Import and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study

Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study

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Summary

Background International travel contributes to the dissemination of antimicrobial resistance. We investigated the acquisition of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) during international travel, with a focus on predictive factors for acquisition, duration of colonisation, and probability of onward transmission.

Methods Within the prospective, multicentre COMBAT study, 2001 Dutch travellers and 215 non-travelling household members were enrolled. Faecal samples and questionnaires on demographics, illnesses, and behaviour were collected before travel and immediately and 1, 3, 6, and 12 months after return. Samples were screened for the presence of ESBL-E. In post-travel samples, ESBL genes were sequenced and PCR with specific primers for plasmid-encoded β-lactamase enzymes TEM, SHV, and CTX-M group 1, 2, 8, 9, and 25 was used to confirm the presence of ESBL genes in follow-up samples. Multivariable regression analyses and mathematical modelling were used to identify predictors for acquisition and sustained carriage, and to determine household transmission rates. This study is registered with ClinicalTrials.gov, number NCT01676974.

Findings 633 (34·3%) of 1847 travellers who were ESBL negative before travel and had available samples after return had acquired ESBL-E during international travel (95% CI 32·1–36·5), with the highest number of acquisitions being among those who travelled to southern Asia in 136 of 181 (75·1%, 95% CI 68·4–80·9). Important predictors for acquisition of ESBL-E were antibiotic use during travel (adjusted odds ratio 2·69, 95% CI 1·79–4·05), traveller's diarrhoea that persisted after return (2·31, 1·42–3·76), and pre-existing chronic bowel disease (2·10, 1·13–3·90). The median duration of colonisation after travel was 30 days (95% CI 29–33). 65 (11·3%) of 577 remained colonised at 12 months. CTX-M enzyme group 9 ESBLs were associated with a significantly increased risk of sustained carriage (median duration 75 days, 95% CI 48–102, p=0·0001). Onward transmission was found in 13 (7·7%) of 168 household members. The probability of transmitting ESBL-E to another household member was 12% (95% CI 5–18).

Interpretation Acquisition and spread of ESBL-E during and after international travel was substantial and worrisome. Travellers to areas with a high risk of ESBL-E acquisition should be viewed as potential carriers of ESBL-E for up to 12 months after return.

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Introduction

Antimicrobial resistance constitutes an increasingly important human health hazard worldwide.[1] The use of antibiotics in human beings and food animals is a well established driving force behind increasing resistance.[2] Given the enormous growth of international tourism, from 25 million travellers in 1950 to 1·133 billion in 2014,[3] international travel might also contribute substantially to the rise in resistance because resistant bacteria or bacterial mobile genetic elements carrying resistance genes (eg, plasmids) may be rapidly transported between regions.[4] An important part of antimicrobial resistance genes is found on plasmids and codes for extended-spectrum β lactamase enzymes ([ESBLs] eg, TEM, SHV, and CTX-M) and carbapenemases that confer resistance to most β-lactam antibiotics.[2,4] Additionally, ESBL-producing Enterobacteriaceae (ESBL-E) and carbapenemase-producing Enterobacteriaceae (CPE) are typically resistant to multiple other antibiotic classes, which leaves few to no effective antimicrobial agents for prevention and treatment of infections.[4,5]

Previous studies have reported frequent acquisition of ESBL-E associated with various predictors and sporadic acquisition of CPE among international travellers.[6–10] However, data on ESBL-E colonisation after travel and assessment of associated predictors for sustained carriage and onward transmission within households are very limited. Such data are needed to establish the public health risk of the introduction and spread of antimicrobial resistance by travellers, and the potential needs and measures to monitor or manage these risks. Identifying individuals at risk of ESBL-E carriage enables appropriate measures to be taken to prevent introduction and spread of ESBL-E or CPE and for empirical adjustment of antibiotic treatment in individuals to optimise clinical care. We investigated the acquisition of ESBL-E during

Research in context

Evidence before this study

We searched PubMed on Aug 17, 2015, with the search terms "Gram negative bacteria", "Enterobacteriaceae", "Escherichia", "Klebsiella", "Salmonella", "Shigella", "Yersinia", "travel", "tourist", "tourism", "turista", "aviation", "air transport", "airport", "resistance", "colonisation", "antibiotic", "susceptibility", "carriage", and "carrier". We did a systematic review and identified 11 eligible studies. We updated this search on April 14, 2016, and found no new prospective studies. The results of the 11 prospective cohort studies showed high acquisition rates of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) among travellers who had returned from southern Asia and northern Africa. Four travellers who visited India acquired carbapenemase-producing Enterobacteriaceae (CPE). However, whether antibiotic use and traveller's diarrhoea are predictors for ESBL-E acquisition was unclear. Moreover, these studies did not sufficiently address duration of ESBL-E carriage among travellers or onward transmission within households. One study asked travellers to provide stool samples up to 12 months after return, but duration of carriage was defined by ESBL phenotype. One other study looked at household transmission, but because only 11 household contacts were included, no reliable conclusion could be inferred about the risk of household transmission.

