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Frequent acquisition of low-virulence strains of ESBL-producing *Escherichia coli* in travellers

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Objectives: International travel is a risk factor for intestinal colonization with ESBL-producing Enterobacteriaceae (EPE). This prospective cohort study focuses on molecular features of and risk factors for travel-acquired EPE.

Methods: Rectal swabs and survey data were collected from 188 Swedes travelling to four regions of high EPE prevalence. Samples were plated onto selective agars. ESBL producers were determined using phenotypic methods. Molecular characterization regarding virulence factors and phylogenetic grouping of ESBL-producing *Escherichia coli* was done using PCR. Isolates were also screened for the plasmid-mediated colistin resistance gene *mcr-1*.

Results: Among 175 pre-travel EPE-negative participants, 32% were positive upon return. No carbapenemase-producing Enterobacteriaceae were found, but one CTX-M-producing *E. coli* harboured *mcr-1* (travel to Thailand). Most *E. coli* strains (43.1%) belonged to phylogroup A and were rarely associated with extraintestinal infections and a few (9.2%) expressed uropathogenicity *pap* genes. During 10–26 months of follow-up, no clinical infections were observed. Colonization rates varied by visited region: the Indian subcontinent, 49.2%; northern Africa, 44.0%; South-East Asia, 19.1%; and Turkey, 9.5%. Travellers' diarrhoea (OR 2.5, $P=0.04$) or antimicrobial treatment during the trip (OR 5.9, $P=0.02$) were both independent risk factors for EPE colonization.

Conclusions: EPE acquired during travel have seemingly low pathogenicity, possibly indicating a low risk of clinical infection. Pre-travel advice should emphasize avoiding unnecessary antibiotic treatment during travel.

Introduction

ESBLs can be produced by all Gram-negative bacteria belonging to the family Enterobacteriaceae, most notably *Escherichia coli* and *Klebsiella pneumoniae*. *E. coli* is part of the commensal intestinal microbiota, but is also the most common pathogen causing urinary tract infection (UTI) and Gram-negative bloodstream infection (BSI).[1] ESBL-producing Enterobacteriaceae (EPE) are a major concern worldwide and are increasing rapidly. Rates of resistance to extended-spectrum cephalosporins >50% have now been described in all WHO regions, albeit with considerable variation within the regions.[2] Infections caused by EPE are associated with higher mortality, longer hospitalizations and higher costs compared with non-EPE.[3,4] Sweden is still considered a low-prevalence country. In 2014, the Public Health Agency in Sweden published a study on intestinal EPE colonization among healthy Swedes; the prevalence was 4.8% for samples collected in 2012–13.[5] The risk of intestinal EPE acquisition when travelling to high-prevalence areas ranges between 20% and 70%, depending on the region visited.[6–9] Besides geographical region, travel-associated diarrhoea, antimicrobial treatment and high age are considered risk factors for colonization.[7,9]

With phylogenetic analyses, *E. coli* can be divided into four major phylogroups (A, B1, B2 and D). Commensal strains mostly belong to phylogroups A and B1, while phylogroup B2 and, to some extent, phylogroup D are connected to longer duration of intestinal colonization and are more often found causing extraintestinal infections.[10–12]

Expression of virulence factors and antimicrobial resistance genes differ among *E. coli* strains.[11] Virulence factors related to uropathogenicity have been shown to have an impact also on the ability of bacteria to colonize the intestine. Different type I fimbriae (*fim*) and P fimbriae (pili associated with pyelonephritis; *pap*) are associated with adhesins, enhancing the possibility for bacteria to adhere to mucosal surfaces.[11,13] High relative abundance (RA) of EPE in the faecal microbiota seems to be correlated to prolonged carriage,[14] but biomolecular features of EPE colonizing the

intestine of healthy individuals in contrast to isolates found after clinical infections have been poorly studied.

In this study, we investigated the molecular features of EPE strains colonizing the intestinal microbiota after travel, as strain factors could affect both the length of carriage and the risk of symptomatic infections caused by EPE strains from the gut microbiota. We also determined risk factors for EPE colonization when travelling and risk of subsequent infections caused by EPE.

