# Health problems and risks encountered among healthy and vulnerable Dutch travelers

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# PART II

## Travel and antimicrobial resistance

# 6

# Extended-spectrum β-lactamase-producing *Enterobacteriaceae* among travelers from the Netherlands

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## Abstract

**Background** A prospective cohort study was performed among travelers from the Netherlands to investigate the acquisition of carbapenemase-producing *Enterobacteriaceae* (CP-E) and extended-spectrum ß-lactamase producing *Enterobacteriaceae* (ESBL-E) and associated risk factors.

**Methods** Questionnaires were administered and rectal swabs were collected and tested before and after return.

**Results** Of 370 travelers, 32 (8.6%) were colonized with ESBL-E before travel, 113 (31%) acquired an ESBL-E during travel and 26 were still colonized six months after return. No CPE were found. Independent risk factors for ESBL-E acquisition were travel to South and East Asia. Multilocus sequence typing showed extensive genetic diversity among *Escherichia coli*. Predominant ESBLs were CTX-M enzymes.

**Conclusion** The acquisition rate, 30.5%, of ESBL-E in travelers from the Netherlands to all destinations studied was high. Active surveillance for ESBL-E and CP-E and contact isolation precautions may be recommended at admission to medical facilities for patients who traveled to Asia during the previous six months.

## Introduction

The effect of international travel on the spread of multidrug resistant *Enterobacteriaceae* (MDR-E) became more evident during 2007-2010. Data obtained during that time from prospective studies among returning travelers from Australia, Canada, Sweden and the United States (New York, New York) revealed high rates of extended-spectrum ß-lactamase producing *Enterobacteriaceae* (ESBL-E) carriage, varying from 18 to 25% after foreign travel (1-4). Two of these studies also reported a pre-travel ESBL-E carriage rate of 7.8%.

The identification of carbapenemase-producing *Enterobacteriaceae* (CP-E) produced another set of challenges. Carbapenemases, such as *Klebsiella pneumoniae* carbapenemases (KPC), New Delhi metallo-β-lactamase (NDM), OXA-48, VIM and IMP, are plasmid-encoded enzymes, which have emerged worldwide. The rate of acquisition of CP-E during foreign travel is unknown; no surveillance system to date tracks these rates, and such as the situation recently reviewed by Van der Bij and Pitout (5). In the Netherlands, CP-E were found for the first time in 2010 (6).

No data were available on the pre-and post travel carriage rates among travelers from the Netherlands. Our objective was to investigate whether these travelers are at risk of MDR-E (ESBL-E and/or CP-E) by use of a prospective cohort study design. Because detailed microbiological data of the isolates and epidemiological data are crucial for assessing the real public health impacts of these organisms, we also investigated the persistence of intestinal colonization and possible spread to household contacts six months after the travelers returned.

## Materials and Methods

### Study design

A prospective cohort study was conducted at the travel clinic at the Leiden University Medical Center (LUMC) and at the Hollands Midden Municipal Health Services (MHS) in Leiden, the Netherlands. During March - September 2011, all adults who made an appointment for travel advice and had the intention to travel to areas outside Europe, North America, and Australia were invited to participate in the study.

Travelers < 18 years of age and those who traveled >3 months were excluded. Only one person in a couple or travel group was included.

Participants were asked to complete an electronic questionnaire and to deliver a rectal swab sample immediately before and immediately after travel. Questionnaires were used to collect demographic data, previous medical history, and travel information. Travelers who acquired MDR-E after foreign travel were asked to fill out a third questionnaire and deliver a third rectal swab 6 months after return.

If travelers were positive for MDR-E 6 months after return, their household contacts were also requested to submit a rectal swab and questionnaire. Household contacts were defined as persons who shared the same household with a participant on a regular basis. MDR-E–positive participants were asked to deliver a fourth rectal swab at the same time. The study was approved by the Leiden University Medical Center medical ethics committee.

### Bacterial isolates

Rectal swab samples were collected with Stuart Agar Gel Medium Transport Swabs (Copan Diagnostics, Corona, CA). The swabs were inoculated in trypticase soy broth supplemented with cefotaxime 0.25 mg/L and vancomycin 8 mg/L (MP products, Groningen, the Netherlands) and incubated for 24 hours at 37°C. After overnight incubation, the trypticase soy broth samples were subcultured on chromogenic ESBL screening agar (ESBL-ID; bioMérieux, Marcy-l'Étoile, France) and sheep blood agar as a growth control. All gram-negative rods growing on the ESBL-ID were identified by using MaldiTof-MS with BioTyper software version 3.0 (Bruker Daltonics, Breman, Germany), and antimicrobial drug susceptibility testing was performed by using the VITEK2 system (BioMérieux). All isolates underwent ESBL confirmatory disk testing by disk diffusion for ceftazidime and cefotaxime or cefepime (in cefoxitin resistant isolates), with and without clavulanic acid, as recommended by Clinical and Laboratory Standards Institute guidelines (www.clsi.org).

MICs for meropenem and ertapenem were determined using Etests (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. MICs were interpreted using EUCAST criteria (http://www.eucast.org/clinical_breakpoints/).

### Molecular characterization of β-lactamases

Molecular characterization of the β-lactamase genes in ESBL-E was performed by using Check-MDR CT103 microarray, version 1.1 (Checkpoints B.V., Wageningen, The Netherlands) to test microarrays. The principals of the microarray system and interpretation software have been described (7). Concisely, the system combines ligation-mediated amplification with the detection of amplified products on a microarray to detect the various carbapenemase genes: OXA-48, NDM-1, IMP, VIM and KPC; CTX-M groups: CTX-M group 1, 2, 9 or combined 8/25; and the most prevalent ESBL-associated single-nucleotide polymorphisms (SNPs) in TEM and SHV-variants. Furthermore, the six plasmid-mediated AmpC β-lactamases can be identified (www.lahey.org/studies).

