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ORIGINAL RESEARCH

Risk factors associated with prolonged intestinal colonization of ESBL-producing *Enterobacteriaceae* – a prospective cohort study

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Background: Extended spectrum β-lactamase-producing *Enterobacteriaceae* (EPE) are responsible for a major part of the widespread antimicrobial resistance (AMR). Increased understanding of risk factors associated with intestinal colonization of EPE is crucial to implement adequate actions against AMR. The aim of this study was to define potential risk factors for prolonged intestinal colonization with EPE. A secondary aim was to analyze if patients were adequately informed about being infected or colonized by antibiotic-resistant bacteria.

Methods: Patients with a positive clinical EPE culture from urine, blood or feces were recruited in a region in the south of Sweden. Selective EPE fecal cultures were obtained at least three months after the initial positive culture. Prolonged intestinal colonization was defined as the prevalence of any EPE in the follow-up fecal sample. Risk factors for prolonged intestinal colonization were evaluated by using a questionnaire and by retrospective review of medical records. A univariate model and a multivariate regression analysis were performed to identify possible risk factors for intestinal EPE colonization.

Results: Out of 143 patients included in the study, 57% remained positive for EPE at the second sampling. In a multivariate regression model, urological intervention, history of EPE infection and travel to Africa and/or Asia within 2 years were found to be significantly associated with prolonged intestinal colonization of EPE. Before being approached by us, 50% of patients displayed inadequate knowledge of EPE infection or colonization.

Conclusion: In this prospective cohort study, urological intervention within 6 months and a history of EPE infection are independently associated with prolonged intestinal colonization with EPE. In contrast, travel to Africa and/or Asia within 2 years is associated with a decreased risk of prolonged intestinal colonization with EPE. There is room for improvement when it comes to patient information regarding EPE to decrease of spread.

Keywords: antibiotic resistance, risk of infection, one health, gram-negative bacteria, antibiotic therapy, patient information

Introduction

A growing incidence of extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* (EPE), and consequently a rapidly increasing antimicrobial resistance (AMR) is highly worrying.[1,2] It is mainly driven by excessive antimicrobial use in humans and animals in addition to inadequate infection prevention and control practices.[3] Humans as well as domestic and wild animals harbor EPE in the intestine, and those bacteria are more often found in the environment as prevalence increases.[4] It is crucial to study the pathogenesis, virulence and spread of EPE in order to implement adequate preventive measures.

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It has previously been described that *E. coli* belonging to the phylogenetic group B2 has a greater capacity to colonize the human gut compared to the other groups including A, B1 and D. These strains are associated with carriage of specific virulence genes, such as p-fimbriae and aerobactin.[5–7] EPE belonging to phylogroup B2, including the globally disseminated ST131 clone, is highly virulent, and associated with prolonged intestinal carriage of EPE.[8–12] It has been suggested that strain-specific virulence factors may impact the persistence of EPE in the intestine.[9]

The long-term duration of intestinal colonization of EPE is unknown and only a few studies exist that study prolonged colonization.[8,10,11,13,14] Most of these studies focus on molecular characterization of EPE, and only a few examine risk factors associated with prolonged colonization. In contrast, numerous investigations on risk factors for acquisition and the short-term loss of EPE have been published.[15–23] It is at present unclear whether a failure to detect EPE in fecal cultures from patients previously colonized with EPE reflects an absolute loss of EPE or merely poor sensitivity of detection methods.

International travel to endemic areas is a risk factor for EPE carriage.[23] The acquisition rate varies depending on geographical regions visited, and the highest rates are associated with Africa, the Middle East and Asia.[24] However, data suggest that travel-associated EPE colonization might be short-lived.[25] In addition to international travel and infection with *E. coli* strains from phylogroup B2, it has previously been demonstrated that immobilization, antibiotic consumption 4 and 12 months prior to colonization, treatment with proton-pump inhibitors and urinary catheter use are independently associated with prolonged intestinal EPE carriage.[14,26,27] Previous studies have focused on bacterial characteristics to determine risk factors for intestinal colonization of EPE.[8,10] The main goal of this study was to study patient characteristics and putative risk factors for prolonged EPE carriage. Furthermore, we wanted to investigate whether patients were adequately informed about their EPE infection/colonization.

Materials and methods

Study design and data collection

We performed a prospective cohort study in Skåne County, a region in the south of Sweden. Inclusion of patients started in January 2016 and ended in April 2017. Patients 18 years of age or older with a verified culture of EPE in urine, blood, feces or any other location during the inclusion period were eligible for inclusion. Medical records were reviewed using the software Melior (Melior, Siemens Healthcare Services, Upplands Väsby, Sweden) for the following exclusion criteria; alcohol or substance abuse, severe psychiatric disorder, immunosuppressed patients (eg, immunodeficiency, ongoing cancer treatment, neutropenia/leukopenia, treatment with TNF-α-inhibitors), inpatient care, patients with chronic venous catheters, dementia or failure to sign informed consent and newly arrived refugees. These exclusion criteria were established as this study used the same cohort of patients as a prospective randomized clinical trial performed by the same group (not yet published).

