (55.5)	37 (56.1)	11 (50.0)	22 (59.5)
	Low SES	103 (19.6)	13 (19.7)	6 (27.3)	3 (8.1)
Age, years	0–4	35 (6.6)	2 (3.0)	2 (9.1)	0 (0)
	5–12	37 (7.0)	2 (3.0)	1 (4.5)	1 (2.6)
	13–19	13 (2.5)	2 (3.0)	1 (4.5)	0 (0)
	20–39	48 (9.1)	6 (9.1)	0 (0)	5 (13.2)
	40–64	217 (40.9)	34 (51.5)	11 (50.0)	22 (57.9)
	65–79	153 (28.9)	16 (24.2)	7 (31.8)	10 (26.3)
	80+	27 (5.1)	4 (6.1)	0 (0)	0 (0)
Proton pump inhibitor use in the past 6 months	Yes	85 (16.2)	10 (16.1)	3 (15.8)	12 (32.4)
	No	441 (83.8)	52 (83.9)	16 (84.2)	25 (67.6)
Medication use in the past 6 months	Yes	331 (62.9)	39 (62.9)	13 (68.4)	29 (78.4)
	No	195 (37.1)	23 (37.1)	6 (31.6)	8 (21.6)
Antibiotic use in the past 12 months	Yes	102 (20.1)	18 (29.5)	3 (15.0)	11 (28.9)
	No	406 (79.9)	43 (70.5)	17 (85.0)	27 (71.1)
Eat in a restaurant	≥20 times/year	53 (10.0)	14 (21.5)	3 (14.3)	8 (21.1)
	<20 times/year	475 (90.0)	51 (78.5)	18 (85.7)	30 (78.9)
Time before groceries are put in the fridge	≥15 minutes	404 (77.2)	46 (71.9)	14 (70.0)	24 (63.2)
	<15 minutes	119 (22.8)	18 (28.1)	6 (30.0)	14 (36.8)
Cleaning the fridge	≥monthly	245 (46.4)	24 (36.9)	11 (52.4)	20 (52.6)
	<monthly	283 (53.6)	41 (63.1)	10 (47.6)	18 (47.4)
Washing hands before cooking	Always	327 (62.3)	42 (64.6)	13 (61.9)	19 (50.0)
	Not always	198 (37.7)	23 (35.4)	8 (38.1)	19 (50.0)
Changing the kitchen towel	Daily	139 (26.6)	15 (23.4)	4 (19.0)	4 (10.5)
	Not daily	384 (73.4)	49 (76.6)	17 (81.0)	34 (89.5)
Changing the bathroom towel	Daily	96 (20.6)	11 (20.0)	3 (16.7)	3 (8.8)
	Not daily	370 (79.4)	44 (80.0)	15 (83.3)	31 (91.2)
Eat meat	Yes	503 (98.1)	60 (95.2)	20 (100.0)	37 (100.0)
	No	10 (1.9)	3 (4.8)	0 (0)	0 (0)
A diet with chicken meat	Yes	499 (97.7)	59 (95.2)	20 (95.2)	35 (94.6)
	No	12 (2.3)	3 (4.8)	1 (4.8)	2 (5.4)
A diet with pork	Yes	464 (92.1)	50 (83.3)	19 (90.5)	26 (81.2)
	No	40 (7.9)	10 (16.7)	2 (9.5)	6 (18.8)
Children in the household	Yes	218 (41.3)	25 (38.5)	8 (38.1)	12 (32.4)
	No	310 (58.7)	40 (61.5)	13 (61.9)	25 (67.6)
Daycare attendance in the household	Yes	27 (6.3)	4 (8.0)	2 (11.8)	1 (3.3)
	No	403 (93.7)	46 (92.0)	15 (88.2)	29 (96.7)
Diapers usage in the household	Yes	49 (11.4)	4 (8.0)	2 (11.8)	2 (6.7)
	No	381 (88.6)	46 (92.0)	15 (88.2)	28 (93.3)
Hospitalization in the past 12 months	Yes	38 (7.2)	5 (7.8)	5 (23.8)	5 (13.2)
	No	487 (92.8)	59 (92.2)	16 (76.2)	33 (86.8)
Hospitalization of a household member in the past 12 months	Yes	48 (10.4)	7 (12.5)	3 (17.6)	3 (8.8)
	No	412 (89.6)	49 (87.5)	14 (82.4)	31 (91.2)