### Molecular typing of *Escherichia coli* isolates

Multilocus sequence typing (MLST) was performed on all *E. coli* isolates by using seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA* and *recA*) to determine the corresponding sequence type (ST) and to designate sequence type complex (STC) using the MLST Databases at the Environmental Research Institute, University College Cork website (http://mlst.ucc.ie/mlst/dbs/Ecoli).

### Data analysis

A logistic regression model was used to determine risk factors for the acquisition of ESBL-E/CP-E after foreign travel in a total of 338 participants. Associations between acquiring an ESBL-E/CP-E after travel and different variables are calculated as odds ratios and p-values. Participants who were positive for ESBL-E/CP-E before travel were analyzed separately. Database processing and statistical analyses (univariate and multivariate analysis) were performed using the SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). MLST analysis was performed using BioNumerics software v.6.6 (Applied Maths, St-Martens-Lathem, Belgium).

## Results

### Study population and travel characteristics

In total, 521 participants were invited to participate in the study; 370 travelers completed two questionnaires and sent in two rectal swabs and were included in the analysis (Figure 1). The median age of the study population was 33 years (range 19-82), and 234 (63.2%) were women. The median length of stay abroad was 21 days (range 6-90 days). The most common reason for travel was vacation (n=277). Of the 370 participants, 113 (30.5%) whose pre-travel swab samples were negative, acquired MDR-E during foreign travel. Of these 113 participants, 19 (16.8%) still carried MDR-E six months after return. In 32 of the 370 travelers (8.6%), MDR-E was identified before travel. Twenty of these 32 participants (62.5%) returned with MDR-E, seven of whom were still colonized after six months (35%). No MDR-E was found before or after travel in 225 participants (60.8%).

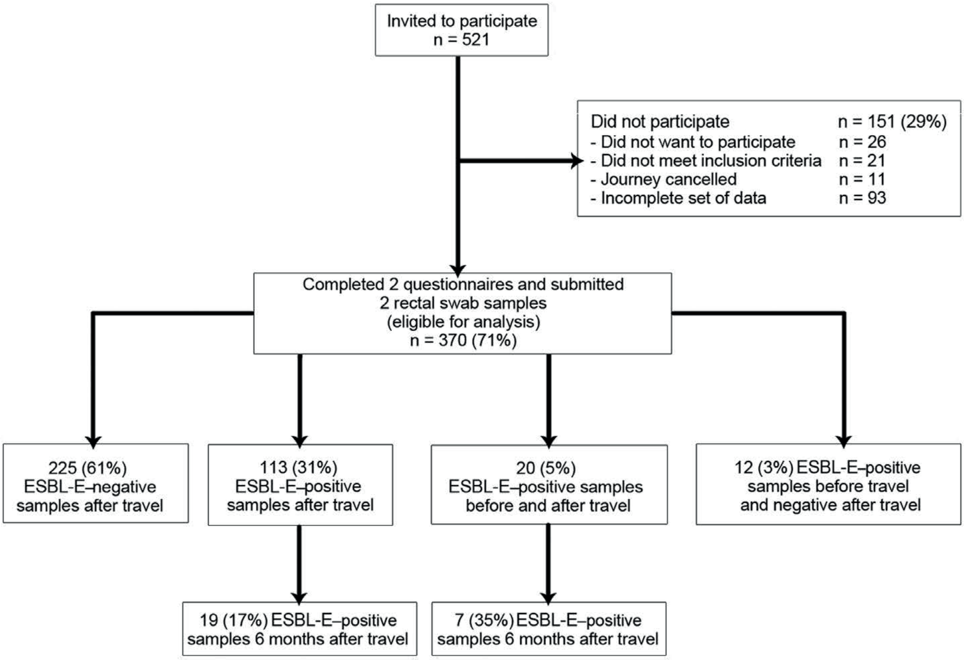

**Figure 1**. Flowchart of participants in the study.

### Travel-associated risk factors for ESBL-acquisition in returning travelers

For the analysis of travel-associated risk factors, data for 338 participating returning travelers with negative pre-travel rectal swab sample test results were used (Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/8/13-0257-Techapp1.pdf). In total, 65 countries were visited; these are subdivided in 10 subcontinents. The most common destinations were Indonesia (n=62), Thailand (n=30), Malaysia (n=27), Cambodia (n=21), People's Republic of China (n=39), Kenya (n=30), Tanzania (n=24), Surinam (n=20), and South Africa (n=19).

The highest ESBL-E acquisition rates were identified among participants who visited countries in Asia: 73% in South Asia and 67% in East Asia. Univariate and multivariate analysis showed that the travel destinations South and East Asia were significant risk factors for the acquisition of ESBL-E (p<0.001). Participants traveling to Asia (all subcontinents) were more likely to return with ESBL-E colonization after a self-arranged trip (odds ratio 1.7; p=0.07) or if they stayed in hostels/lodges (odds ratio 1.9; p= 0.08), although this finding was not statistically significant. There were no other risk factors for the acquisition of ESBL-E after foreign travel. The incidence proportions of ESBL-E after foreign travel are listed in Table 1.