Added value of this study

In this large-scale, longitudinal cohort study, we followed up travellers and their non-travelling household members for up to 12 months after travel. The large sample size meant that we could investigate ESBL-E acquisition among travellers who had returned from a large number of countries across the world, including those such as Uganda, for which community carriage rates of ESBL-E were previously unknown. We identified several predictors (some new) for ESBL-E acquisition, including factors specific to subregions. Moreover, we were able to ascertain duration of ESBL-E carriage and associated resistance genes, identify predictors for sustained colonisation, and to model transmission rates mathematically within households.

Implications of all the available evidence

High frequencies of ESBL-E acquisition during travel, subsequent sustained carriage, and evidence of onward transmission within households show that travellers contribute to the emergence and spread of ESBL-E on a global scale. Active screening for ESBL-E and CPE and adjustment of empirical antimicrobial therapy should be considered for returning travellers at increased risk of ESBL-E carriage. However, implications for infection prevention and antibiotic treatment policies will differ locally because the degree of consequence of acquisition and spread of ESBL-E by travellers is highly dependent on local ESBL-E prevalence in the country of origin.

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international travel, the associated predictive factors for acquisition, duration of colonisation, and onward transmission to household members.

Methods

Study design and participants

The study design and methods have been described in detail elsewhere.[11] Briefly, we did a multicentre, longitudinal, prospective cohort study involving travellers who were followed up from 1–3 weeks before travel departure until 12 months after return. To study household transmission, we also assessed non-travelling household members in the same period.

Eligible participants were adults (age ≥18 years) planning to travel for at least 1 week and up to 3 months. They were recruited at three outpatient travel clinics across the Netherlands from November, 2012, to November, 2013. The study was approved by the Medical Research Ethics Committee, Maastricht University Medical Centre (METC 12-4-093). All participants provided written informed consent.

Procedures

Participants were provided with faeces collection kits and instructed to self-collect faecal swabs (appendix) before and immediately and 1 month after travel. If any of these samples contained ESBL-E, the traveller and his or her household members were asked to provide further samples at 3, 6, and 12 months after travel. If no samples were positive for ESBL-E, no additional samples were collected. Questionnaires were also collected at all timepoints to obtain information on potential risk factors for ESBL-E acquisition, including demographics, illnesses, and behaviour before, during, and after travel.

Samples were processed immediately after receipt. They were inoculated in tryptic soy broth supplemented with vancomycin (50 mg/L) to select for Enterobacteriaceae. The broth was then subcultured on chromID ESBL (bioMérieux, Marcy l'Etoile, France). All morphologically distinct colonies were characterised to the species level with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Bruker Microflex LT, Bruker, London, UK). Antibiotic minimum inhibitory concentrations were measured with the automated susceptibility testing system Vitek 2 (bioMérieux) for all Enterobacteriaceae. ESBL production was phenotypically confirmed by the combination disc diffusion test, according to current national Dutch guidelines.[12]

All phenotypically confirmed ESBL-E isolates acquired during travel were screened for the presence of ESBL

See Online for appendix

genes with microarray, as described previously (appendix). The presence of ESBL genes was confirmed by PCR with primers specific for CTX-M enzyme groups 1, 2, 8, 9, and 25 and in-house primer sets. Further characterisation by sequencing was done for the most prevalent and largest CTX-M groups, 1 and 9. PCR confirmation and sequencing of genes for TEM and SHV ESBLs were limited to isolates that had negative microarray results for all CTX-M genes. A generic CTX-M PCR was done if no ESBL genes were detected by microarray, and, if positive, was followed by specific PCR and sequence confirmation for the different CTX-M groups (appendix). Sequences were compared with those in the NCBI GenBank and Lahey databases.

Acquisition was defined as the absence of ESBL-E in faecal samples before travel and the presence of ESBL-E in those obtained immediately after travel, as identified by phenotypic tests. Duration of carriage was defined by the last positive sample harbouring an ESBL of the same group (TEM, SHV, or CTX-M group 1, 2, 8, 9, or 25, or a combination) as detected immediately after travel. Participants with consecutive samples positive for ESBL-E were classified as being persistent carriers and those with ESBL-E-positive samples interspersed with at least one negative sample were classified as being intermittent carriers.

