Clinical Microbiology and Infection 24 (2018) 908.e9–908.e16

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

A prospective study of travellers' diarrhoea: analysis of pathogen findings by destination in various (sub)tropical regions

T. Lääveri [1], K. Vilkman [1, 2], S.H. Pakkanen [3], J. Kirveskari [4], A. Kantele [1, 2, 5, 6, *]

[1)] *Inflammation Center, Division of Infectious Diseases, Helsinki University Hospital, Finland and University of Helsinki, Finland*
[2)] *Aava Travel Clinic, Medical Centre Aava, Helsinki, Finland*
[3)] *Department of Bacteriology and Immunology, University of Helsinki, Finland*
[4)] *Department of Bacteriology, Helsinki University Hospital Laboratory (HUSLAB), Finland*
[5)] *Clinicum, University of Helsinki, Finland*
[6)] *Unit of Infectious Diseases, Department of Medicine/Solna, Karolinska Institutet, Stockholm, Sweden*

ARTICLE INFO

Article history:
Received 19 June 2017
Received in revised form
12 September 2017
Accepted 24 October 2017
Available online 10 November 2017

Editor: E. Bottieau

Keywords:
Campylobacter
Diarrhoea
EAEC
EHEC
EIEC
EPEC
ETEC
Region
Travel
Travellers' diarrhoea

ABSTRACT

Objectives: Eighty million travellers visiting (sub)tropical regions contract travellers' diarrhoea (TD) each year, yet prospective data comparing the prevalence of TD pathogens in various geographical regions are scarce. Our recent study using modern molecular methods found enteropathogenic (EPEC) and enteroaggregative (EAEC) *Escherichia coli* to be the most frequent pathogens, followed by enterotoxigenic *E. coli* (ETEC) and *Campylobacter*. We revisited our data to compare the findings by geographical region.
Methods: A total of 459 prospectively recruited travellers provided stool samples and completed questionnaires before and after visiting destinations in various geographical regions. A multiplex quantitative real-time PCR assay was used to analyse *Salmonella, Yersinia, Campylobacter jejuni/Campylobacter coli, Shigella, Vibrio cholerae,* EPEC, EAEC, ETEC, enterohaemorrhagic *E. coli* and enteroinvasive *E. coli*.
Results: TD was contracted by 69% (316/459) of the subjects; EPEC and EAEC outnumbered ETEC and *Campylobacter* in all regions. Multiple pathogens were detected in 42% (133/316) of the samples. The proportions of all pathogens varied by region. The greatest differences were seen for *Campylobacter*: while relatively frequent in South Asia ($n = 11$; 20% of the 55 with TD during travel) and Southeast Asia (15/84, 15%), it was less common in East and West Africa (5/71, 7% and 1/57, 2%) and absent in South America and the Caribbean (0/40).
Conclusions: EPEC and EAEC outnumbered ETEC and *Campylobacter* everywhere, yet the proportions of pathogen findings varied by region, with ETEC and *Campylobacter* rates showing the greatest differences. The high frequency of multibacterial findings in many regions indicates a need for further investigation of the clinical role of each pathogen. **T. Lääveri, Clin Microbiol Infect 2018;24:908.e9–908.e16**

Introduction

Travellers' diarrhoea (TD) accounts for more than 80 million cases each year [1]. It is not only the disease most commonly encountered by travellers to (sub)tropical destinations [2,3] but also the most common health complaint at posttravel consultations [2,4]. Bacterial pathogens are known to predominate as aetiologic agents. However, the pathogen remains unidentified in up to half of cases [5,6]. Over the past decade the field of TD research has experienced a renaissance, with new molecular methods covering a larger variety of pathogens than traditional approaches. However, few data are available comparing the incidence of the various pathogens by geographical area.