Table 1. Continued

		Non-Carriers	Incidental Carriers	Short-term Carriers	Long-term Carriers
Household has a dog	Yes	93 (17.7)	8 (12.3)	4 (19.0)	6 (16.2)
	No	432 (82.3)	57 (87.7)	17 (81.0)	31 (83.8)
Household has a cat	Yes	113 (21.5)	7 (10.8)	4 (19.0)	3 (8.1)
	No	412 (78.5)	58 (89.2)	17 (81.0)	34 (91.9)
Household has a horse	Yes	15 (2.9)	1 (1.5)	0 (0)	1 (2.7)
	No	510 (97.1)	64 (98.5)	21 (100.0)	36 (97.3)
Stayed at a camping site in the past 12 months	Yes	180 (36.5)	21 (36.8)	7 (43.8)	10 (27.0)
	No	313 (63.5)	36 (63.2)	9 (56.2)	27 (73.0)
Visited a farm in the past 12 months	Yes	161 (33.6)	17 (29.8)	3 (18.8)	11 (29.7)
	No	318 (66.4)	40 (70.2)	13 (81.2)	26 (70.3)
Swimming in sea/ocean in the past 12 months	Yes	163 (34.5)	26 (42.6)	6 (40.0)	21 (58.3)
	No	310 (65.5)	35 (57.4)	9 (60.0)	15 (41.7)
Swimming in river/lake in the past 12 months	Yes	121 (25.6)	15 (26.3)	4 (26.7)	6 (17.1)
	No	352 (74.4)	42 (73.7)	11 (73.3)	29 (82.9)
Travelled in the past 12 months	Yes	365 (69.7)	48 (73.8)	11 (52.4)	31 (81.6)
	No	159 (30.3)	17 (26.2)	10 (47.6)	7 (18.4)
Travelled to	None	159 (30.8)	17 (26.6)	10 (47.6)	7 (18.4)
	Africa	6 (1.2)	1 (1.6)	1 (4.8)	3 (7.9)
	Asia	25 (4.8)	8 (12.5)	1 (4.8)	8 (21.1)
	Europe	316 (61.1)	37 (57.8)	9 (42.9)	19 (50.0)
	North America	11 (2.1)	1 (1.6)	0 (0)	1 (2.6)
Based on the isolate level					
ESBL-E type	*Escherichia Coli*	NA	65 (84.4)	41 (90.9)	113 (100.0)
	Klebsiella Pneumoniae	NA	11 (14.3)	2 (4.5)	0
	Enterobacter cloacae	NA	1 (4.5)	1 (2.3)	0

Abbreviations: ESBL-E, extended-spectrum beta-lactamase–producing *Enterobacteriaceae*; NA, not applicable; SES, socioeconomic status.

ST131 was the most prevalent ST (n = 9 [13.6%], n = 8 [36.4%], n = 17 [44.7%] for incidental, short-term, and long-term carriers, respectively). The IncFIB and IncFII plasmid replicon types were the most prevalent in all carrier groups. Individuals with incidental, short-term, and long-term carriage were plotted in a canonical correspondence analysis together with the genotypes, STs, and plasmid types (Figure 3). The plasmid replicons Col and Col156 were more frequently observed in the long-term carriers (Figure 3; Supplementary Table 4).

Transmissibility of ESBL-E

For quantifying the transmissibility of ESBL-E, we also used data from the Livestock Farming and Neighbouring Residents' Health (VGO) study (Study 2), which was performed from March 2014 to February 2015 in the Netherlands [9], with 325 participants and 4 and 6 rectal samples per participant (mean of 5.8) obtained. The timing between cultures differed between participants, but the mean time between the first and the last culture was 242 days, with a standard deviation of 26 days.