Between July 2016 and March 2018, 2148 patients with cultures positive for EPE in the region of Skåne were screened for inclusion. Out of these, 820 adult patients with at least 1 documented positive ESBL-culture from blood, wound, cervix, urine or feces were contacted by mail and asked to submit one fecal sample for microbiological analysis. Prolonged EPE colonization was defined by the group as the prevalence of EPE in the fecal culture obtained with a minimum time period of 3 months from the first positive EPE-culture. Of the 820 adult patients invited to take part in the study, 677 declined to submit a fecal sample. Definition of variables, ie, possible risk factors, was designed prior to data collection. The steering document containing definition of variables is attached in the Supplementary materials.

To assess possible risk factors associated with prolonged intestinal carriage of EPE, medical and personal information was collected by sending out questionnaires to the participants, and by reviewing the patients' medical records. Some information on potential risk factors relied solely on the answers from the questionnaires, eg, travel habits, social factors, etc. The queries on the questionnaire were tested on a small number of patients before the questionnaires were distributed. Three weeks after sending out the questionnaires, patients who had not responded to the questionnaires were contacted by phone and interviewed according to the same questionnaire. During the period of which the data were collected, three patients were diseased.

Study setting

The Skåne County is situated in South Sweden with a total population of 1,324,565 (December 2016).[28] All samples from primary, secondary as well as tertiary health care suppliers in Skåne County were analyzed at Clinical Microbiology (Laboratory Medicine, Lund, Sweden).

Laboratory methods

All samples were selectively cultivated for EPE at the Clinical Microbiology laboratory according to standard protocols. Briefly, the sample material was plated on URI-Select four agar plates with vancomycin (BioRad) complemented with two antimicrobial susceptibility discs containing ceftazidime (10 µg/mL, Oxoid) and meropenem (10 µg/mL, Oxoid) followed by incubation at 37°C overnight. Sample material was also plated on chromogenic agar plates ChromID ESBL (BioMerieux) and incubated as above. Colonies of presumptive EPE were subcultivated on horse blood agar (HBA) and typed to bacterial species using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF).

The phenotype of EPE was characterized by susceptibility to cloxacillin (AmpC) or clavulanic acid (ESBL). All EPE were tested for susceptibility against the following antibiotics using the EUCAST methodology and breakpoints; piperacillin-tazobactam, cefotaxime, ceftazidime-avibactam, ceftazidime, ceftolozane-tazobactam, imipenem, meropenem, gentamicin, tobramycin, amikacin, trimethoprim-sulfamethoxazole, ciprofloxacin and, finally, temocillin.

Statistical analyses

Fisher's exact test was used to perform univariate analyses. A two-sided exact significance of ≤0.05 was considered significant. These analyses were conducted using dichotomized data. All dichotomized data were analyzed and unevenly distributed variables were excluded for the multiple logistic regression after analyzing the cross-tabulation tables and the Fisher's exact test. The variable antibiotic use was highly correlated with a history of EPE infection and was thus excluded from the multiple comparisons. The statistical importance of the risk factors was estimated using a binary multiple logistic regression model. Patients willing to submit a fecal sample and not willing to submit a fecal sample were compared with regard to baseline characteristics and risk factors. Due to observed differences regarding resistance against both ciprofloxacin and trimethoprim/sulfamethoxazole and EPE-infected compared to EPE-colonized, statistical weighting with respect to these variables was used. A model of stepwise forward multiple regression was used to determine the most significant risk factors for prolonged intestinal EPE carriage. Figure 1, Tables 1, S1, S3–S5 are based on actual data, whereas Tables 2–4 and S2 are based on weighted data.

Association between the potential risk factors and prolonged carriage was quantified with odds ratio (OR) with a 95% confidence interval (CI). A *P*-value of ≤0.05 was considered statistically significant. The statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. Additionally, the statistical analyses were validated by a statistician using the same data.

Ethical considerations

This study was granted ethical approval from the Regional Ethical Review Board at Lund's Tingsrätt with reference number 2016-304 and 2018-143. All patients included in the study provided written informed consent and this study was conducted in accordance with the Declaration of Helsinki.