**Table 1.** Incidence proportions and incidence rates for ESBL-E* colonization in 338 travelers from the Netherlands.

| Destination | No. of travelers | No. (%) of travelers with ESBL-E after return | Incidence proportion, % (SE) | Person days, all travelers | Mean duration of travel, all travelers | ESBL incidence rate per 100 pdt* (SE†) |
|---|---|---|---|---|---|---|
| Southeast Asia | 110 | 37 (34) | 34 (4.5) | 2,980 | 27 | 1.24 (0.20) |
| East Asia | 33 | 22 (67) | 67 (8.3) | 776 | 24 | 2.83 (0.60) |
| South Asia | 25 | 18 (72) | 72 (9.2) | 599 | 24 | 3.01 (0.70) |
| Central Asia | 3 | 1 (30) | 33 (33.3) | 94 | 31 | 1.06 (1.06) |
| North Africa | 10 | 4 (40) | 40 (16.3) | 112 | 11.2 | 3.57 (1.76) ‡ |
| Middle Africa | 56 | 17 (30) | 30 (6.2) | 1,637 | 29 | 1.04 (0.25) |
| Southern Africa | 25 | 3 (12) | 12 (6.6) | 631 | 25 | 0.48 (0.27) |
| Middle East | 15 | 2 (13) | 13 (9.1) | 222 | 14.8 | 0.90 (0.64) |
| Central America and the Caribbean | 28 | 7 (25) | 25 (8.3) | 544 | 19 | 1.29 (0.48) |
| South America | 32 | 2 (6) | 6 (4.4) | 922 | 29 | 0.22 (0.15) |
| Total | 338 | 113 (33) | 33 (2.6) | 8,536 | 25 | 1.32 (0.12) |

[*] ESBL-E: extended-spectrum β-lactamase *Enterobacteriaceae;* Pdt: person days of travel;

† SE standard error.

‡ The ESBL incidence rate per 100 person days of travel is represented by 4 ESBL-E carrying returning travelers from North Africa. Three of them had traveled for 7 days and one traveler had a 25-day stay abroad, which accounts for the high SE.

## Microbiological results and molecular characterization

A total of 133 participants were colonized with MDR-E after travel. This group consisted of 113 travelers who had initially negative pre-travel swab samples. In addition, twenty participants who had positive pre-travel samples also returned colonized with MDR-E. The ESBL-E of these 133 post-travel swab samples consisted of 146 *E. coli*, 10 *K. pneumoniae,* and two *Enterobacter cloacae* isolates.

No carbapenemase-producing MDR-E were found among the pre-and post travel isolates. Molecular characterization of the post-travel isolates demonstrated that CTX-M group 1 ESBL (n=110) predominated (CTX-M-1 like, n=4; CTX-M-3 like, n=1; CTX-M-15 like, n=85; CTX-M-32 like, n=20), followed by CTX-M group 9 ESBL (n=42), CTX-M group 2 (n=2) and CTX-M group8/25 (n=1). One *E. coli* isolate carried an SHV-ESBL (238S+240K). In addition, some isolates coproduced plasmid-mediated AmpC β-lactamase, ACT/MIR (n=1) or CMY-2 (n=2).

Thirty-four ESBL-E were isolated from pre-travel rectal swab samples from 32 participants: 29 samples (85.3%) of them were positive for *E. coli,* four *Klebsiella pneumoniae* (11.8%), and one *Citrobacter freundii* (2.9%). The CTX-M group 1 ESBL (n=22) comprised (CTX-M-1 like, n=4; CTX-M-15 like, n=16; CTX-M-32 like, n=2); the remaining ESBL isolates belonged to CTX-M group 9 ESBL (n=8) and CTX-M group 2 (n=1). Two *E. coli* isolates carried an SHV-ESBL (238S+240K).

Co-resistance to other classes if antimicrobial drugs was common in pre- and post-travel isolates; 67% displayed resistance to trimethoprim/sulphamethoxazole, 36% to ciprofloxacin, 37% to tobramycin, 35% to gentamicin, and 29% to nitrofurantoin. All isolates were susceptible to colistin and carbapenems.

## MLST of ESBL-producing *E. coli* isolates

MLST of 146 *E. coli* isolates from the post-travel samples identified 86 different sequence types (ST); 31 new STs were found. The most prevalent STs were: ST38 (12%; n=17), ST10 (7%; n=10), and ST131 (4%; n=9). The distribution of the CTX-M groups and types and STs is displayed in Figure 2. There was no association between ST and ESBL-type, nor were STs associated with specific travel destinations. Pre-travel isolates showed a similar diversity of STs, of which three were ST131.