Methods

Ethical considerations

The study protocol followed the guidelines of the Declaration of Helsinki and ethics approval was obtained from the Karolinska Institutet Regional Ethics Committee of Stockholm (recordals 2012/1265-31/4, 2013/1844-32 and 2015/1620-32). Written consent was collected from the study subjects.

Study design

A prospective cohort study was performed between 15 April 2013 and 31 May 2015. Swedish travellers aged ≥18 years, travelling to either South-East Asia, the Indian subcontinent, northern Africa or the Middle East (Turkey), were enrolled when receiving pre-travel advice at a travel consultation clinic in Stockholm. For details on countries see Table S1 (available as Supplementary data at *JAC* Online). Before and after the trip, the participants submitted rectal swabs and data on potential risk factors. Samples were sent to the Department of Clinical Microbiology, Karolinska University Hospital, Solna, Sweden (Figure S1). Travellers not providing both samples were excluded from the study.

Characterization of bacterial isolates

ChromID ESBL agar (bioMérieux, Marcy-l'Étoile, France) was used for screening of EPE. Additionally, all samples ($n=188$) were subjected to broth enrichment with LB broth (Thermo Fisher Scientific, Waltham, USA) containing 0.25 mg/L meropenem, followed by cultivation on an in-house agar on a chromogenic base (Liofilchem, Roseto degli Abruzzi, Italy) supplemented with 200 mg/L cloxacillin and 0.25 mg/L meropenem. Samples were inoculated onto agar plates and incubated overnight at 35°C and MALDI-TOF Biotyper (Bruker, Billerica, MA, USA) was used for species identification of unique colony morphologies growing on the selective plates. Vitek 2 (bioMérieux) was used for phenotypic ESBL testing. Further testing for concomitant production of AmpC was done according to EUCAST guidelines with Rosco kits (Rosco Diagnostica, Taastrup, Denmark).[15] Susceptibility testing of confirmed EPE was performed with the disc diffusion method (Oxoid, Basingstoke, UK) and interpreted according to EUCAST guidelines.

ESBL subtyping was performed with Check-MDR (Checkpoints, Wageningen, The Netherlands)[16] and epidemiological typing was performed with the automated rep-PCR system DiversiLab (bioMérieux) according to the manufacturer's protocol.[17]

Phylogrouping of *E. coli* was performed using real-time PCR according to a previously described method.[10]

Virulence factors (*fimAMT78*, *fimH*, *papAH*, *papC*, *papEF* and *papG* alleles I, II and III) and resistance genes (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS*) were analysed using real-time PCR. The prevalence of the plasmid-mediated fluoroquinolone resistance gene aminoglycoside-(6)-*N*-acetyltransferase [*aac(6′)-Ib*] and its point mutant *aac(6′)-Ib-cr* were analysed according to previously published protocols.[18,19] All EPE isolates were screened for the recently emerged plasmid-mediated colistin resistance gene *mcr-1* according to a published protocol.[20] Next-generation sequencing was carried out in one isolate, using the Ion Torrent S5 XL system (Thermo Fisher Scientific, Waltham, MA, USA). ResFinder was used to retrieve resistance genes.[21] MLST ST was retrieved as described previously.[22] PlasmidFinder was used to detect the replicon sequence for the sequenced isolate.[23]

Factors associated with the study participants

Study participants filled in a questionnaire on personal data and potential risk factors such as underlying chronic diseases, time spent abroad, intake of antibiotics and contact with local healthcare facilities (questionnaire in detail in Figure S2) before and after travel. Travellers' diarrhoea was defined as more than three loose stools per day during ≥1 day. Antibiotic treatment during the trip included all antibiotic classes.

Participants with EPE colonization were followed for 10–26 months to detect clinical EPE infections by searching both the database at the Department of Clinical Microbiology (Karolinska University Hospital) and SmiNet-2 (a national microbiological database).[24] Together, these databases cover microbiological culture results in the Stockholm area.

To compare differences in resistance rates between isolates causing invasive infection and colonizing strains, antibiograms from blood culture isolates at Karolinska University Hospital 2013–15 were retrieved (covers ∼80% of the Stockholm population) and compared with the antibiotic resistance pattern in EPE isolates found among the travellers.