Results

Characteristics of EPE and rate of prolonged colonization

We included a total number of 143 patients in the study, and these individuals carried in total 147 strains of EPE, of which 134 (91%) were *E. coli*, 12 (8%) were *Klebsiella* spp. and 1 (1%) was *Proteus* spp. Out of the 147 strains of EPE, 134 (91%) were characterized as having an ESBL A phenotype (according to the Ambler classification; inhibited by clavulanic acid), 10 (7%) as AmpC (ampicillinase C; inhibited by cloxacillin) and 3 (2%) as having both. Of the 147 strains, 24 (16%) were resistant to ciprofloxacin, 24 (16%) were resistant to trimethoprim/sulfamethoxazole and 71 (48%) were resistant to both antimicrobials. In contrast, 28 (19%) EPE were susceptible to both ciprofloxacin and trimethoprim/sulfamethoxazole. The characteristics of EPE strains are displayed in detail in Table S1.

Eighty-one patients (57%) of the study cohort remained positive for EPE in the follow-up fecal sample (Figure 1). A minority of patients (n=62; 43%) were negative for EPE in feces on follow-up. Of the 81 patients who remained positive for EPE in the follow-up fecal sample, an identical EPE was found as determined by phenotype and species when compared to the original sample. However, 19 patients (23%) had at least one antibiotic that diverged compared to the antibiogram of the original culture.

Baseline characteristics of the study population

Baseline characteristics of the study population based on aggregate of medical record and questionnaires are as outlined in Table 1. Out of the 143 patients included in our