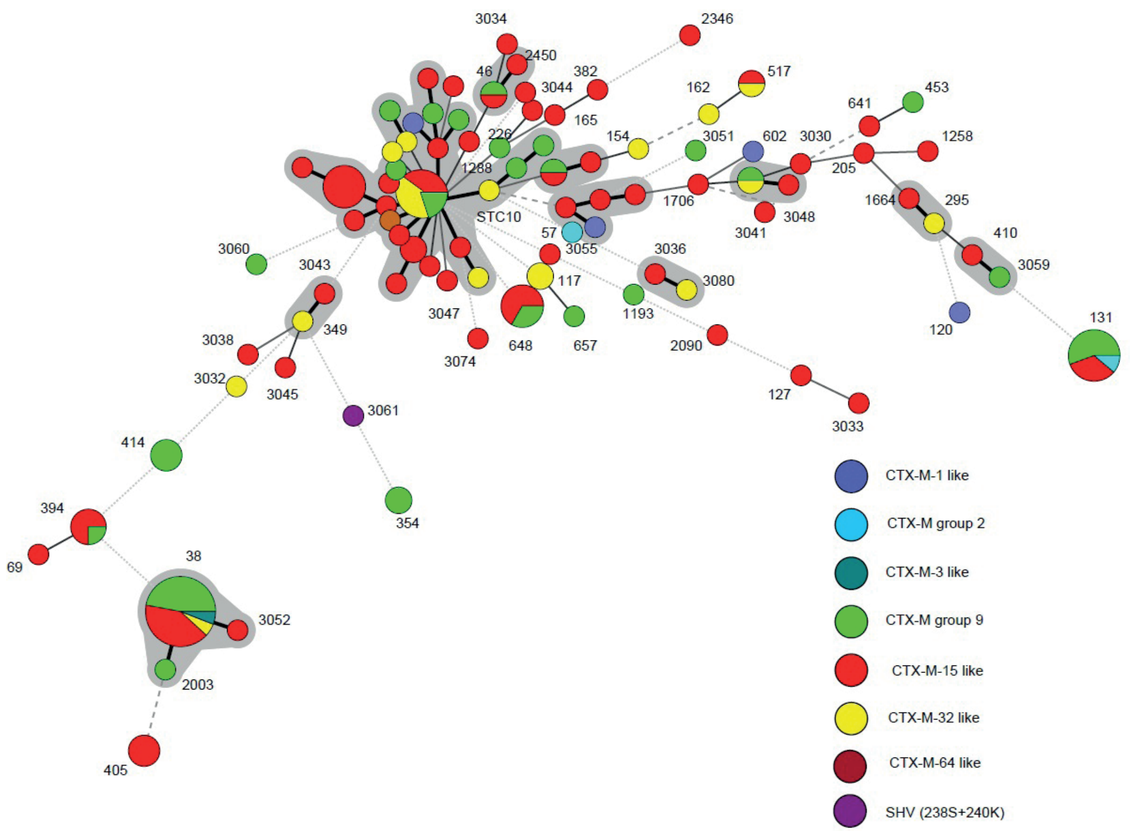

**Figure 2**. Multiocus sequence typing of *Escherichia coli* (n=146) from the post-travel isolates of 133 travelers from the Netherlands. The numbers indicate the most prevalent sequence types (STs). Grey shadow indicates that more than one ST belongs to the same complex. The following sequences belong to STC10: ST4,10, 34,43, 44,48, 167, 193, 215,218,227 and 617. Thick connecting lines indicate single locus variants; thin connecting lines indicate variants with two or three loci difference; dashed connecting lines indicate variants with four loci difference; dotted connecting lines indicate five to seven loci differences.

### Prolonged carriage and household contacts

Of the 133 participants whose samples were positive for ESBL-E after return, 127 (95.4%) completed the follow-up survey and provided samples after six months.

ESBL-E was isolated from 26 samples (20.4%) (Table 2). None of these participants reported the use of antimicrobial drugs or were hospitalized during the previous six months; none were health care workers, and none reported contact with farm animals. Diarrhea was reported by seven participants.

Of 113 participants who had initially negative pre-travel samples and positive samples immediately after return, 19 (16.8%) were still colonized after six months. Of these, seven participants had samples that were positive for *E. coli* with the same ST six months after return. Nine participants were positive for *E. coli* and had a different ST six months after return; three were positive for a different species six months after return. Eleven household contacts of four MDR-E-positive participants agreed to cooperate and submitted a rectal swab sample. ESBL-producing *E. coli* was isolated from two household contacts (18.1%), each from different households. The first household contact carried a different ESBL-producing *E. coli* than the associated traveler before and after the trip. Both isolates carried a CTX-M group 9 enzyme. The second household contact was positive for SHV-ESBL producing *E. coli* ST2599. The associated traveler's samples were positive for *E. coli* ST617 and ST38 immediately after the trip, *K. pneumoniae* six months after return, and the fourth rectal swab sample was positive for a CTX-M-15 like *E. coli* ST3363.

Of 20 participants who samples were positive before and after return, seven participants (35.0%) were still colonized six months after return. Of these seven participants, five (20%) carried a similar strain: two carried a CTX-M- group 9-producing *E. coli* with an identical ST as before the trip, two carried a similar ST but with a different CTX-M group enzyme as before the trip, and one participant carried a CTX-M group 1 producing *K. pneumoniae* during the study period; two participants returned with an *E. coli* with a different ST. No household contacts were included in this subgroup of travelers.

**Table 2**. Microbiological and molecular characteristics of rectal swab samples collected from travelers from the Netherlands immediately pre-and post-travel and six months after return.