Until recently, enterotoxigenic *Escherichia coli* (ETEC) has been reported to outnumber all other pathogens causing TD in most regions [5,6], with the exception of Southeast Asia (Thailand), where *Campylobacter* has been considered the primary pathogen [5–9]. Studies using modern molecular methods have decreased the proportion of unexplained TD cases to only 4% to 24% [10–12]. These investigations have also revealed diarrhoeagenic *E. coli* (DEC) to be even more prevalent than previously thought. The rates for enteropathogenic *E. coli* (EPEC) and enteroaggregative *E. coli* (EAEC) have been at least as high as those for ETEC even after visiting Central America [11,13], South Asia [10] or Africa [10,12,14], where earlier findings have been predominated by ETEC. Other bacterial pathogens such as *Shigella,*

* Corresponding author. A. Kantele, Inflammation Center, Division of Infectious Diseases, Helsinki University Hospital, POB 348, FIN-00029 HUS, Finland.
E-mail address: anu.kantele@hus.fi (A. Kantele).

https://doi.org/10.1016/j.cmi.2017.10.034
1198-743X/

Salmonella spp., enteroinvasive and enterohaemorrhagic *E. coli* (EIEC and EHEC respectively), *Aeromonas* spp., *Plesiomonas shigelloides, Arcobacter* spp., enterotoxigenic *Bacteroides fragilis* and *Vibrio* spp. have been detected less frequently [5,6].

We found only a handful of reports providing data on TD pathogens by region in a single study using modern molecular methods. EPEC has only been covered in a few investigations. Moreover, EAEC prevalence data among travellers to Southeast Asia and East Africa remain virtually nonexistent.

In our prospective study of 459 Finnish travellers, we applied multiplex quantitative real-time PCR (qPCR) methodology to analysis of various TD pathogens by region, seeking to challenge the current understanding of regionally predominant pathogens.

Materials and methods

Study population and recruitment

The participants for this prospective study were initially enrolled at the Travel Clinic of Aava Medical Centre, Helsinki, Finland, over a 12-month period in 2009–2010 before their journey outside the Nordic countries for more than four nights. Of the 526 volunteers, 459 (63%) completed questionnaires and provided stool samples before and after travel (Supplementary Fig. S1). Ethical approval was obtained from the ethics committee of the department of medicine at Helsinki University Hospital. All study subjects provided written informed consent. We earlier reported the findings of resistant *Enterobacteriaceae* [15] and travel-related health problems [3] in the same volunteers. Recently we described the stool pathogens of 382 travellers who did not use antimicrobials [16].

Definition of TD

TD was defined according to World Health Organization criteria as passage of loose or liquid stools more frequently than is normal for the individual (http://www.who.int/topics/diarrhoea/en/). Severe TD was defined as six or more diarrhoeal stools per day, TD accompanied by fever or haemorrhagic stools, or TD requiring hospitalization.

Questionnaires

The pretravel questionnaire included demographic data and information on possible diarrhoeal symptoms at the time of the first faecal sample. The posttravel questionnaire assessed the travel itinerary, diarrhoeal and other symptoms, and medications during or immediately after travel.

Travel destinations

The countries visited were grouped into regions as modified from the United Nations classification: South Asia; Southeast Asia; East Asia; North Africa and Middle East; East Africa; West Africa (Western and Middle Africa); Southern Africa; Latin America (South and Central America and the Caribbean); and Europe, Australia and North America. The 37 travellers (8%) who visited more than one region were grouped by longest stay; 16 (43%) of these visited Europe or the United States on their way to the (sub)tropics. This report focuses on the five most popular destinations: South Asia, Southeast Asia, East Africa, West Africa and Latin America.

Collection and laboratory analysis of stool samples

Briefly, stool samples were collected before departure and from the first (or second) stool passed after returning home as swabs in Copan M40 Transystem tubes (Copan Diagnostics, Brescia, Italy) and mailed in special boxes, reaching the laboratory within 1 to 3 days. Once the samples arrived, total nucleic acids were extracted using the standard semiautomated protocol of easyMAG (bioMérieux, Marcy l'Etoile, France). The analyses were carried out with a multiplex qPCR method [1] which covers the following pathogens: *Campylobacter jejuni* and *Campylobacter coli, Salmonella* spp., *Yersinia, Vibrio cholerae,* EPEC, EAEC, ETEC, EHEC and EIEC/*Shigella* spp. Here we focus on the findings of pathogens found to associate with ongoing TD symptoms discussed in our previous report (EPEC, EAEC, ETEC and *Campylobacter*) [16].