Statistics

For categorical variables, Fisher's exact test and the χ^2 test were used. The Mann–Whitney test was used to compare continuous variables. For all tests, a two-sided P value <0.05 was considered significant. Multivariable logistic regression was performed using Stata 12.0 software (StataCorp, USA). For the multivariable model, factors previously correlated to EPE colonization (travel destination, travellers' diarrhoea, antimicrobial treatment and age), sex and other factors with a P value <0.2 in univariate analysis were included in the first model. We thereafter excluded factors backwards using a P value of 0.05 and comparing models with the likelihood ratio test. In the final model, significant risk factors and the previously described important risk factors were kept.

Results

A total of 376 faecal samples were analysed from 188 study subjects (Figure S1). Thirteen of 188 (6.9%) were colonized with EPE before travelling and excluded concerning analysis of risk factors, thus leaving 175 participants. The majority (119/175, 68.0%) were women and the median age was 49 years.

Characterization of isolates

In Table 1, bacterial characteristics are presented in relation to geographical region. In total, 67 different EPE strains were identified from the pre-travel negative participants. Most isolates were *E. coli* ($n=65$), followed by *K. pneumoniae* ($n=1$) and *Citrobacter freundii* ($n=1$). Nine travellers were colonized with more than one EPE strain upon return, at most three different strains in one traveller. No carbapenemase-producing Enterobacteriaceae were detected.

In total, 61 (91.0%) of the EPE strains were of the CTX-M type, most commonly CTX-M-1 ($n=49$, 73.1%), followed by CTX-M-9 ($n=12$, 17.9%). Most CTX-M-9 strains (8 out of 12) were acquired in South-East Asia (Figure 1).

Virulence factors

The type 1 fimbriae-encoding gene *fimH* was found in 50 (76.9%) *E. coli* strains and gene *fimAMT78* was found in 15 (23.1%) strains.

Table 1. Bacterial characteristics in relation to geographical region

Characteristic	South-East Asia ($N=15$) [*E. coli* ($N=15$)], *n* (%)	Indian subcontinent ($N=38$) [*E. coli* ($N=36$)], *n* (%)	Northern Africa ($N=12$) [*E. coli* ($N=12$)], *n* (%)	Turkey ($N=2$) [*E. coli* ($N=2$)], *n* (%)	P^a
Phylogroup A	7 (46.7)	10 (27.8)	9 (75.0)	2 (100.0)	**0.01**
Phylogroup B1	2 (13.3)	10 (27.8)	0 (0.0)	0 (0.0)	0.11
Phylogroup B2	2 (13.3)	6 (16.7)	0 (0.0)	0 (0.0)	0.45
Phylogroup D	4 (26.7)	10 (27.8)	3 (25.0)	0 (0.0)	1.00
CTX-M-1	7 (46.7)	31 (81.6)	9 (75.0)	2 (100.0)	0.06
CTX-M-9	8 (53.3)	2 (5.3)	2 (16.7)	0 (0.0)	**<0.001**
CMY II	1 (6.7)	2 (5.3)	0 (0.0)	0 (0.0)	1.00
SHV[b]	0 (0.0)	5 (13.2)	1 (8.3)	0 (0.0)	0.42
TEM	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.00
Antibiotic resistance (intermediate and resistant)					
cefotaxime	15 (100.0)	38 (100.0)	12 (100.0)	2 (100.0)	1.00
ceftazidime	14 (93.3)	35 (92.1)	11 (91.7)	2 (100.0)	1.00
piperacillin/tazobactam	3 (20.0)	9 (23.7)	1 (8.3)	2 (100.0)	
gentamicin	9 (60.0)	10 (26.3)	1 (8.3)	0 (0.0)	**0.01**
amikacin	0 (0.0)	5 (13.2)	0 (0.0)	0 (0.0)	0.21
ciprofloxacin	5 (33.3)	20 (52.6)	2 (16.7)	0 (0.0)	0.07
trimethoprim/sulfamethoxazole	12 (80.0)	20 (52.6)	7 (58.3)	0 (0.0)	0.19
imipenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.00
meropenem	0 (0.0)	1 (2.6)	0 (0.0)	0 (0.0)	1.00
ertapenem	0 (0.0)	4 (10.5)	0 (0.0)	0 (0.0)	0.48
MDR (intermediate or resistant to at least three classes of antibiotics)	11 (73.3)	20 (52.6)	2 (16.7)	0 (0.0)	**0.01**
Virulence factors in *E. coli*					
fim	13 (86.7)	28 (77.8)	9 (75.0)	2 (100.0)	0.76
pap	0 (0.0)	6 (37.5)	0 (0.0)	0 (0.0)	0.12
Several (at least three) virulence factors	0 (0.0)	5 (13.9)	0 (0.0)	0 (0.0)	0.22
Quinolone resistance genes					
qnr	8 (53.3)	14 (36.8)	2 (16.7)	0 (0.0)	0.06
aac(6′)-Ib-CR	1 (6.7)	9 (23.7)	0 (0.0)	0 (0.0)	0.09
16S methylases	0 (0.0)	2 (5.3)	0 (0.0)	0 (0.0)	1.00