| | | | | **Immediate post-travel samples** | | | | | | **Post-travel sample six mo. after return†** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Pre-travel samples** | | | **Isolate 1** | | | **Isolate 2** | | | | | |
| **ID** | **Species** | **CTX-M group** | **ST** | **Species** | **CTX-M group** | **ST** | **Species** | **CTX-M group** | **ST** | **Species** | **CTX-M group** | **ST** |
| 25 | Neg | NA | NA | *E. coli* | 9 | 131 | Neg | NA | Na | *E. coli* | 9 | 131 |
| 45 | Neg | NA | NA | *E. coli* | 1 | 405 | *E. coli* | 9 | 338 | *E. coli* | 1 | 405 |
| 56 | Neg | NA | NA | *E. coli* | 1 | 3036 | *E. coli* | 1 | 517 | *E. coli* | 1 | 3267 |
| 60 | Neg | NA | NA | *E. coli* | 1 | 648 | *K. p.* | 1 | Nd | *E. coli* | 1 | 648 |
| 61 | Neg | NA | NA | *E. coli* | 1 | 648 | *E. coli* | 9 | 227 | *E. coli* | 1 | 131 |
| 62 | Neg | NA | NA | *E. coli* | 9 | 3037 | Neg | NA | NA | *E. coli* | 9 | 501 |
| 80 | Neg | NA | NA | *E. coli* | 1 | 131 | Neg | NA | NA | *E. coli* | 9 | 1177 |
| 86 | Neg | NA | NA | *E. coli* | 1 | 93 | *E. coli* | 1 | 2090 | *E. cloacae* | 9 | ND |
| 137 | Neg | NA | NA | *E. coli* | 1 | 155 | *E. coli* | 1 | 617 | *E. coli* | 9 | 131 |
| 204 | Neg | NA | NA | *E. coli* | 1 | 38 | Neg | NA | NA | *K. p.* | 1 | ND |
| 211 | Neg | NA | NA | *E. coli* | 1 | 3044 | Neg | NA | NA | *K. p.* | 1 | ND |
| 222 | Neg | NA | NA | *E. coli* | 9 | 2003 | Neg | NA | NA | *E. coli* | 9 | 2003 |
| 238 | Neg | NA | NA | *E. coli* | 9 | 414 | Neg | NA | NA | *E. coli* | 9 | 10 |
| 251 | Neg | NA | NA | *E. coli* | 1 | 34 | Neg | NA | NA | *E. coli* | 1 | 450 |
| 309 | Neg | NA | NA | *E. coli* | 1 | 3045 | Neg | NA | NA | *E. coli* | 1 | 3045 |
| 373 | Neg | NA | NA | *E. coli* | 1 | 38 | Neg | NA | NA | *E. coli* | 1 | 3266 |
| 387 | Neg | NA | NA | *E. coli* | 1 | 131 | Neg | NA | NA | *E. coli* | 1 | 131 |
| 454 | Neg | NA | NA | *E. coli* | 9 | 10 | Neg | NA | NA | *E. coli* | 9 | 10 |
| 474 | Neg | NA | NA | *E. coli* | 1 | 154 | Neg | NA | NA | *E. coli* | 1 | 131 |

|  |  |  |  | Immediate post-travel samples |  |  |  |  |  | Post-travel sample six mo. after return† |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Pre-travel samples |  |  | Isolate 1 |  |  | Isolate 2 |  |  |  |  |  |
| ID | Species | CTX-M group | ST | Species | CTX-M group | ST | Species | CTX-M group | ST | Species | CTX-M group | ST |
| 12 | *E. coli* | 9 | 38 | *E. coli* | 1 | 3074 | Neg | NA | NA | *E. coli* | 1 | 38 |
| 105 | *E. coli* | 1 | 191 | *E. coli* | 1 | 120 | *E. coli* | 1 | 38 | *E. coli* | 1 | 120 |
| 255 | *E. coli* | 9 | 131 | *E. coli* | 1 | 617 | Neg | NA | NA | *E. coli* | 9 | 131 |
| 269 | *K. p.* | 1 | ND | *K. p.* | 1 | ND | Neg | NA | NA | *K. p.* | 1 | ND |
| 283 | *E. coli* | 9 | 131 | *E. coli* | 1 | 46 | Neg | NA | NA | *E. coli* | 9 | 131 |
| 505 | *E. coli* | 1 | 1163 | *E. coli* | 1 | 69 | Neg | NA | NA | *E. coli* | 9 | 3268 |
| 512 | *E. coli* | 9 | 657 | *E. coli* | 9 | 657 | Neg | NA | NA | *E. coli* | 1 | 657 |

*ID, participant identification number; CTX-M, extended-spectrum β-lactamase enzyme; ST, sequence type; Neg, no species were isolated from sample; *E. coli*, *Escherichia coli*; *K.P.*, *Klebsiella pneumoniae*; NA, not applicable; ND, no sequence type data available; Neg, negative pre-travel swab sample.

† None of the participants with a positive rectal swab sample after six months reported antimicrobial drug use during the six months after return.

## Discussion

The results of this study show a high ESBL-E carriage rate of 30.5% among healthy participating travelers from the Netherlands after return. This finding is worrisome, because this ESBL-E carriage rate is higher compared with those in recent studies that identified international travel as an independent risk factor for ESBL-E colonization (1-4). It is striking that none of the potential travel-associated risk factors investigated in the present study, other than traveling to South and East Asia, were found to contribute to this high ESBL-E carriage rate. Additional risk factors were not revealed by including in the univariate analysis the 13 participants who had a positive pre-travel sample and acquired an ESBL-producing *E. coli* during travel with a different ST than before the trip.

Tangden *et al*. associated gastroenteritis during travel with the risk for ESBL-E acquisition among travelers from Sweden (3). That association was not found in this study, which may reflect less fecal-oral contamination while traveling. Baaten *et. al*. reported that diseases transmitted by the fecal-oral route among travelers to non-industrialized countries have declined because of improved hygiene standards at the destination as measured by the human developmental index, sanitation index, and the water source index (8). The sanitation index (SI) levels, which represent the proportion of the population that has access to sanitation, were the lowest for Sub-Saharan Africa and the Indian subcontinent. On the basis of these indices, we would expect the incidence of ESBL-E acquisition to be similar among travelers in countries in Asia and Africa. Nonetheless, participating travelers to Asia had the highest post-travel colonization rates. Travelers to Asia most likely differ in their eating habits compared with travelers to African countries, since the former are more likely to eat in individual establishments outside of hotels or from street vendors. Thus, the high incidence rate found for returning travelers from Asia in this study may result from the increased risk of food-borne exposure.