Statistical analysis

Incidence proportions and incidence per 100 person-days of travel and accompanying 95% CIs for ESBL-E acquisition were calculated for each subregion (appendix) and country of destination. Incidence per 100 person-days of travel was calculated with a maximum likelihood method that was based on a constant acquisition rate with right-censored and interval-censored data.

Predictors for ESBL-E acquisition were determined by logistic regression models that were based on the method proposed by Bursac and colleagues[13] (appendix) and analysed with IBM SPSS Statistics (version 21.0). Results are presented as odds ratios (ORs) and 95% CIs. We did separate analyses for the subregions of southeast Asia, southern Asia, and eastern Africa, as several dietary variables (eg, consumption of chicken, barbecue meat, or pork) interacted with specific travel destination subregions.

Time to decolonisation was assessed with Kaplan-Meier survival analyses with right censoring for participants whose last provided sample was ESBL-E positive. Univariable and multivariable Cox's regression analyses were done to identify predictors associated with decolonisation (appendix). Results are presented as hazard ratios (HRs) and 95% CIs (HRs <1·00 indicate decreased risk of decolonisation and, therefore, increased duration of carriage).

A Markov model was used to calculate the probability of transmission within households. For computational reasons, this model was based on ESBL-E as defined by phenotypic confirmation, and only data from households consisting of at most five people were included, but these accounted for 98% of households. The model took into account false-negative results, missing culture results, and unobserved colonisation times. The method of calculation was as follows. ESBL-E-positive people

	Travellers (n=2001)*	Non-travelling household members (n=215)†
Sex		
Male	920 (46·0%)	80 (37·2%)
Female	1081 (54·0%)	135 (62·8%)
Age (years)	50·5 (32·8–60·7)	46·9 (25·7-55·8)
Education level		
No education, elementary school, or prevocational secondary education	243 (12·4%)	78 (36·4%)
Vocational secondary education	280 (14·2%)	37 (17·3%)
Senior general secondary education or education up to university	200 (10·2%)	45 (21·0%)
Higher professional education	642 (32·7%)	53 (24·7%)
Academic (university) education	595 (30·3%)	38 (17·8%)
Antibiotic use in previous 3 months		
No	1760 (90·1%)	189 (88·3%)
Yes	194 (9·9%)	25 (11·7%)
Travel in past year		
None	185 (9·5%)	27 (12·6%)
In Europe	915 (46·9%)	124 (57·7%)
Outside Europe	852 (43·6%)	64 (29·8%)
Chronic disease‡		
No	1500 (77·2%)	173 (82·0%)
Yes	443 (22·8%)	38 (18·0%)
Chronic bowel disease‡		
No	1912 (97·4%)	212 (99·1%)
Yes	51 (2·6%)	2 (0·9%)
Continent visited during travel§		
Asia	1016 (50·8%)	NA
Africa	633 (31·6%)	NA
America	326 (16·3%)	NA
Europe	21 (1·0%)	NA
Oceania	5 (0·2%)	NA
Duration of index travel (days)	20 (15·0–25·0)	NA
Purpose of index travel		
Holiday	1655 (84·2%)	NA
Work or internship	161 (8·2%)	NA
Visiting family or relatives	82 (4·2%)	NA
Other reason	66 (3·4%)	NA

Data are number (%) or median (IQR). NA=not applicable. *Some numbers do not add up to 2001 because of missing data. †Some numbers do not add up to 215 because of missing data. ‡Self-reported by traveller or household member. §If travellers visited multiple continents, only the main continent visited is presented in this table.

Table 1: **Baseline characteristics of travellers and non-travelling household members**