P values<0.05 are shown in bold.
[a]Univariate analysis in 3×2 table including South-East Asia, the Indian subcontinent and northern Africa.
[b]CTX-M-negative isolates carrying SHV.

Pyelonephritis-associated pili-encoding genes were found in six (9.2%) isolates. All of these isolates harboured *papEG*, two had additional carriage of *papAH*, *papC* and *papGII* and two isolates had additional carriage of *papC* and *papGII*. All strains harbouring *pap* were acquired when travelling to India.

Phylogroups

Most *E. coli* isolates (28/65, 43.1%) belonged to phylogroup A, followed by phylogroup D (17/65, 26.2%), phylogroup B1 (12/65, 18.5%) and phylogroup B2 (8/65, 12.3%). Out of the five strains harbouring several virulence factors, four belonged to phylogroup B2 and one to phylogroup D. Isolates belonging to phylogroups B2 and D were in a higher proportion multiresistant than isolates belonging to phylogroups A and B1. Phylogroup A was more commonly detected in travellers from northern Africa compared with South-East Asia and the Indian subcontinent ($P=0.01$).

Thirteen travellers were colonized with EPE before travelling and only six of them after return. From these six travellers, DiversiLab revealed identical strains among four persons before and after the trip, all of these strains belonged to phylogroup B2. Two travellers were colonized with new EPE strains, whereas the original strains (phylogroups A and B1, respectively) were not found. The strains from the remaining travellers positive before travelling and negative upon return ($n=8$ strains) belonged to phylogroup A ($n=2$), phylogroup B2 ($n=3$) and phylogroup D ($n=3$).