No CP-E were found despite the fact that countries were visited where CP-E are prevalent in hospitals and in the environment (5). Other known risk areas besides India for the acquisition of CP-E, such as the United States, Greece, Italy, and the Balkan region were not included in this study, because these travelers do not visit the Travel Clinic of the LUMC. Many citizens from the Netherlands have relatives in

North African countries or Turkey whom they visit frequently. OXA-48 producing bacteria are endemic in these countries (9). These travelers do not consult travel clinics and may well return carrying OXA-48 producing isolates unnoticed.

Peirano *et. al.* (2) reported that the prevalence of ST131, an uropathogenic *E. coli* notorious for its worldwide expansion and spread of CTX-M-15, was similar among travelers and non-travelers from the Calgary region. The most prevalent ESBL among the travelers participating in this study was the CTX-M-15-like enzyme. However, this enzyme was found in a plethora of different sequences types of *E. coli*. Participants in the Leiden area not only showed a great heterogeneity of STs, but also harbored different CTX-M-types after travel and six months after return. The majority of the *E. coli* strains identified in the participants in this study were of STs that clustered around ST10 and belonged to sequence type complex 10 (STC10). STC10 strains essentially belong to the non-virulent, commensal phylogenetic group A (10). In a recent study based in French, isolates belonging to STC10 were found to be the most prevalent among fecal samples from healthy carriers of nalidixic acid resistant (but ESBL-negative) *E. coli* (11). It is also the most prevalent STC in the MLST database. Data from this study show transmissible genetic elements containing resistance genes are exchanged with naïve *E. coli* strains of the human intestinal microbiota during foreign travel combined with foodborne exposure.

Although 26 participants had positive results for ESBL-E six months after travel, they were not all positive for the same strain enterobacterial strain that was identified immediately after travel. In eight travelers colonized with *E. coli,* an ESBL of the same CTX-M group was identified in the immediate post-travel sample as after six months, but *E. coli* with a different ST was detected. In 11 travelers, the strain persisted during the study period. It is possible that more strain types were present in the rectal samples where colony morphology of different strains was not discriminative. However, it is also possible that the transfer of ESBL genes between strains within a host is a frequent occurrence. Or, the acquisition of a new ESBL-E occurs at the expense of the resident strain.

Inter-household transmission of ESBL-E has been demonstrated in the community setting (12,13). Clonally related strains could be found for 66% of the isolates from infected community patients and their corresponding household contacts (13). Because of the limited data on household contacts in the present study, the transmission dynamics of ESBL-E in households after foreign travel remains to be discovered.

The high pre-travel ESBL-E carriage rate among our study participants (8.6%) was an unexpected finding. Two recent studies on the ESBL-E carriage rate in the community have been conducted in the Amsterdam area. In the first study, 10.1% of the fecal samples from outpatients with gastrointestinal discomfort being assessed by their general practitioners yielded ESBL-E, predominantly CTX-M-15 producing *E. coli* (14). In a second study, investigating the prevalence of ESBL-E carriage in the general community, a carriage rate of 8.5% was found (15). Although no data on travel history were given, the investigators pointed out that foreign travel might be responsible for at least part of ESBL-E carriage rate among outpatients from the Netherlands. This finding is supported by data from our study: 50% of participants who had a positive pre-travel sample had traveled during the previous 12 months. This high percentage of carriers identified in this study before travel points toward ongoing importation of ESBL-E. Other potential reservoirs for ESBL-E are poultry and retail meats, which have been found to be contaminated with ESBL-producing *E. coli* strains harboring the genes on identical plasmids as found in human isolates (16,17).

International travel is growing and the number of intercontinental flights has increased during the past decade. The findings in this study support the role of international travel on the ESBL-E acquisition and carriage rate in travelers from the Netherlands, especially to South and East Asia. The high pre- and post travel carriage rates among persons traveling from the Netherlands indicate that the consequences of increased foreign travel are already manifest in this country. The lack of apparent travel-associated risk factors, the spread of CTX-M enzymes through a highly diverse population of *E. coli,* the association of ESBL-production with multidrug resistance and the possible role of other sources make containing the spread difficult. These factors also complicate the implementation of other strategies, such as pre-travel advice, and imply that all travelers to Asia should be considered for carriage of ESBL-E. Although CP-E were not found in this study, CP-E have been introduced into the Netherlands by returning travelers (6,18-20), and introduction by asymptomatic travelers to the Netherlands from countries where CP-E are endemic may largely go unnoticed. There is no reason to assume that, after CP-E are introduced, their spread will be less dynamic than that of ESBL-E. This interference has serious implications for the implementation of screening methods and effective infection control strategies. On the basis of the results of this study, we recommend active surveillance of CP-E and ESBL-E and at least temporary contact isolation precautions for patients being admitted to hospitals after travel to Asia during the previous six months.

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Dr Paltansing is a clinical microbiologist in the Department of Medical Microbiology at the Leiden University Medical Center, the Netherlands. Her research interests include the molecular characterization and epidemiology of MDR-E.