Model simulations revealed similar measures of transmissibility for both data sets. The estimated median decolonization rates were 2.83/year (95% credibility intervals [CrI], 2.23–3.24) and 2.32 year (95% CrI, 1.89–2.84) for the ESBLAT study and the VGO study, respectively (Table 2). This corresponds to estimated average durations of carriage of 0.35 year (95% CrI, .31–.45) and 0.43 years (95% CrI, .35–.53) for both studies. The estimated acquisition rates at the start of each study (T = 0) were 0.34/year (95% CrI, .35–.54) and 0.57/year (95% CrI, .39–.78) for both studies. Finally, we did not observe either an increase or decrease in the acquisition rate during the study periods of either study (−0.04 [95% CrI, −0.07 to −0.007] in the current study and −3e−4 [95% CrI, −9e−4 to 2e−4] in Study 2).

DISCUSSION

In this study, we found that intestinal ESBL-E carriage in 656 community-dwelling subjects was mostly incidental, with about 25% of initial carriers being categorized as long-term carriers. This carrier status was associated with travelling and hygiene habits. The long-term carrier status was associated with ST131, Col, and Col156. At the population level, the average duration of carriage was estimated to be around 5 months, and the observed balanced decolonization and acquisition rates suggest that the population prevalence is in equilibrium.

There were 57 (59.4%) ESBL-E carriers that were still carriers after 1 month and 35 (36.5%) that were still carriers after 6 months. In the current study, the majority of noncarriers remained noncarriers (94.6%); this confirmed findings from another longitudinal study in a farm-dense area in the Netherlands, in which 87.6% of noncarriers remained noncarriers over a