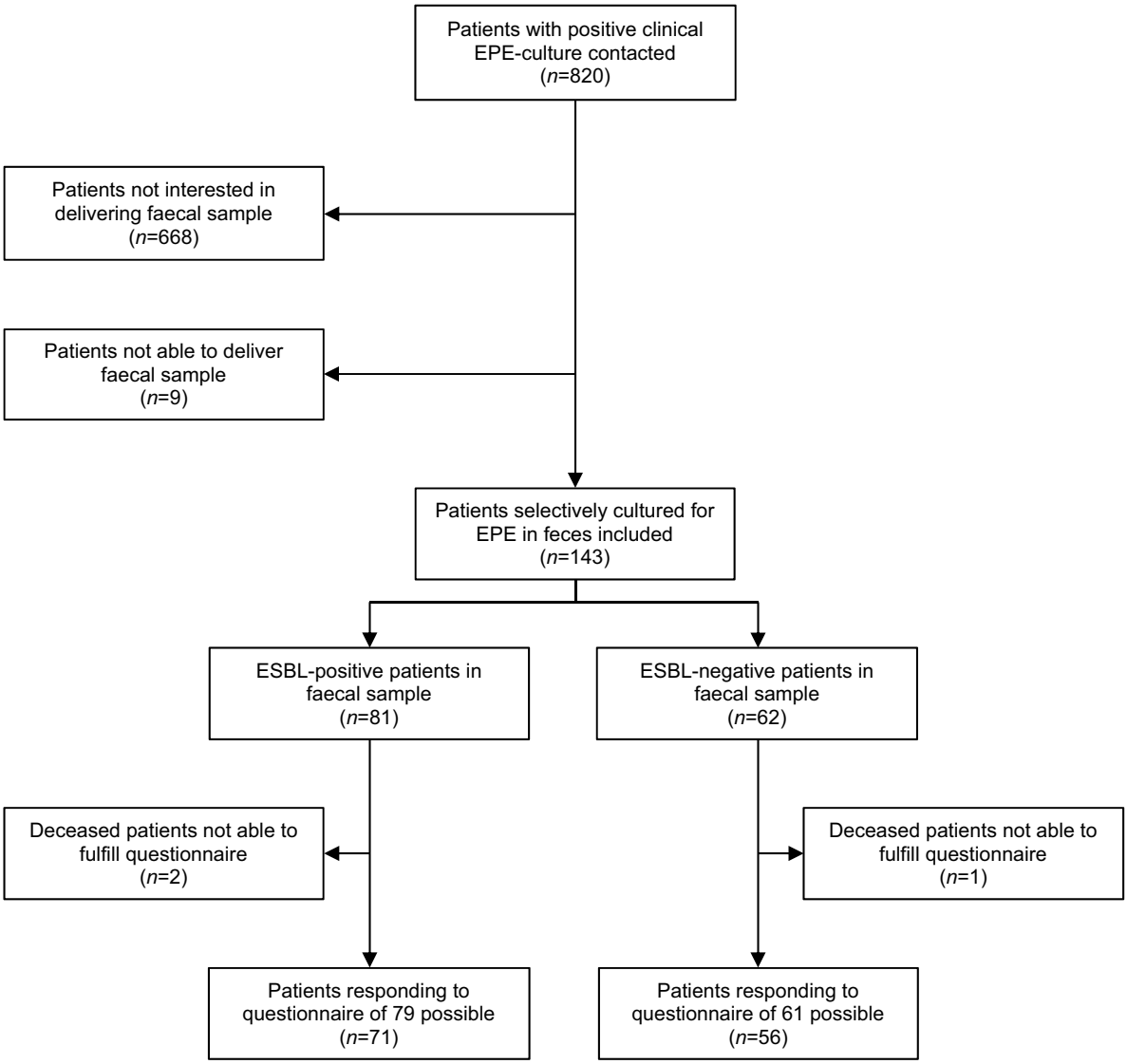

Figure 1 Flow chart of study patient inclusion and questionnaire distribution.
Abbreviation: EPE, extended spectrum β-lactamase producing *Enterobacteriaceae*.

study, 92 (64%) were women. The median age was 67 years (range 19–87). The median Charlson comorbidity index was 0 (range 0–7). At the time of inclusion (ie, submission of fecal sample), median duration of known EPE carriage was at least 7 months in both groups. Out of the 143 patients, 106 (74%), 10 (7%) and 27 (19%) had an original culture positive for EPE in urine, blood and rectum, respectively. Furthermore, 116 (81%) of patients had a history of an EPE infection, whereas 27 (19%) patients were merely colonized with EPE. During the last 2 years, 70 (49%) of the 143 patients had been hospitalized and 122 (85%) had received at least one prescription of antibiotics.

The two groups, ie, EPE positive and EPE negative as determined by a follow-up fecal sample, were similar regarding underlying medical conditions. There were no statistical differences in comorbidity as determined by Charlson comorbidity score. Characteristics of the patients' comorbidity index according to this score are presented in Table S2.

Analyses of putative risk factors for prolonged EPE colonization

In the univariate model, urological intervention within 6 months (P=0.000), an original EPE-positive culture in urine (P=0.009), a history of EPE infection (P=0.007) and travel to Asia and/or Africa within two years (P=0.020) were associated with a follow-up fecal sample positive for EPE. Primary outcome for dichotomized data

Table 1 Baseline characteristics of the study population based on medical records and questionnaires

Baseline characteristic	EPE-positive (n=81)	EPE-negative (n=62)
Age, median years (range)	69 (19–87)	66 (31–86)
Female sex (%)	51 (63)	41 (66)
Original culture positive for EPE, source (%)		
Urine	66 (81)	40 (65)
Blood[1]	7 (9)	3 (5)
Rectal	8 (10)	19 (30)
Duration of known carriage, median months (range)	7 (3–108)	8 (3–56)
History of any EPE infection (%)		
Yes	72 (89)	44 (71)
No, ie, colonization	9 (11)	18 (29)
EPE infection and/or colonization prior to January 2016 (%)	14 (17)	6 (10)
Urological intervention within 6 months[2] (%)	29 (36)	7 (11)
Inflammatory bowel disease (%)	0 (0)	2 (3)
Inpatient hospital care within 2 years[3] (%)	44 (54)	26 (42)
Urinary catheter or clean intermittent catheterization (%)	9 (11)	2 (3)
Antibiotic treatment within 2 years[4] (%)	69 (85)	53 (85)
Overall medications (%)		
No medication	14 (17)	13 (21)
1 or 2 medications	15 (19)	19 (31)
3 or more medications	52 (64)	30 (48)
Ongoing proton pump inhibitor treatment (%)	18 (22)	11 (18)
Ongoing immunosuppressive treatment[5] (%)	4 (5)	3 (5)

Notes: All data were collected at the time of inclusion, ie, when fecal culture was obtained. [1]If positive in both blood and urine, only blood was chosen. [2]Minor or major invasive procedure on bladder, prostate and/or kidney and cystoscopy. [3]In Sweden and/or internationally. [4]Any systemic antibiotic. [5]Including any dosage of corticosteroids and/or methotrexate. All percentages are rounded up to the nearest integer.

Abbreviations: ESBL, extended spectrum β-lactamase; EPE, extended spectrum β-lactamase producing *Enterobacteriaceae*.

is presented in Table 2. In the multiple logistic regression model, history of EPE infection (P=0.002, OR 4.12, 95% CI: 1.36–12.51), urological intervention within 6 months (P=0.005, OR 4.53, 95% CI: 1.43–14.38) and one or two medications (P=0.028, OR 0.31, 95% CI: 0.05–0.77) were associated with prolonged intestinal EPE colonization.

Other putative risk factors for prolonged intestinal EPE carriage, ie, age over 70 years, female sex, urinary catheter/intermittent clean catheterization, antibiotic resistance and travel to Africa and/or Asia within two years were not associated with prolonged intestinal EPE colonization. Secondary outcomes from a multiple logistic regression model are displayed in Table 3.