	Number of travellers (n=1847)*	Number of travellers who acquired ESBL-E (n=633)†	ESBL-E incidence proportion (95% CI)‡	Number of travel-days	Mean (SD) duration of travel (days)	ESBL-E incidence per 100 person-days of travel (95% CI)§
Southern Asia	181 (9·8%)	136 (21·5%)	75·1 (68·4–80·9)	3727	20·6 (11·0)	7·2 (5·9–8·6)
Central and eastern Asia	84 (4·5%)	41 (6·5%)	48·8 (38·4–59·3)	1712	20·4 (10·8)	3·5 (2·5–4·7)
Western Asia	28 (1·5%)	12 (1·9%)	42·9 (26·5–60·9)	305	10·9 (7·5)	5·8 (3·0–9·9)
Northern Africa	81 (4·4%)	34 (5·4%)	42·0 (31·8–52·9)	981	12·1 (5·7)	4·5 (3·1–6·2)
Southeastern Asia	540 (29·2%)	200 (31·6%)	37·0 (33·1–41·2)	12 493	23·1 (11·6)	2·1 (1·8–2·4)
Caribbean and Central America	86 (4·7%)	24 (3·8%)	27·9 (19·5–38·2)	1653	19·2 (12·4)	1·7 (1·1–2·5)
Middle and eastern Africa	205 (11·1%)	57 (9·0%)	27·8 (22·1–34·3)	4060	19·8 (14·3)	1·6 (1·2–2·1)
Western Africa	106 (5·7%)	20 (3·2%)	18·9 (12·6–27·4)	1638	15·5 (11·1)	1·4 (0·8–2·0)
South America	180 (9·7%)	33 (5·2%)	18·3 (13·4–24·6)	4778	26·5 (14·7)	0·8 (0·5–1·1)
Southern Africa	116 (6.3%)	7 (1·1%)	6·0 (2·5–12·0)	2522	21·7 (8·6)	0·3 (0·1–0·6)
Northern America, Europe, and Oceania	17 (1·0%)	1 (<1·0%)	5·9 (1·1–27·0)	292	17·2 (11·3)	0·4 (0–1·6)

ESBL-E=extended-spectrum β-lactamase-producing Enterobacteriaceae. *Numbers do not add up to 1847 because 221 travellers visited more than one subregion (66 with ESBL-E acquisition) and destination information was missing for two. †Numbers do not add up to 633 because 66 travellers visited multiple subregions and destination information was missing for two. ‡Based on binomial distribution (Wilson's score interval). §Calculated with the maximum likelihood estimation method based on a constant acquisition rate with right-censored and interval-censored data.

***Table 2*: Incidence proportion and incidence per 100 person-days of travel for ESBL-E acquisition in Dutch travellers, by subregion**

(travellers or non-travelling household members) transmit ESBL-E to household members with rate β. Transmission from other sources was incorporated by the background transmission parameter α. De-colonisation of ESBL-E occurred with rate γ. Negative cultures could be false negative and affect the estimate of the sensitivity (ϕ). The specificity of culture was assumed to be 100%. Thus, the probability of transmission from an ESBL-E-positive to an ESBL-E-negative person, given that the ESBL-E-negative household member did not acquire ESBL-E via another route, could be calculated as β/(β+γ). Model parameters were simultaneously estimated with a maximum likelihood method in Mathematica version 9.0. This study is registered with ClinicalTrials.gov, number NCT01676974.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

2737 travellers were screened for eligibility, of whom 2001 were included in the study (appendix), with median age 50·5 years (IQR 32·8–60·7) and good health before travelling in most (table 1). 49 travellers were lost to follow-up.

The main purpose for travel was tourism (1655 [84·2%] of 1965 travellers) and the median travel duration was 20 days (IQR 15·0–25·0; table 1). The subregions most frequently visited were southeast Asia (n=650), eastern Africa (n=287), South America (n=228), and southern Asia (n=217). 122 (6·1%) of 2001 travellers were carrying ESBL-E before travel, leaving 1879 at risk of ESBL-E acquisition. 1847 (98·3%) of these submitted faecal samples after travel, among whom 633 had acquired at least one ESBL-E during travel (table 2), giving an acquisition rate of 34·3% (95% CI 32·1–36·5). From these 633 travellers, 859 morphologically different ESBL-E strains were isolated (759 *Escherichia coli*, 67 *Klebsiella pneumoniae*, and 33 other species). *CTX-M-15* was the most frequently acquired ESBL gene, being found in 338 (53·4%) of 633 travellers (appendix).

ESBL-E were most frequently acquired in southern Asia (75·1%, 95% CI 68·4–80·9), followed by central and eastern Asia (48·8%, 38·4–59·3; table 2, figure 1), but the frequency of acquisition varied widely between countries. Among the 22 most frequently visited countries, acquisition was highest in India (88·6%, 95% CI 79·8–93·9) and lowest in Suriname (3·6%, 1·0–12·1; appendix). Acquisition was also common after travel to eastern African countries, such as Uganda (44·4%, 27·6–62·7, appendix).