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**Technical Appendix Table**. Person and travel characteristics and risk factors for ESBL-E acquisition in a cohort of 338 travelers from the Netherlands*

| Variable | No. (%) negative pre- and post-travel N=225 | No. (%) positive post-travel only N=113 | Univariate analysis OR (95% CI) | p-value † | Multivariate analysis OR (95% CI) | p-value |
|---|---|---|---|---|---|---|
| **Gender, female** | 144 (64.0) | 69 (61.1) | 0.88 (0.55-1.41) | 0.60 | | |
| **Age** | | | | | | |
| 18-25 y | 54 (24.0) | 28 (24.8) | 1.0 | | | |
| 26-33 y | 66 (29.3) | 24 (21.2) | 0.39 (0.17-0.92) | 0.03 | | |
| 34-51 y | 56 (24.9) | 29 (24.8) | 064 (0.28-1.61) | 0.37 | | |
| ≥52y | 43 (21.8) | 33 (29.2) | 1.46 (0.60-3.54) | 0.41 | | |
| **Vegetarian** | 13 (5.8) | 6 (5.3) | 0.91 (0.34-2.47) | 0.86 | | |
| **Health care worker** | 59 (26.2) | 27 (23.9) | 0.88 (0.52-1.49) | 0.64 | | |
| **Daily contact with farm animals** | 8 (3.6) | 4 (3.5) | 1.0 (0.29-3.38) | 0.99 | | |
| **Visit to identified risk areas during previous 12 mo.** | | | 1.01 (0.64-1.61) | 0.96 | | |
| None | 138 (61.3) | 69 (61.1) | | | | |
| Africa | 26 (11.6) | 15 (13.3) | | | | |
| Asia | 21 (9.3) | 12 (10.6) | | | | |
| India | 5 (2.2) | 4 (3.5) | | | | |
| Middle East | 26 (11.6) | 13 (11.5) | | | | |
| Central America and Caribbean Region | 17 (7.6) | 7 (6.2) | | | | |
| South America | 6 (2.7) | 5 (4.4) | | | | |
| **Medical problem‡** | | | | | | |
| None | 161 (71.6) | 84 (74.3) | | | | |
| Inflammatory Bowel Disease | 2 (0.9) | 1 (0.9) | | | | |
| Chronic diarrhea | 3 (1.3) | 0 | | | | |

| Variable | No. (%) negative pre- and post-travel N=225 | No. (%) positive post-travel only N=113 | Univariate analysis OR (95% CI) | p-value † | Multivariate analysis OR (95% CI) | p-value |
|---|---|---|---|---|---|---|
| Chronic constipation | 3 (1.3) | 1 (0.9) | | | | |
| Irritable bowel syndrome | 17 (3.1) | 7 (6.2) | | | | |
| Diabetes mellitus | 3 (1.3) | 1 (0.9) | | | | |
| Gastro esophageal reflux | 12 (5.3) | 4 (3.5) | | | | |
| Recurrent urinary tract infections | 4 (1.8) | 1 (0.9) | | | | |
| Autoimmune disease | 7 (3.1) | 2 (1.8) | | | | |
| Abdominal pain with unknown origin | 5 (2.2) | 2 (1.8) | | | | |
| Gallbladder problems | 4 (1.7) | 1 (0.9) | | | | |
| Transplantation | 1 (0.4) | 0 | | | | |
| Coeliac disease | 0 | 2 (1.8) | | | | |
| Other | 30 (13.3) | 18 (15.9) | | | | |
| **Antibiotic use during 12 mo. before travel** | 47 (20.9) | 17 (15.1) | 0.85 (0.56-1.29) | 0.45 | | |
| **Hospitalization during 12 mo. before travel** | | | | | | |
| <3 mo prior | 5 (2.2) | 3 (2.7) | | | | |
| 3-6 mo prior | 2 (0.9) | 1 (0.9) | | | | |
| 6-9 mo prior | 1 (0.4) | 1 (0.9) | | | | |
| 9-12 mo prio | 2 (0.9) | 2 (1.8) | | | | |
| **Travel destination, by subcontinents§** | | | | | | |
| Southeast Asia | 73 (32.4) | 37 (32.7) | 1.01 (0.63-1.64) | 0.96 | | |
| East Asia | 11 (4.9) | 22 (19.5) | 4.70 (2.19-10.1) | <0.001 | 3.95 (1.78-8.73) | 0.001 |
| South Asia | 7 (3.1) | 18 (15.9) | 5.90 (2.39-14.60) | <0.001 | 5.09 (2-12.92) | 0.001 |
| Central Asia | 2 (0.9) | 1 (0.9) | 1.0 (0.089-1.11) | 0.99 | | |