In the stepwise forward multiple regression model, the risk factor one to two medications was no longer of significance. Urological intervention within 6 months (P=0.007, OR 4.32, 95% CI: 1.50–12.42), history of EPE infection (P=0.028, OR 2.82, 95% CI: 1.12–7.10) and travel to Africa and/or Asia within the last 2 years (P=0.04, OR 0.42, 95% CI: 0.18–0.96) remained significant risk factors. The results of the stepwise forward multiple regression model are displayed in Table 4.

Data obtained by the questionnaire

Thus, 140 patients received the questionnaire of which 127 (91%) submitted the form. The remaining 13 study subjects did not submit the questionnaire and could neither be reached by the investigators. The proportion of questions answered is displayed in Table S3.

Out of the 140 patients receiving the questionnaire, 122 (86%) answered the closed question if they had knowledge of EPE before being contacted by the study group. Sixty-one

Table 2 Univariate statistic model of putative risk factors for prolonged intestinal EPE carriage using Fisher's exact test

Variable	EPE-positive (n=76)	EPE-negative (n=67)	*P*-value
Age >70 years	32	19	0.115
Female sex	50	45	1.000
Escherichia coli[1]	73	61	0.149
ESBL phenotype A[1]	71	60	0.229
Bacteria resistant to Ciprofloxacin and/or Trimethoprim/Sulfamethoxazole[1]	62	51	0.538
Isolate in urine[1]	57	36	**0.009**
History of EPE infection	60	40	**0.007**
Carriage prior to January 2016	12	6	0.218
Urological intervention within 6 months[2]	26	6	**0.000**
Inflammatory bowel disease	0	2	0.218
Charlson comorbidity index >0	39	26	0.131
Inpatient hospital care within 2 years[3]	37	29	0.506
Urinary catheter or clean intermittent catheterization	7	1	0.066
Antibiotic use within 2 years[4]	60	52	1.000
Overall drug treatment >0	57	53	0.699
Proton pump inhibitor treatment	15	12	0.831
Immunosuppressive treatment[5]	3	3	1.000
Travel to Africa and/or Asia within 2 years#	15	26	**0.020**
Abdominal pain and/or altered bowel habits#	23	27	0.358
Domestic animal#	20	22	0.570
Currently working#	22	24	0.454
>1 person in household#	50	42	0.275
Known carrier of EPE in household[6]#	2	2	1.000
Vegetarian#	1	0	1.000
Post-secondary education#	33	32	1.000

Notes: Statistical analysis using Fisher's exact test with exact 2-sided significance. A *P*-value of ≤0.05 was considered significant. All data were collected originating at time of inclusion, ie, when fecal culture was collected, except [1] which denotes original EPE-positive culture. [2]Minor or major invasive procedure on bladder, prostate and/or kidney and cystoscopy. [3]Both in Sweden and internationally. [4]Any systemic antibiotic. [5]Including any dosage of corticosteroids or methotrexate. [6]Other than patient included in the study. All calculations are based on the weighted data. All counts are rounded to the nearest integer. Due to rounding, there are small variations in the number of patients included in each test. All percentages are rounded up to the nearest integer. #Based on the total number of questionnaire responses according to Table S3. In this article, a *P*-value of ≤0.05 highlighted in bold was considered significant.

Abbreviations: ESBL, extended spectrum β-lactamase; EPE, extended spectrum β-lactamase producing *Enterobacteriaceae*.

patients (50%) were unaware of prior EPE infection or colonization. When contacted by the research group, 61 patients (50%) had knowledge of EPE infection or colonization. Of the EPE-positive cohort, 37 of 80 (45%) and in the EPE-negative cohort 24 of 61 (39%) were aware of EPE infection or colonization.

Accounting for missing data

Out of 820 patients approached in this study, only 143 (17%) were willing to submit a fecal sample. If the patients who chose not to provide a fecal sample in the study diverged from the patients included in the study, there could be a problem with the external validity of our results. To investigate this, the medical records of the patients (n=677) not willing to submit a fecal sample for the study were reviewed with regard to age, sex and Charlson comorbidity score. Also, the risk factors significantly associated with prolonged colonization with EPE that fell out before weighing were examined – history of EPE infection, urological intervention within 6 months and resistance against both ciprofloxacin and trimethoprim/sulfamethoxazole. These data were compared to the same characteristics of the patients included in our study, and Table S4 shows the background characteristics of the groups. The median age, female sex, median Charlson comorbidity score or percentage of urological intervention within 6 months did not differ in the patients included in our study and the patients not included. Thus, our findings could be generalized to the public. Antibiotic consumption could not, however, be compared since these data relied on the submitted questionnaires. However, there was a difference in the percentage of patients with resistance against both ciprofloxacin and trimethoprim/sulfamethoxazole (P=0.01) and infected compared to colonized (P=0.001) in the two groups. To rectify this, statistical weights were computed with regard to resistance and history of EPE infection. Computed statistical weights are presented in Table S5.