In the multivariable logistic regression, antibiotic use during travel was the strongest independent predictor for ESBL-E acquisition (table 3). To assess the effects of different antibiotic classes in the model, we exchanged the variable antibiotic use during travel (no *vs* yes) for a variable indicating antibiotic class (no antibiotics *vs* β-lactam, or quinolone, or other). Quinolone use was most strongly associated with ESBL acquisition (adjusted OR 6·0, 95% CI 2·9–12·4), whereas associations were non-significant for use of β-lactam (2·2, 0·95–5·14) or other antibiotics (1·7, 0·59–2·35). We also detected strong associations between ESBL-E acquisition and diarrhoea during travel and, particularly, traveller's diarrhoea that persisted on return (table 3). Travellers who had occasionally consumed food from street vendors

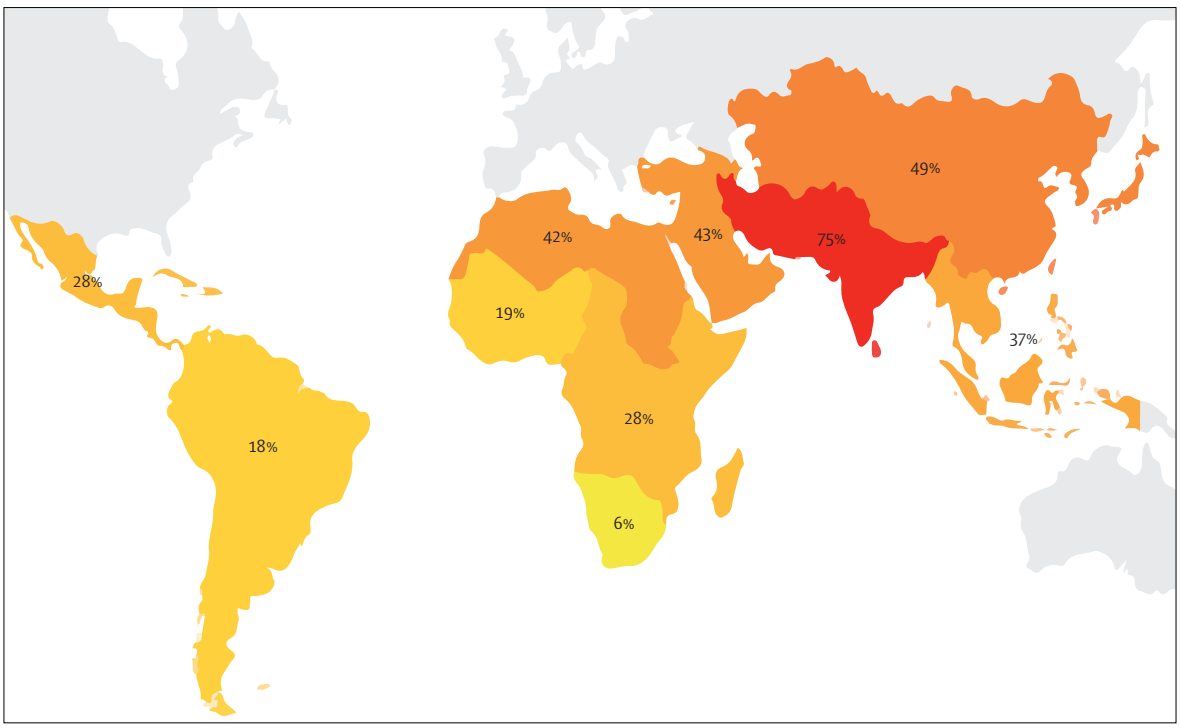

Figure 1: **Percentages of travellers that acquired β-lactamase-producing Enterobacteriaceae per subregion, according to the United Nations geoscheme**

were at increased risk of acquiring ESBL-E compared with those who had avoided street food vendors, and the risk increased further in travellers who consumed food from street vendors daily (table 3). Self-reported pre-existing chronic bowel disease was another notable risk factor for ESBL-E acquisition (table 3).

In the separate analyses for three of the visited subregions, the consumption of raw vegetables and antibiotic use were predictors of ESBL-E acquisition in southeastern Asia. In southern Asia, the strongest predictors were contact with orphan children and daily food consumption at a hostel or guesthouse. In eastern Africa, the strongest associations were daily visits to the local markets and staying in rural areas (appendix).

Sustained ESBL-E carriage (persistent and intermittent) after acquisition was seen in 42·9%, 25·1%, 14·3%, and 11·3% of travellers at 1, 3, 6, and 12 months after return, respectively. Most of these participants were continuously colonised (appendix). The median duration of post-travel colonisation was 30·0 days (95% CI 28·9–33·1, figure 2). ESBL-producing *K pneumoniae* and travel to western Asia were associated with the shortest times to decolonisation. Travellers who acquired a CTX-M group 9 ESBL had a significantly increased risk of sustained carriage compared with travellers who acquired a CTX-M group 1 ESBL (appendix).