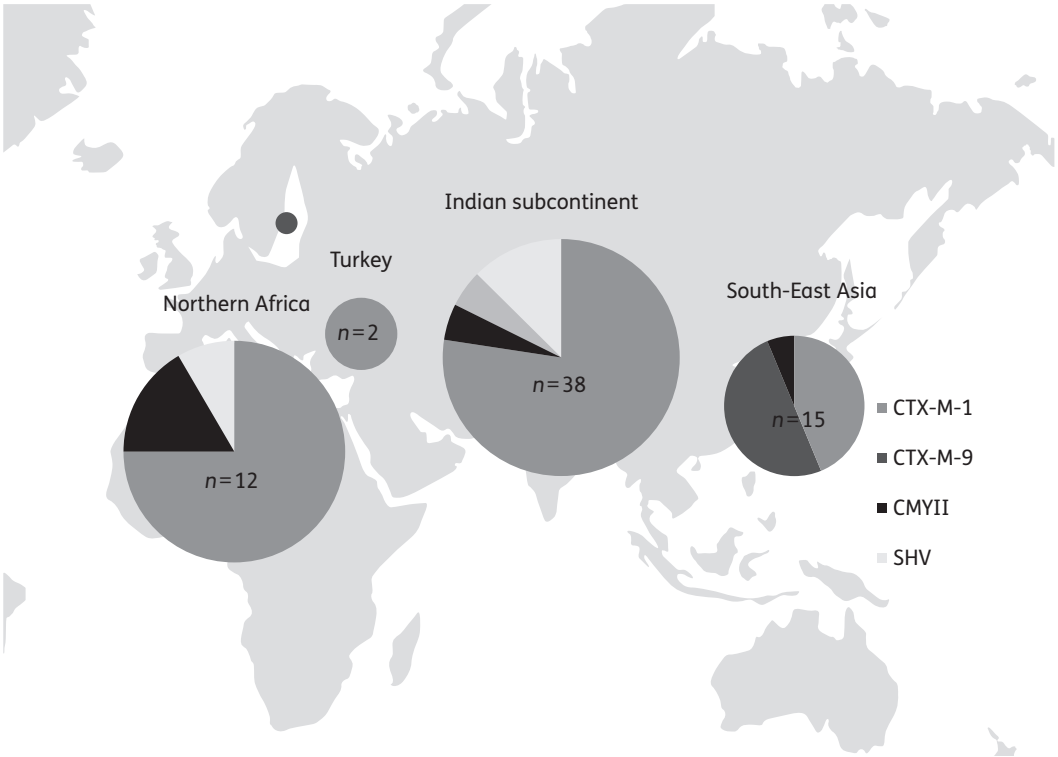

Figure 1. Geographical distribution of ESBL enzymes and total number of strains. Circle sizes correspond to acquisition rate.

Table 2. Antimicrobial resistance among EPE in the travellers' study versus EPE strains identified among *E. coli* in blood, Karolinska University Hospital, 2013–15

Antibiotic	Travellers' study ($n=67$), number intermediate and resistant (%)	Karolinska University Hospital ($n=327$), number intermediate and resistant (%) *E. coli*	*P*, travellers versus BSI
Cefotaxime	67 (100.0)	323 (98.8)	1.00
Ceftazidime	62 (92.5)	307 (93.9)	0.68
Piperacillin/tazobactam	15 (22.4)	134 (41.0)	**0.006**
Imipenem	0 (0.0)	2 (0.6)	
Meropenem	1 (1.5)	5 (1.5)	1.00
Ertapenem	4 (6.0)	10 (3.1)	0.27
Gentamicin	20 (29.9)	127 (38.8)	0.17
Amikacin	5 (7.5)	20 (6.5)[a]	0.79
Ciprofloxacin	27 (40.3)	237 (72.5)	**<0.001**
Trimethoprim/sulfamethoxazole	39 (58.2)	226 (69.1)[b]	0.08
MDR (intermediate or resistant to at least three classes of antibiotics)	33 (49.3)	265 (81.3)	**<0.001**
Carbapenemase-producing Enterobacteriaceae	0 (0.0)	1 (0.3)	1.00

P values<0.05 are shown in bold.
[a]Eighteen strains not analysed.
[b]One strain not analysed.

Resistance genes and antimicrobial resistance

Table 2 shows resistance rates among the EPE strains isolated from the travellers as well as ESBL-producing *E. coli* in blood cultures analysed at the Department of Clinical Microbiology, Karolinska University Hospital during 2013–15. Significantly more invasive isolates were non-susceptible to piperacillin/tazobactam and ciprofloxacin and were MDR (defined as resistance

to at least three classes of antibiotics).[25] All isolates from the travellers were resistant to cefotaxime and most (62/67, 92.5%) to ceftazidime. Of the other antimicrobial classes, trimethoprim/sulfamethoxazole showed the highest resistance rates (39/67, 58.2%), followed by ciprofloxacin (27/67, 40.3%), gentamicin (20/67, 29.9%), piperacillin/tazobactam (15/67, 22.4%), amikacin (5/67, 7.5%) and ertapenem (4/67, 6.0%). Multidrug resistance was detected in 33/67 (49.3%) strains.