Table 3 Multiple logistic regression model of putative risk factors for prolonged intestinal EPE carriage

Variable	EPE-positive (n=62)	EPE-negative (n=58)	OR	CI 95%		*P*-value
				Lower	Upper	
Age ≥70 years	27	18	1.19	0.43	3.27	0.957
Female sex	45	38	2.57	0.82	8.07	0.089
Antibiotic resistance[1]						0.180
Ciprofloxacin	15	9	1.54	0.35	6.71	0.341
Trimethoprim/sulfamethoxazole	11	16	0.55	0.15	2.07	0.714
Ciprofloxacin + trimethoprim/sulfamethoxazole	25	23	1.88	0.55	6.40	0.124
History of EPE infection	52	35	4.12	1.36	12.51	**0.002**
Urological intervention within 6 months[2]	23	5	4.53	1.43	14.38	**0.005**
Urinary catheter or intermittent clean catheterization[#]	5	1	2.10	0.23	19.15	0.544
Overall medications[#]						0.089
1–2 medicines	11	19	0.19	0.05	0.77	**0.028**
3 or more medicines	36	27	0.31	0.09	1.06	0.145
Travel to Africa and/or Asia within 2 years[#]	15	26	0.40	0.15	1.04	0.100

Notes: Statistical analysis using binary logistic regression model. *P*-value ≤0.05 was considered significant. Odds ratio for prolonged intestinal EPE carriage. All data were collected originating at the time of inclusion, ie, when fecal culture was collected, except [1] which denotes original EPE-positive culture. [2]Minor or major invasive procedure on bladder, prostate and/or kidney and cystoscopy. All calculations are based on the weighted data. All counts are rounded to the nearest integer. Due to missing data, the regression was calculated on 120 observations. All percentages are rounded up to the nearest integer. [#]Information based on questionnaires. In this article, a *P*-value of ≤0.05 highlighted in bold was considered significant.

Abbreviations: OR, odds ratio; CI, confidence interval; ESBL, extended spectrum β-lactamase; EPE, extended spectrum β-lactamase producing *Enterobacteriaceae*.

Discussion

In this prospective cohort study, factors independently associated with prolonged intestinal colonization with EPE were urological intervention within 6 months and a history of EPE infection. Travel to Africa and/or Asia within the last 2 years was also significantly associated with a decreased risk of becoming a long-term carrier. Furthermore, 50% of the patients in this study were not properly informed of infection/colonization of EPE.

Only a few studies exist that investigate why and in whom prolonged EPE colonization occurs.[8,10,14] This study adds new information to the knowledge-void that exists in this field of research. The strength of this study is the prospective study design and robust methods of measurement. The statistical method of weighing data allowed for a somewhat sizable cohort of patients affected of EPE to be investigated. Overall, more than 90% submitted the questionnaire, either by sending it in or responding over the telephone, which is a strength in the present study. Furthermore, we show that patients affected by EPE are underinformed by health care givers, something which has not priorly been investigated in a similar setting.

Urological intervention at a maximum 6 months prior to inclusion was associated with prolonged intestinal colonization of EPE. Apart from transrectal prostate biopsy, which is a common cause of nosocomial infection both with and without EPE, it is unlikely that the urological intervention itself is associated with prolonged intestinal colonization with EPE. However, antibiotic prophylaxis is common prior to urological interventions. The recommended prophylaxis according to guidelines in Skåne is one dose of either trimethoprim/sulfamethoxazole or ciprofloxacin prior to the biopsy. It is a well-known fact that quinolones, including ciprofloxacin, are drivers of antibiotic resistance.[29] Importantly, EPE isolates from urological patients are less susceptible to ciprofloxacin than isolates from non-urological patients.[30] Furthermore, fecal carriage of EPE is common after transrectal prostate biopsy. The presence of EPE after biopsy is associated with fluoroquinolone consumption before biopsy.[31] In addition, transrectal prostate biopsy is a common cause of antibiotic-resistant bloodstream infection, and the *E. coli* clone CTX-M-15 ST131 is a major contributing

Table 4 Stepwise forward multiple regression model of potential risk factors for prolonged intestinal EPE carriage