Of 215 non-travelling household members included in the study, 63 were ESBL-E negative at baseline and shared households with people who acquired ESBL-E while travelling. Additionally, 105 co-travellers who were ESBL-E negative immediately after return shared households with travellers who acquired ESBL-E. Thus, 168 household members (in 152 households) were at risk of ESBL-E transmission. Evidence of onward transmission within households was found in 13 (7·7%) of these 168 household members (ten co-travellers and three non-travelling household members, appendix), who had one or more follow-up isolates with the same ESBL group (TEM, SHV, CTX-M group 1, 2, 8, 9, or 25) as had been acquired by the index traveller.

We subsequently used a Markov model to estimate the transmission rate of ESBL-E after introduction into a household. We included 3330 people from 1542 households in the estimation of probability of transmission of ESBL-E after introduction. 381 households consisted of one person, 774 of two people, 187 of three, 160 of four, and 40 of five. Person-to-person transmission was estimated to occur at a rate of 0·0013 (95% CI 0·0005–0·0024) per colonised person per day, with background transmission occurring at a rate of 0·00073 (0·00054–0·0009) per day. The decolonisation rate was 0·010 (0·0092–0·011) per day. The sensitivity of the screening process was 90% (86–93). Thus, the probability of transmission from an ESBL-E-positive to an ESBL-E-negative person in the household was 12% (5–18).

Discussion

Results from this large cohort study of travellers indicated that the risk of ESBL-E acquisition during travel is high, especially during travel to Asia and northern Africa. 11·3%

	Number of travellers at risk (n=1847)*	Number of travellers who acquired ESBL-E (n=633)†	Odds ratio (95% CI)‡	p value	Adjusted odds ratio (95% CI)§	p value
Pre-existing bowel disease						
No	1793 (97·3%)	606 (33·8%)	1·00	..	1·00	..
Yes	50 (2·7%)	24 (48·0%)	2·34 (1·26–4·34)	0·007	2·10 (1·13–3·90)	0·019
Beach holiday						
No	1404 (76·1%)	504 (35·9%)	1·00	..	1·00	..
Yes	441 (23·9%)	127 (28·8%)	0·72 (0·55–0·93)	0·010	0·73 (0·56–0·95)	0·021
Traveller's diarrhoea¶						
No	1085 (60·1%)	329 (30·3%)	1·00	..	1·00	..
During travel	593 (32·8%)	235 (39·6%)	1·56 (1·24–1·96)	<0·001	1·42 (1·12–1·80)	0·003
Immediately after travel	41 (2·3%)	14 (34·1%)	1·19 (0·58–2·44)	0·640	1·3 (0·63–2·68)	0·477
During travel and immediately after travel	87 (4·8%)	44 (50·6%)	2·42 (1·50–3·91)	<0·001	2·31 (1·42–3·76)	0·001
Antibiotic use during travel‖						
No	1697 (92·8%)	553(32·6%)	1·00	..	1·00	..
Yes	132 (7·2%)	73 (55·3%)	2·65 (1·80–3·91)	<0·001	2·69 (1·79–4·05)	<0·001
Attendance of large (religious) gathering						
No	1744 (94·6%)	595 (34·1%)	1·00	..	1·00	..
Yes	100 (5·4%)	36 (36·0%)	0·56 (0·34–0·92)	0·020	0·57 (0·34–0·94)	0·028
Daily hand hygiene before meals						
None	782 (42·4%)	265 (33·9%)	1·00	..	1·00	..
Clean with alcohol	161 (8·7%)	69 (42·9%)	1·03 (0·71–1·51)	0·870	0·97 (0·66–1·44)	0·885
Clean with soap	666 (36·1%)	200 (30·0%)	0·82 (0·64–1·04)	0·100	0·77 (0·60–0·99)	0·044
Clean with alcohol and soap	235 (12·7%)	97 (41·3%)	1·03 (0·74–1·44)	0·860	1·12 (0·79–1·59)	0·518
Meal at street food stalls during travel						
Never	1248 (67·7%)	386 (30·9%)	1·00	..	1·00	..
Occasionally	513 (27·8%)	205 (40·0%)	1·37 (1·08–1·73)	0·010	1·33 (1·04–1·71)	0·022
Daily	83 (4·5%)	40 (48·2%)	2·09 (1·30–3·38)	0·003	1·78 (1·07–2·95)	0·025

ESBL-E=extended-spectrum β-lactamase-producing Enterobacteriaceae. *Numbers do not add up to 1847 because of missing values. Valid percentages are reported after removal of missing values, which were assumed to be random. †Numbers do not add up to 633 because of missing values. The denominators for percentages are the numbers of travellers at risk given in the previous column. ‡Only adjusted for travel destination subregion, defined according to the United Nations geoscheme: Caribbean and Central America, middle and eastern Africa, central and eastern Asia, North America, Europe, and Oceania, southern Asia, southeastern Asia, western Asia, northern Africa, southern Africa, western Africa, and South America. §Adjusted for travel destination and travel variables shown in table. ¶Defined as ≥3 unformed stools within 24 h, with or without accompanying symptoms. ‖Most frequently used to treat gastroenteritis (41 [31·1%] of 132 travellers), of whom 17 (41·5%) took them without consulting a doctor.