Table 3. EPE colonization and risk factors; univariate analysis

Characteristic	All travellers (*N*=175), *n* (column %)	EPE positive (*N*=56), *n* (row %)	EPE negative (*N*=119), *n* (row %)	OR (95% CI)	*P*
Male	56 (32.0)	22 (39.3)	34 (60.7)	1.61 (0.83–3.15)	0.16[a]
Age (years), median (IQR)	49 (33–58)	49 (37–56)	49 (32–58)	NA	0.87
Age group (years)					
18–29	30 (17.1)	7 (23.3)	23 (76.7)	1.00	NA
30–49	59 (33.7)	22 (37.3)	37 (62.7)	1.95 (0.72–5.30)	0.19[b]
50–64	63 (36.0)	21 (33.3)	42 (66.7)	1.64 (0.61–4.44)	0.33[b]
≥65	23 (13.1)	6 (26.1)	17 (73.9)	1.16 (0.33–4.08)	0.82[b]
Geographical region					
South-East Asia	68 (38.9)	13 (19.1)	55 (80.9)	1.00	NA
Indian subcontinent	61 (34.9)	30 (49.2)	31 (50.8)	4.09 (1.87–8.98)	**<0.001**[c]
northern Africa	25 (14.3)	11 (44.0)	14 (56.0)	3.32 (1.23–8.99)	**0.018**[c]
Turkey	21 (12.0)	2 (9.5)	19 (90.5)	0.44 (0.09–2.16)	0.32[c]
Trip duration (days), median (IQR)	14 (8–20)	14.5 (11–21)	14 (8–18)	NA	0.32
Trip duration (days)					
≤7	33 (18.9)	7 (21.2)	26 (78.8)	1.0	NA
8–15	78 (44.6)	27 (34.6)	51 (65.4)	1.98 (0.76–5.12)	0.16[d]
16–30	39 (22.3)	13 (33.3)	26 (66.7)	1.86 (0.64–5.40)	0.30[d]
>30	25 (14.3)	9 (36.0)	16 (64.0)	2.09 (0.65–6.72)	0.21[d]
Oral cholera vaccine before trip[e]	102 (59.0)	38 (22.0)	64 (78.0)	1.89 (0.96–3.71)	0.064
Travellers' diarrhoea[f]	50 (28.7)	23 (46.0)	27 (54.0)	2.35 (1.19–4.66)	**0.013**
Use of antibiotics[f]	15 (8.6)	10 (66.7)	5 (33.3)	4.91 (1.59–15.16)	**0.003**
Attended healthcare facility during trip[f]	14 (8.0)	8 (57.1)	6 (42.9)	3.11 (1.02–9.45)	**0.037**
Hospitalized during trip[f]	4 (2.3)	3 (75.0)	1 (25.0)	6.62 (0.67–65.16)	0.10
Use of proton pump inhibitor ≤30 days pre-travel	15 (8.6)	2 (13.3)	13 (86.7)	0.30 (0.07–1.39)	0.15
Chronic disease[g]	36 (20.6)	6 (16.7)	30 (83.3)	0.36 (0.14–0.91)	**0.027**
Time to stool sample after return (days), median (IQR)	12 (7–23)	10 (6–21)	13 (8–26)	NA	0.089
Stool sample after return					
day 0–7	50 (28.6)	22 (44.0)	28 (56.0)	1.00	NA
day 7–14	55 (31.4)	15 (26.8)	40 (73.2)	0.48 (0.21–1.08)	0.073[h]
day 15–30	31 (17.7)	9 (29.0)	22 (71.0)	0.52 (0.20–1.35)	0.18[h]
>30 days	39 (22.3)	10 (25.6)	29 (74.4)	0.44 (0.18–1.09)	0.073[h]

NA, not applicable. *P* values<0.05 are shown in bold.
[a]In relation to female.
[b]In relation to group 18–29 years old.
[c]In relation to risk when travelling to South-East Asia.
[d]In relation to travel length 0–7 days.
[e]Two persons did not answer the question, so the calculation is based on 173 persons.
[f]One person did not answer the question, so the calculation is based on 174 persons.
[g]Diseases specified in Table S3.
[h]In relation to stool sample within 7 days after return.

Table 4. EPE colonization and risk factors; multivariable analysis based on 172 persons (due to missing data for 3 travellers)

Characteristic	*P*	OR (95% CI)
Male	0.063[a]	2.11 (0.96–4.65)
Age	0.80	1.00 (0.98–1.03)
Travel destination		
South-East Asia	1.00	NA
Indian subcontinent	**<0.001**[b]	5.62 (2.27–13.89)
northern Africa	**0.003**[b]	5.50 (1.78–16.94)
Turkey	0.81[b]	0.81 (0.15–4.32)
Travellers' diarrhoea	**0.040**	2.50 (1.04–6.03)
Antibiotics during trip	**0.024**	5.92 (1.27–27.20)
Chronic disease	**0.014**	0.27 (0.10–0.76)

NA, not applicable. *P* values<0.05 are shown in bold.
[a]In relation to female.
[b]In relation to South-East Asia.