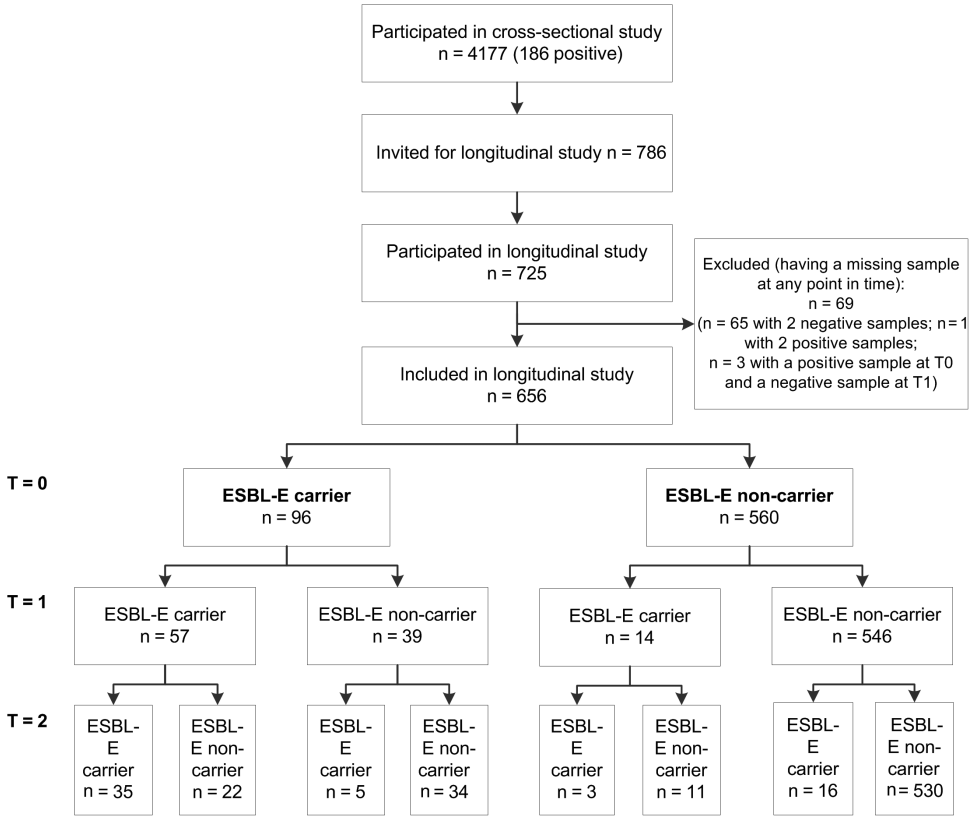

Figure 1. Flowchart of the ESBL-E status per sampling moment. T = 0 is the baseline sampling moment (monthly new inclusions were added from 14 February 2016). T = 1 is the sampling moment after 1 month from baseline. T = 2 is the sampling moment after 6 months from baseline. Abbreviations: ESBL-E, extended-spectrum beta-lactamase–producing *Enterobacteriaceae*; T, time point.

period of 8 months, with 5 sampling moments [9]. These findings indicate that ESBL-E acquisition occurs sporadically over a 6-month period in persons from the general community and that 40.6% of the colonized persons lose ESBL-E within 1 month.

Persistent carriage might have resulted from repetitive decolonization and new acquisitions might have been due to continuous exposure to the same sources and/or risk factors that increased the chance of new ESBL-E acquisitions. Indeed, we previously identified travel-related risk factors, including swimming in the sea/ocean, and hygiene-related risk factors, like not changing the kitchen towel daily, for ESBL-E carriage [11]. These factors are behavioral and were probably either repeated (traveling) or not changed (hygiene) during the course of the study. Travelling has also been associated with prolonged carriage [8, 19], as was antimicrobial usage [9]. The latter, though, was not identified as a risk factor in the current study, which might be due to limited antibiotic usage by the study population and, therefore, limited power to identify such a relationship. Yet in the current study, all short-term carriers had identical ESBL-E isolates at T = 0 and T = 1, as did 81.6% of the long-term carriers at the different time points. This suggests that long-term carriage after 6 months was through the persistence of ESBL-E, rather than the acquisition of new strains.

Persistent carriage might also be associated with, or caused by, specific molecular characteristics. In the current study, ST131 was more frequently observed in short- and long-term carriers than in incidental carriers. Yet ST131 was the most prevalent sequence type among community-dwelling ESBL-E carriers [11], not only in the current study, but also in other studies [2, 9, 10, 20, 21]. Similarly, comparable high-prevalence figures were derived for the most common ESBL gene, $bla_{\text{CTX-M-15}}$, but were equally distributed among incidental, short-term, and long-term carriers. The plasmid types Col and Col156 were more often found in long-term carriers, which might be explained by the frequent co-existence of ST131 and Col plasmids, as observed by Liu et al [22]. Also, the genotype $bla_{\text{CTX-M-27}}$ was more often observed in long-term carriers, but we considered that association to not be statistically significant because of multiple testing. However, in our previous analyses of this study, ST131 and $bla_{\text{CTX-M-27}}$ were associated, which is in accordance with findings in other studies [3, 6, 9]. In a single-center study in a long-term care facility in the Netherlands, the observed half-life of ESBL-E carriage was 13 months for ST131, as compared to 2 to 3 months for other STs. Based on a mathematical model used in that study, it was suggested that a prolonged duration of carriage was the main reason for the high prevalence, as estimated