***Table* 3: Predictors for ESBL-E acquisition among travellers in the final adjusted logistic regression model**

of travellers who acquired EBSL-E remained colonised at 12 months after return, and the estimated probability of onward transmission within households was 12%. Other important predictors for ESBL-E acquisition during travel were antibiotic use, traveller's diarrhoea that persisted after return, and pre-existing chronic bowel disease.

The frequency with which ESBL-E was imported by travellers is worrisome. 75·1% of travellers to southern Asia and 40–50% of those to central or eastern Asia, western Asia, and northern Africa acquired ESBL-E while travelling. Additionally, in central and eastern Africa, frequency of ESBL-E acquisition was substantial in some countries, particularly Uganda (44·4%). So far, data on acquisition among travellers to countries in central and eastern Africa have been very limited. Additionally, we have previously shown acquisition of carbapenemases and plasmid-mediated *mcr-1* colistin-resistance genes in, respectively, five and six travellers in this study cohort.[14,15]

Only two of six studies that previously did multivariable risk factor analysis identified antibiotic use and traveller's diarrhoea as significant travel-associated predictors for ESBL-E acquisition,[6–8] which probably reflects limited power to do extensive risk factor analysis. Self-reported pre-existing chronic bowel disease (mainly inflammatory bowel disease, irritable bowel syndrome, and coeliac disease) was a new predictor for ESBL-E acquisition in this study. Antibiotic use, traveller's diarrhoea, and chronic bowel disease have well established associations with dysbiosis of the gut microbiota.[16–18] A dysbiosis-induced reduction in colonisation resistance being the underlying biological mechanism through which these factors predispose to ESBL-E acquisition is, therefore, conceivable. Antimicrobial agents have substantial effects on the gut microbiota, which mainly manifest as decreased colonisation resistance resulting in consequent emergence of pathogenic or antibiotic-resistant strains.[19] In this study we found that,

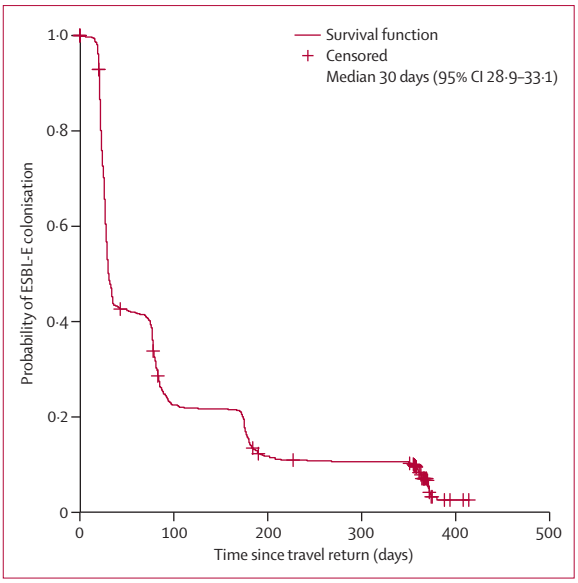

Figure 2: **Kaplan-Meier estimate of time to decolonisation of ESBL-E in travellers**
ESBL-E=extended-spectrum β-lactamase-producing Enterobacteriaceae.

second to travel destination, antibiotic use was the strongest predictor for ESBL-E acquisition, particularly quinolone use during travel. Pervasive disturbance in the human microbiota has been reported after ciprofloxacin treatment.[16,20] For amoxicillin, although the effect on the human microbiota is moderate, an increase in the abundance of resistant Enterobacteriaceae has been reported after its use.[16,19] Similar to other studies,[21] antibiotics were mostly used to treat gastroenteritis. Counselling before travel to refrain from the use of antibiotics to treat self-limiting infections could reduce the import of ESBL-E by travellers. Kantele and colleagues,[22] for example, showed that use of loperamide alone to treat mild traveller's diarrhoea was not associated with an increased risk of ESBL-E colonisation.