Step	Variable	OR	CI 95%		*P*-value
			Lower	Upper	
Step 1	Urological intervention within 6 months[1]	5.72	2.05	15.92	**0.001**
Step 2	Urological intervention within 6 months	4.69	1.65	13.33	**0.004**
Step 3	History of EPE infection	2.77	1.13	6.83	**0.027**
	Urological intervention within 6 months	4.32	1.50	12.42	**0.007**
	History of EPE infection	2.82	1.12	7.10	**0.028**
	Travel to Africa and/or Asia within 2 years[#]	0.42	0.18	0.96	**0.040**

Notes: Statistical analysis using binary logistic regression model. *P*-value ≤0.05 was considered significant. Odds ratio for prolonged intestinal EPE carriage. All data were collected originating at the time of inclusion, ie, when fecal culture was collected. [1]Minor or major invasive procedure on bladder, prostate and/or kidney and cystoscopy. All calculations are based on the weighted data. All counts are rounded to the nearest integer. Due to missing data, the regression was calculated on 120 observations. Included variables in the stepwise regression model as presented in Table 3. [#]Information based on questionnaires. In this article, a *P*-value of ≤0.05 highlighted in bold was considered significant.

Abbreviations: OR, odds ratio; CI, confidence interval; EPE, Extended spectrum β-lactamase producing *Enterobacteriaceae*.

pathogen in this context.[32,33] Hence, administration of antibiotics in relation to urological interventions could explain why we found an association with prolonged intestinal colonization with EPE.

However, a more plausible explanation for the association between urological intervention and prolonged intestinal colonization of EPE is that cystoscopy is routinely performed in patients with recurrent UTIs. In Sweden, a nonendemic country of EPE, repeated urinary cultures with EPE is an indication for urological intervention, to exclude a treatable cause for recurrent UTIs.

We suggest that this at least to a certain extent could explain the association.

When compared to mere colonization, a history of EPE infection was associated with a higher risk for prolonged intestinal colonization with EPE. This is in accordance with a previous study where colonization was associated with clearance of EPE, when compared to clinical samples.[18] A study from Sweden revealed that 43% of the patients still being colonized 12 months after EPE infection.[9] This colonization rate is higher compared to what Östholm-Balkhed et al and Arcilla and collaborators presented, 11.0% and 11.3%, respectively, found 12 months after EPE-colonization diagnosed after foreign travel.[25,34] In addition, Ruppé et al observed an even less frequent prevalence (4.7%) of EPE three months after being colonized/infected abroad.[15]

Subsequent EPE infection in patients previously colonized with EPE is rare.[33] On the other hand, recurrent infection with EPE is common, especially within the first 6 months of the first infection and in patients ≥65 years of age.[35] Hence, it is reasonable to assume that more virulent strains to a higher extent possess virulence factors giving them a survival advantage, and the ability to colonize the intestine. Strains merely colonizing the intestine lack important virulence genes needed to trigger infection. This is in accordance with Ny et al who found strains of low pathogenicity in fecal samples in randomly selected Swedish citizens compared to strains causing invasive infection.[36]

The ST131 clone has managed to disseminate globally due to its ability to frequently colonize the intestine, its enhanced virulence and broader antibiotic resistance compared to other EPE clones.[37] Strains originating from this clone are more prone to possess antibiotic resistance against ciprofloxacin and trimethoprim/sulfamethoxazole.[38] We did not find any association between EPEs with resistance against ciprofloxacin and trimethoprim/sulfamethoxazole, and prolonged EPE colonization.

Unfortunately, we were not able to conduct any molecular analysis such as whole genome sequencing (WGS) of the EPE isolates. WGS could have given us useful information on EPE characteristics. Furthermore, without WGS, multiple-locus variable number tandem repeat or pulse-field gel electrophoresis, the strain obtained in the fecal culture could not be determined to be exactly the same as the strain in the original culture. In fact, 23% of patients had disconcordant antibiograms when the original culture was compared to the follow-up fecal culture regarding at least one antibiotic. We believe that the main reason for this is that patients can carry many different EPEs, which can be impossible to distinguish when selecting colonies on a blood agar. A previous study, with a longer follow-up time, using MLVA showed that the majority of long-term EPE carriers had either a different ESBL-producing species or a ESBL-producing *E. coli*

with a different MLVA-profile in original culture compared to the follow-up sample.[10]

Furthermore, the methodology in the Clinical Microbiology laboratory may differ and the probability that the same strain can yield different susceptibility patterns increases as many antibiotics are tested. In addition, there is a slight risk that patients become colonized with new EPE strains, in which the term prolonged intestinal EPE colonization is misused in this context. This risk was estimated low by the study group in this low endemic EPE setting.