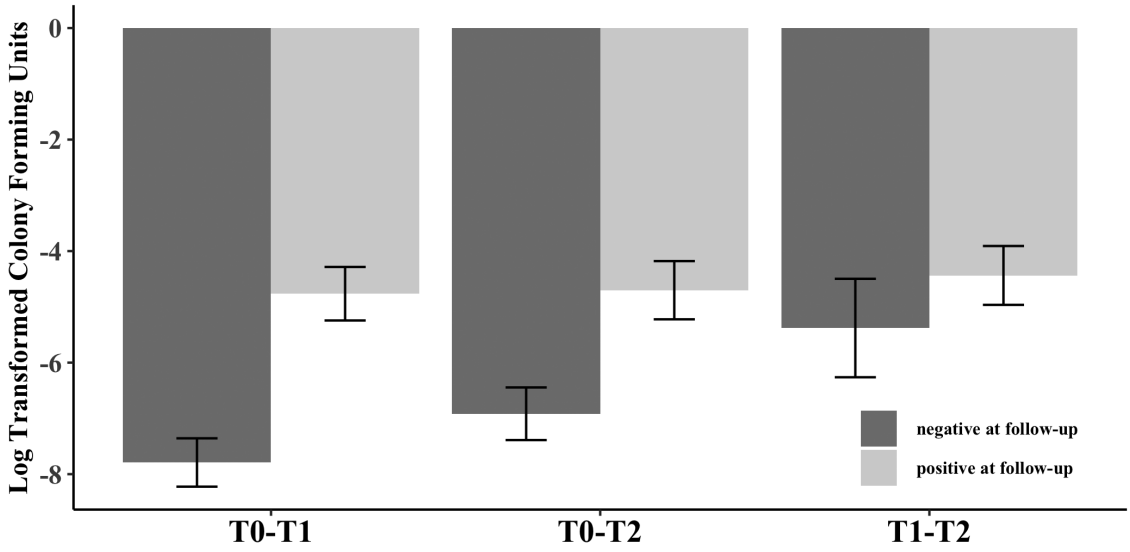

Figure 2. The log-transformed CFU ratio at baseline was higher in participants with ESBL-E carriage, as compared to non-carriage, after 1 and 6 months. Participants with persistent ESBL-E carriage at T = 1 and/or T = 2 had, on average, higher CFU counts at T = 0 (Y-axis). The same trend was observed between T = 1 to T = 2, although statistical significance was not reached. Abbreviations: CFU, colony forming units; ESBL-E, extended-spectrum beta-lactamase–producing *Enterobacteriaceae*; T, time point.

transmission rates were comparable for ST131 and other STs. Furthermore, ST131 was less often detected in environmental samples [6]. In contrast, ST131 with $bla_{\text{CTX-M-27}}$ appeared to be transmitted more efficiently than ST131 with $bla_{\text{CTX-M-15}}$ in a hospital setting in 2 rehabilitation wards in Israel [23]. Whether this combination of ST131 and $bla_{\text{CTX-M-27}}$ is associated with persistent carriage remains to be determined, and the same holds for associations between different plasmid replicon types and carriage.

The log-transformed CFU ratio had predictive value for the short-term and long-term persistence of ESBL-E carriage in our study population. However, a ratio of −4 had a PPV of 90% and an NPV of 55%, hampering accurate prediction for individuals. In a previous study performed in households, children with relatively high CFUs of ESBL-E/AmpC had higher odds of having ESBL-E/AmpC–carrying parents [10]. These findings suggest that semi-quantitative loads might provide more granular information on the dynamics of carriage that could be used in the mathematical modeling of transmission events within households or hospital wards. In addition, more frequent sampling (eg, weekly or even daily) might provide more insights into the dynamics of and individual prediction for ESBL-E carriage. Importantly, we considered carriage with ESBL-E at 2 successive time points as persistent carriage, and