The significantly higher frequency of ESBL-E acquisition among travellers to Asia than other regions is probably due to the widespread dissemination of ESBL-E in these regions and high risk of food contamination. Diet-associated predictors, therefore, might differ by travel destination, and might have been missed in previous studies that did not stratify data by destination. In the overall analysis, food consumption from street vendors was associated with an increased risk of ESBL-E acquisition, but in the stratified analysis in southern Asia daily food consumption at a hostel or guesthouse and in southeastern Asia consumption of raw vegetables were predictive factors.

While the frequency of acquisition of ESBL-E by travellers is fairly consistent across studies, duration of carriage has varied from 5% to 24% at 6 months after return.[6] In our study, we found that 65 (11·3%) of 577 travellers who acquired ESBL-E during travel had sustained colonisation (persistent or intermittent) 12 months after return. Although our study focused on asymptomatic carriage of ESBL-E, international travel has also been associated with ESBL-E infection among patients in the community and in hospital.[23,24] Depending on the local policies, therefore, empirical adjustment of antimicrobial therapy should be considered in patients recently returned from international travel.

Our findings suggest that strains or plasmids carrying CTX-M group 9 ESBL genes have a colonisation advantage that results in sustained carriage. This finding agrees with those from other studies showing sustained carriage associated with these genes in travellers returning from Asia, in the community and in hospital.[25,26] Moreover, colonisation in this study was longer in travellers who acquired ESBL-producing *E coli* than in those with ESBL-producing *K pneumoniae*. These observations might be explained by accessory colonisation factors, such as P-fimbriae or aerobactin, or differences in fitness costs and plasmid stability between *E coli* and *K pneumoniae*.[27,28]

Our mathematical model of onward transmission of ESBL-E in households of travellers, which took into account factors such as total number of household members, estimated 12% probability of transmission. In households of recently discharged patients, Hilty and colleagues[29] reported transmission of ESBL-E to 20 (22·7%) of 88 household contacts. This higher risk might be due to more frequent and longer exposure times of caregiving household members to discharged patients. Practising hand hygiene at home might lessen the risk of household transmission of ESBL-E.[30]

Our study has some potential limitations. First, as in most epidemiological studies, our study population was probably more affluent and healthy than the average for the general population, which could have led to selection that affected the frequency of ESBL-E acquisition and the statistical power and generalisability of the results. However, for bias to occur, selection would have to affect both the exposure and the outcome, which is unlikely in prospective cohort studies. Inferences drawn from our study are also unlikely to be affected by (selective) attrition, since loss to follow-up was minimal and 12-month follow-up was achieved in 92·2% of participants after travellers returned. Second, faecal cross-contamination during collection of stool samples could theoretically have affected the estimations of colonisation and transmission. We aimed to keep the risk of cross-contamination to a minimum by providing participants with clear instructions for sample collection, including graphics. Lastly, although our results showed very low background transmission rates, in the absence of molecular typing of strains or mobile genetic elements harbouring ESBL genes, some overestimation of the duration of colonisation and household transmission due to novel ESBL-E acquisition from outside the household cannot be completely excluded.

320 million people visit Asia, northern Africa, and the Middle East per year[3] and, therefore, international travel is expected to contribute substantially to the emergence and

spread of ESBL-E in travellers' countries of origin. Taking into account the total number of Dutch travellers visiting these regions annually, we estimate that each year between 3·0% and 7·1% of the Dutch population acquires an ESBL-E during travel to destinations outside Europe, northern America, and Oceania (appendix). Overall, with acquisition of 34·3% and sustained carriage after acquisition seen in 11·3% of travellers 12 months after return, plus a 12% probability of household transmissions, our findings support the substantial contribution of international travel to the spread of ESBL-E and antimicrobial resistance worldwide. The degree of consequence of the emergence and spread of antimicrobial resistance by travellers, however, differs by region, and is highly dependent on local prevalence of antimicrobial resistance in the country of origin.

Contributors

MSA and JMvH did the study, collected the data, and contributed to the study design. PJJvG, CS, HAV, MDdJ, DCM, and JP designed the study and are members of the supervising board. MRH, MCJB, AG, MPG, AMOL, NM, and EES contributed to the study design, data collection, or both. MSA, JMvH, MRH, MCJB, PJJvG, CS, HAV, MDdJ, DCM, and JP contributed to the data analysis and interpretation. MSA, JMvH, MRH, and JP drafted the Article with help from all authors. MSA, JMvH, MRH, MCJB, PJJvG, AG, MPG, CS, EES, HAV, MDdJ, DCM, and JP contributed to the critical revision of the drafts for important intellectual content. All authors read and approved the final version of the paper.

Declaration of interests

We declare no competing interests.

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