We found an association between EPE that were isolated from urine in original cultures, as opposed to any other anatomical location, and prolonged intestinal colonization with EPE in the univariate analysis. This was, however, not confirmed in the multivariate logistic regression model. We believe that these two risk factors, ie, history of EPE infection and EPE present in urine, are closely connected. An EPE-positive urine culture reflects infection or asymptomatic bacteriuria, whereas an EPE-positive rectal culture is a marker of colonization. As previously mentioned, antibiotic consumption is closely connected to a history of EPE infection and the rate of consumption was high in both groups. We did not find any association between previous antibiotic consumption and prolonged intestinal colonization with EPE. This is in accordance with several studies in non-travelers.[9,10,14] However, Rogers et al found an association between antibiotic consumption abroad and prolonged colonization of EPE post travel.[21]

Traveling to high endemic areas of EPE such as Africa, Asia and Middle East is a known risk factor for EPE acquisition. In our study, we found that previous travel to EPE-high endemic areas was associated with a decreased risk of prolonged intestinal carriage of EPE. This is in accordance with ÖstholmBalkhed, who found no risk factors associated with prolonged intestinal colonization with EPE, but found that diarrhea during travel and a new trip during follow-up was associated with a decreased risk of becoming a long-term carrier.[25] EPE acquisition during travel may thus be unrelated to specific microbiota profile but could affect the duration of colonization.[39]

We did not find correlation between a positive EPE fecal sample and abdominal pain or diarrhea, the latter a known risk factor for EPE acquisition.[24] Papst et al showed that immobilization is a risk factor for prolonged EPE colonization, a risk factor this study did not address.[14] In contrast, Alsterlund et al did not find risk factors associated with prolonged carriage of EPE, but did not perform molecular characterization of the isolates nor multivariable analysis of the data.[40]

With this study, we also wanted to investigate to what extent patients are informed about their EPE infection or colonization. Surprisingly, 50% of the patients were not adequately informed of their EPE status by their health care supplier. The Public Health Agency of Sweden states that it is mandatory for the responsible physician to provide information regarding infection or colonization with EPE.[41] This information is mandatory to prevent EPE from spreading, both in the community and in health care settings, as patients are obliged to disclose their EPE status when seeking health care. Adequate actions are needed by authorities since information is an underestimated but important tool fighting antibiotic resistance.

A limitation with this study is that we used only one follow-up rectal culture, and it is a well-known fact that transient negative samples occur upon EPE-colonization.[8,14] It is important to note that we do not consider that a negative follow-up sample as equal to a permanent loss of EPE colonization. It could be argued, however, that a negative EPE fecal sample reflects quantitatively less EPE shedding. This could lead to reduced risk of spreading EPE in the community and hospital setting. If this is associated with a less risk of EPE infection is not answered in this study.

In general, when using questionnaires to collect information, response bias is a matter of concern. This was taken into consideration when composing the questionnaire. Still, some patients could have agreed to be included in the study and answer the questionnaire because they knew their EPE status. This could be addressed by sending specific questionnaires to a sample of the patients who were invited to the study but declined to provide samples for the study.

We discovered that some questions in the questionnaire tended to be misapprehended. Furthermore, some participants did not fulfill the whole questionnaire and sample collecting was not done immediately, which is why lacking memory could affect the answers. This could be countered by interviewing the patients at the time of inclusion.

A major limitation of this study was the low percentage of inclusion in the study, only 17% (143 of 820 patients) of the patients were willing to submit a fecal sample. A reason for this could be lack of information and implications of EPE colonization. Another reason could be a perceived discomfort of submitting a fecal culture to the study group. However, as weighting was performed, our results gained validity.

Results from this study contribute to knowledge about the long-term carriage of EPE. We did not find that patient characteristics such as severe underlying diseases, age, etc., are risk factors associated with prolonged colonization of EPE. Bacterial features such as the ability to trigger an infection over colonization seem to be of greater importance. More information about colonization with EPE is crucial to implement adequate control measures, thus reducing spread of disease. It is particularly important to work preventive in a time of increasing antibiotic resistance. This is the most important reason to continue with well-designed research within this field.

Conclusion

In this prospective cohort study, we have presented that urological intervention within 6 months and a history of EPE infection are independently associated with prolonged intestinal colonization with EPE. Travel to Africa and/or Asia within 2 years is associated with a decreased risk of prolonged intestinal colonization with EPE. We could not find any association between other risk factors including prior antibiotic consumption, comorbidity, international travel, in hospital care, etc., with prolonged intestinal colonization with EPE. These findings ultimately contribute to knowledge about EPE pathogenesis and stimulate further research within this field. Despite the fact that the Public Health Agency of Sweden states that information regarding EPE is mandatory, 50% of the patients in this study were not properly informed about their EPE status.

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Disclosure

The authors have no conflict of interest to disclose in this work.

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