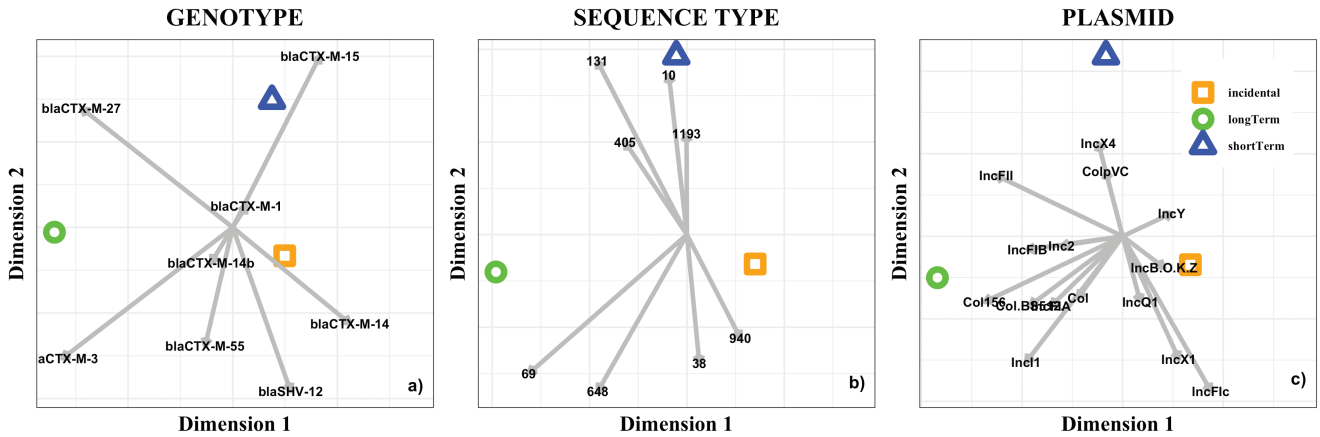

Figure 3. CCA of genotypes, sequence types, and plasmid types versus incidental, long-term, and short-term carriage. $bla_{\text{CTX-M-27}}$ was more frequently present in the long-term carriers (n = 10; 26.3%), as compared to the incidental and short-term carriers (n = 5 [3.0%] and n = 2 [9.1%], respectively). ST131 was more frequently observed in the long- (n = 17; 44.7%) and short-term (n = 8; 36.4%) carriers, as compared to the incidental carriers (n = 9; 13.6%), and ST69 only occurred in the long-term carriers (n = 4; 10.5%). The plasmid types of IncFII, Col, Col156, and Col.BS512 were more frequently present in the long-term carriers. Abbreviations: CCA, canonical correspondence analysis; ST, sequence type.

Table 2. Model Parameters for Studies 1 and 2

Parameter	Symbol	Study 1: Current, ESBLAT Study	Study 2: Previous, VGO Study
Decolonization rate/year for carriers of ESBL-E	α	2.83 (2.33–3.49)	2.32 (1.89–2.84)
Mean duration of colonization in years	$1/\alpha$	.35 (.29–.43)	.43 (.35–.53)
Acquisition rate/year at the start of the study, T = 0	λ_0	.34 (.21–.49)	.57 (.39–.78)
Trend in time/year of acquisition rate	λ_1	−.04 (−.07 to −.007)	−3e−4 (−9e−4 to 2e−4)

Numbers in parentheses represent 95% credibility intervals.

Abbreviations: ESBLAT, ESBL Attribution; ESBL-E, extended-spectrum beta-lactamase–producing *Enterobacteriaceae*; T, time point; VGO, Livestock Farming and Neighbouring Residents' Health.

did not take potential differences in ESBL-E sequence types or plasmids into account.

A simple transmission model, based on the current and a previous study [9], provided evidence for a stable prevalence of ESBL-E carriage in the general community between 2014–2016, with balancing acquisition and decolonization rates. This corroborates the trend of resistance against third-generation cephalosporins among *E. coli*–causing invasive infections in the Netherlands, which has stabilized at around 7% since 2015 [24, 25]. Based on the model, the average duration of colonization was 4.5 months, which would mean that 20% of people would lose ESBL-E within a month. This finding seems to contradict the observed 40.6% from our study, and this may suggest that the assumption of a constant decolonization rate is not valid; that is, the mean duration of carriage is still 4.5 months, but 1 group of individuals may be more prone to lose colonization, while another group is less prone. A limitation of this model is that we assumed that sampling was 100% sensitive, which most likely is not true. However, since sensitivity is constant in time, this assumption did not reduce the validity of our conclusion that there is no evidence for an increased acquisition rate over time.

In conclusion, the persistence of ESBL-E carriage was demonstrated in 59.4% and 36.5% individuals after 1 and 6 months, respectively. Risk factors for long-term carriage were related to travel and hygiene. The findings in this study, and data from a previous longitudinal study, suggest that ESBL-E carriage in the general population is dynamically stable.

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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