Plasmid-mediated quinolone and fluoroquinolone resistance genes [*qnr* and/or *aac(6')-Ib*] were seen in 31/67 (46.3%) isolates, most commonly *qnrS* present in 20 strains, followed by *aac(6')-Ib* in 10 strains, *qnrB* in 4 strains and *qepA* in 1 strain. Ten out of 24 isolates containing *qnr* were non-susceptible to ciprofloxacin. Two isolates were resistant to both gentamicin and amikacin and both isolates were positive for the 16S methylase gene *rmtB*.

One CTX-M-55-producing isolate (1.5%) was positive for the plasmid-mediated colistin resistance gene *mcr-1*, the MIC of colistin was 4 mg/L. The traveller had visited Thailand in late 2013 and had none of the risk factors presented in Table 3. ResFinder analysis identified the *mcr-1* gene and additionally two aminoglycoside resistance genes [*aadA1A2* and *aac(3)-IId*], two β-lactamase genes ($bla_{CTX\text{-}M\text{-}55}$ and $bla_{TEM\text{-}1B}$) and one gene encoding plasmid-mediated fluoroquinolone resistance (*qnrS1*). Genes were also found conferring resistance to macrolides (*mefB*), chloramphenicol (*cmlA1*), sulphonamides (*sul3*), tetracycline (*tetA*) and trimethoprim (*dfrA12*). The isolate belonged to ST10 and contained five known plasmid replicon types: IncFIB, IncFIA(HI1), IncX4, IncX1 and IncFII.

Risk factors for colonization

Table 3 shows the results of the univariate analysis and factors included in the final multivariable model are presented in Table 4.

Colonization rates differed between geographical regions and were highest after visiting the Indian subcontinent (30/61, 49.2%), followed by northern Africa (11/25, 44.0%), South-East Asia (13/68, 19.1%) and Turkey (2/21, 9.5%). Travellers' diarrhoea was reported by 50 persons, of which 23 (46.0%) became EPE colonized. In multivariable analysis, geographical regions were independent risk factors for colonization where travelling to the Indian subcontinent and northern Africa contributed to similar high risk compared with South-East Asia [OR 5.62 (95% CI 2.27–13.89) and 5.50 (1.78–16.94), respectively]. Antibiotic treatment (distribution specified in Table S2) [OR 5.92 (1.27–27.20)] and travellers' diarrhoea [OR 2.50 (1.04–6.03)] were also independent risk factors. Chronic diseases (distribution specified in Table S3) were negatively associated with EPE colonization [OR 0.27 (0.10–0.76)]. There was no significant association with sex, age, length of stay, intake of proton pump inhibitors or use of oral cholera vaccine (Dukoral®). The median time to receiving a faecal sample after return was 14 days among EPE-negative participants versus 11 days among EPE-positive participants ($P=0.06$). During follow-up (10–26 months), no clinical infections caused by EPE were detected among colonized study participants.

Discussion

Among EPE isolated from the travellers in our study, phylogroup B2 was less common while phylogroup A dominated, implying that less-virulent strains are common colonizers in travellers. This is in contrast to a previous study from our research group where B2 was the most common phylogroup in faecal samples and was associated with prolonged carriage among patients with clinical infections caused by EPE.[13] Phylogroups B2 and D have in other studies been primarily found in more pathogenic *E. coli* strains causing extraintestinal infections (UTI and BSI), while commensal strains belong to phylogroups A and B1.[26,27] The low expression of virulence factors in the majority of isolates in our study differs from carriage isolates in patients with clinical infections caused by EPE where strains usually express one or several virulence factors essential for the bacterium to adhere to the infected surface.[13]

The risk among healthy asymptomatic EPE carriers to develop clinical infections due to colonizing strains is not yet well known. A retrospective study by Rottier *et al.*[28] found that prior colonization with third-generation cephalosporin-resistant Enterobacteriaceae had a positive predictive value for the risk of bacteraemia caused by EPE of only 7.4%. The EPE strains found among our travellers, the majority of them community acquired, differ from pathogens causing clinical infections both in terms of phylogroup and virulence characteristics and are therefore presumably less prone to cause clinical infections. Follow-up of EPE-positive travellers up to 26 months after colonization revealed no clinical infections caused by EPE. Despite the apparent risk of acquiring faecal colonization with EPE when travelling to high-prevalence areas, the risk of future infection caused by that EPE strain appears to be low and the duration of faecal carriage in most cases is short.[14] However, dissemination of resistant strains from healthy travellers to vulnerable individuals with comorbidities as well as endogenous bacterial conjugation to other more virulent and persistent strains must still be considered potential risks.