| **Variable** | **No. (%) negative pre- and post-travel N=225** | **No. (%) positive post-travel only N=113** | **Univariate analysis OR (95% CI)** | **p-value †** | **Multivariate analysis OR (95% CI)** | **p-value** |
|---|---|---|---|---|---|---|
| Middle East | 13 (5.8) | 2 (1.8) | 0.29 (0.07-1.33) | 0.11 | 0.28 (0.06-1.30) | 0.103 |
| North Africa | 6 (2.7) | 4 (3.5) | | | | |
| Central Africa | 39 (17.3) | 17 (15) | | | | |
| Southern Africa | 23 (10.2) | 3 (2.7) | 0.24 (0.07-0.82) | 0.02 | 0.24 (0.07-0.85) | 0.027 |
| Central America and the Caribbean | 21 (9.3) | 7 (6.2) | 0.64 (0.26-1.56) | 0.33 | | |
| South America | 30 (13.3) | 2 (1.8) | 0.12 (0.027-0.50) | 0.004 | 0.14 (0.03-0.59) | 0.008 |
| **Median duration of stay in days** (range) | 21 (6-90) | 22 (6-89) | 0.99 (0.976-1.004) | 0.17 | 1.0 (0.97-1.0) | 0.22 |
| **Type of travel** | | | | | | |
| Self-arranged travel | 95 (42.2) | 52 (46) | 1.17 (0.74-1.84) | 0.51 | | |
| Backpacking | 51 (22.7) | 25 (22.1) | 0.97 (0.56-1.67) | 0.91 | | |
| Organized group travel | 62 (27.6) | 27 (23.9) | 0.83 (0.49-1.39) | 0.47 | | |
| Cruise | 1 (0.4) | 0 | | | | |
| Other | 16 (7.1) | 9 (8) | | | | |
| **Reason for travel** | | | | | | |
| Vacation | 166 (73.8) | 83 (73.5) | | | | |
| Visiting family/friends | 8 (3.6) | 8 (7.1) | | | | |
| Business | 15 (6.7) | 9 (8.0) | | | | |
| Study | 18 (8) | 7 (6.2) | | | | |
| Volunteer work | 10 (4.4) | 5 (4.4) | | | | |
| **Travel group composition** | | | | | | |
| Alone | 25 (11.1) | 14 (12.4) | 1.13 (0.56-2.27) | 0.73 | | |
| With one partner | 102 (45.3) | 44 (38.9) | 0.77 (0.49-1.22) | 0.26 | | |

| **Variable** | **No. (%) negative pre- and post-travel N=225** | **No. (%) positive post-travel only N=113** | **Univariate analysis OR (95% CI)** | **p-value †** | **Multivariate analysis OR (95% CI)** | **p-value** |
|---|---|---|---|---|---|---|
| More partners | 44 (19.6) | 30 (26.5) | | | | |
| Group travel | 54 (24) | 25 (22.1) | 1.23 (0.78-1.93) | 0.37 | | |
| **Accommodation during travel** | | | | | | |
| Luxury hotels | 78 (34.7) | 34 (30.1) | 0.81 (0.50-1.32) | 0.40 | | |
| Hostels | 50 (22.2) | 30 (26.5) | 1.27 (0.75-2.13) | 0.38 | | |
| Budget hotels | 49 (21.8) | 27 (23.9) | 1.13 (0.66-1.93) | 0.66 | | |
| Own holiday home | 16 (7.1) | 3 (2.7) | | | | |
| Camping | 10 (4.4) | 6 (5.3) | | | | |
| With family/friends | 8 (3.6) | 5 (4.4) | | | | |
| Locals | 7 (3.1) | 3 (2.7) | | | | |
| Boat | 4 (1.8) | 2 (1.8) | | | | |
| Other | 3 (1.3) | 3 (2.7) | | | | |
| **Diarrhea during travel** | 83 (36.9) | 45 (39.8) | 1.13 (0.71-1.80) | 0.60 | | |
| **Companion travelers with diarrhea** | 115 (51.1) | 61 (54.0) | 1.1 (0.71-1.77) | 0.62 | | |
| **Antibiotic use during travel** | 10 (4.4) | 9 (8.0) | 1.86 (0.73-4.72) | 0.19 | 1.98 (0.72-5.47) | 0.16 |

* Data are presented as no. (%), unless stated otherwise. Blank cells indicate no data available for value. Or, odds ratio; UTI, urinary tract infection.

†Variables with $p < 0.2$ in the univariate analysis were included in the multivariate logistic regression model.

‡Participants could report more than one medical problem

§Travel destinations visited by the travelers who completed the study were divided in 10 subcontinents (n= no. of travelers per destination. One participant could have visited more than one country):

Southeast Asia: Cambodia (n=21), Philippines (n=1), Indonesia (n=62), Laos (n=9), Malaysia, (n=27), Singapore (n=9), Thailand (n=30) and Vietnam (n=17)

East Asia: China (n=39), Japan (n=1), Mongolia (n=4) and Taiwan ( n=1)

South Asia: Bangladesh (n=1), India, (n=20) Maldives (n=2), Nepal (n=8) and Sri Lanka (n=5)

Central Asia: Kazakhstan,(n=2), Kyrgyzstan (n=2) Uzbekistan (n=2) and Turkmenistan (n=1)

Middle East: Iran (n=1), Jordan (n=1), Turkey (n=14) Emirates( n=3)

North Africa: Egypt (n=10) and Morocco (n=5)

Middle Africa: Benin (n=1), Cameroon (n=1), Congo (n=7), Gambia (n=2), Ghana (n=1), Kenya (n=30), Liberia (n=1) , Rwanda (n=1) , Sierra Leone (n=1), Tanzania (n=24) and Uganda (n=9)

Southern Africa: Angola (n=1), Botswana (n=5), Lesotho (n=2), Madagascar (n=3), Malawi (n=5), Mauritius (n=1), Mozambique (n=2), Namibia (n=7) South Africa (n=19), Swaziland (n=6) , Zambia (n=6)and Zimbabwe. (n=1)

Central America and the Caribbean: Belize (n=2), Bonaire (n=1), Costa Rica (n=9), Cuba (n=5), Curacao (n=1), Dominican Republic (n=4), Grenada (n=1) Guatemala (n=4), Honduras (n=2), Mexico (n=9), Nicaragua (n=5) and Panama (n=3)

South-America: Argentina (n=3), Bolivia (n=2), Brazil (n=5), Chile (n=2), Ecuador (n=3), Guyana (n=3, Peru (n=3), Surinam (n=20), Trinidad and Tobago (n=2) and Venezuela (n=1)