Coresistance rates among clinical isolates collected from blood were significantly higher than among the strains colonizing the travellers in our study. Studies of EPE causing UTI show similarly high coresistance rates as our BSI cases and also a high level of virulence factors.[29] Most MDR isolates in this study belonged to phylogroups B2 and D. Studies on *E. coli* resistance and connection to phylogroups show the opposite: higher resistance rates among phylogroups A and B1 than phylogroups B2 and D.[27,30] However, these studies include not only ESBL-producing isolates, but *E. coli* in general. The dominating resistance gene encoding an ESBL in clinical isolates detected in Sweden is $bla_{CTX\text{-}M\text{-}15}$, frequently carried by ST131 of phylogroup B2, known for epidemic dissemination and multidrug resistance, and a strain well adapted to intestinal survival.[31] The alarming prevalence of *mcr-1* in Asia as well as

other regions has recently attracted attention.[32–34] Among the EPE found in our study, one strain producing CTX-M-55 also produced *mcr-1*; this isolate is the first *mcr-1* producer detected in Sweden. It was found in a traveller returning from Thailand in late 2013.

As previously shown, geographical destination is of major importance for EPE colonization when travelling. Herein, the risk of EPE colonization was highest when travelling to the Indian subcontinent and northern Africa. Diarrhoea and antimicrobial treatment during the trip—factors disrupting the endogenous microbiota—were independent risk factors for EPE colonization, which is in concordance with other studies.[6–9,35] A history of chronic diseases appeared to protect from EPE colonization and could not be explained by the travellers' choice of destination. It may be speculated that lower risk-taking behaviour in terms of food intake and hand hygiene in the group reporting chronic diseases is an explanation. In contrast to other studies, high age was not a risk factor for colonization in this study.

In our study, 7 out of 13 travellers were EPE positive before travelling but EPE negative upon return and only 4 were colonized with identical strains. They could have eliminated their carriage during travel or the bacterial load was below laboratory detection limits. As shown previously, one negative faecal sample is not synonymous with elimination of EPE colonization.[13,36] In the two travellers with new EPE strains after travel, either bacterial conjugation or recolonization is possible. Ruppé *et al.*[14] showed that persistence of faecal EPE colonization after travelling was prolonged in travellers with a high RA of EPE. In our pre-travel EPE-positive group, no strains belonging to phylogroups A and B1 could be detected post-travel, with a median time of 51 days between the two samples, while four strains belonging to phylogroup B2 were still detected. One explanation could be that phylogroup B2 is connected to a higher RA when colonizing the intestine. As it is usually easier to investigate strain characteristics than to determine RA, the putative correlation between RA and strain features deserves further investigation.

Some limitations must be mentioned. Studies on the duration of intestinal EPE colonization after clinical infection show that nearly half of colonized patients remain colonized after 1 year and data support faster elimination among carriers without clinical symptoms.[13,14] The EPE-positive and -negative groups showed no significant differences in the time between return from their trip to the second sampling, but the possibility of elimination of faecal carriage during the time to second sample or not detecting EPE due to low sensitivity in one faecal sample cannot be excluded. The results could also have been affected by the quality of self-collected samples and transportation time. If anything, these limitations would contribute to an underestimation of EPE colonization. Another limitation is the fairly low sensitivity of open questions in questionnaires, where e.g. the definition of a chronic disease can vary between travellers. Our follow-up data for infections caused by EPE were limited to 56 persons and the reason for no EPE infections being identified could be low incidence.

In conclusion, EPE found in faecal screening samples of healthy individuals are predominantly of low pathogenicity as indicated by the low frequency of virulence factors and phylogroups associated with extraintestinal infections. The geographical area as well as diarrhoea and antimicrobial treatment during travelling are important risk factors for EPE acquisition, so advising travellers on avoiding unnecessary antibiotic treatment is of importance. The risk of colonization with carbapenemase-producing Enterobacteriaceae (CPE), as measured herein with a very sensitive screening method, is likely still low, even when travelling to high-prevalence areas.

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Transparency declarations

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Supplementary data

Tables S1 to S3 and Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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