Statistical analysis

Statistical analyses were carried out by SPSS 22 (IBM SPSS, Chicago, IL, USA).

The chi-square test or binary logistic regression analysis was used to compare categorical variables when applicable. A binominal regression model was used to obtain profile likelihood confidence intervals (CIs) for the proportions of different pathogens in geographical regions; in cases when computation did not converge, asymptotic Wald CIs were used. Statistical significance was determined as either 95% CIs not overlapping, or ranging only either above or below 1.

Results

Demographic data and occurrence of TD

Background data on the travellers and their travels are shown in Supplementary Table S1. TD was reported by 316 travellers (69%). At the time of the posttravel stool sample, 143 (31%) reported having ongoing symptoms, 173 (38%) reported that symptoms had already resolved and 143 (31%) were asymptomatic through the entire journey. Seventy-two (16%) reported having taken antimicrobial medications during travel. TD was most common among those who visited South Asia (55/69, 80%), Southeast Asia (84/108, 79%) and East Africa (71/96, 74%), while 67% (57/85) of those who travelled to West Africa and 60% (24/40) to Latin America reported TD (Supplementary Table S2). There were no significant differences between regions in the severity of TD (severe vs. nonsevere TD, p 0.118; data not shown).

Pathogen findings in stool samples of all 459 travellers

Nineteen (4%) of 459 pretravel samples were positive for bacterial pathogens; only one subject with EPEC reported mild diarrhoeal symptoms. An analysis of the posttravel stool samples of all 459 subjects (Table 1) revealed EPEC ($n = 194$; 42%) and EAEC (192; 42%) to be the most common pathogens, outnumbering both ETEC (88; 19%) and *Campylobacter* (31; 7%). EHEC was identified in 37 (8%), *Salmonella* in 11 (2%) and EIEC/*Shigella* in six (1%) stool samples. *Vibrio cholerae* and *Yersinia* were not detected. Two or more types of bacterial pathogens were found in 168 (37%) of all posttravel faecal samples; 57 (12%) had three or more pathogens.

EPEC, EAEC, ETEC and Campylobacter findings in stool samples of travellers to five regions

The findings of EPEC, EAEC, ETEC and *Campylobacter* in relation to symptoms among travellers to South Asia, Southeast Asia, East Africa, West Africa and Latin America are presented in Supplementary Table S2 and in Figs. 1–3. Pathogen findings from countries with more than ten visitors are presented in Supplementary Table S3.

Table 1
Bacterial pathogens detected in posttravel stool samples of 459 Finnish travellers in relation to symptoms and geographical region

Characteristic	All travellers	Any pathogen	Multiple pathogens	EPEC	EAEC	ETEC	EHEC	Only DEC	Only non-DEC	*Campylobacter*	*Salmonella*	*Shigella*/EIEC
Total (% of all)	459	326 (71)	168 (37)	193 (42)	192 (42)	88 (19)	37 (8)	223 (49)	12 (3)	31 (7)	11 (2)	6 (1)
Proportion of travellers with TD by pathogen finding												
TD during travel	316 (69)	241 (74)	133 (79)	134 (69)	152 (79)	76 (86)	27 (73)	159 (71)[a]	11 (92)[a]	30 (97)	8 (73)	5 (83)
OR (95% CI) TD during travel vs. no TD[a]		2.2 (1.4–3.3)	2.2 (1.4–3.5)	1.0 (0.7–1.6)	2.4 (1.6–3.7)	3.5 (1.8–6.6)	1.2 (0.6–2.6)	1.6 (1.0–2.4)	8.5 (1.1–67.8)	14.9 (2.0–110.3)	1.2 (0.3–4.6)	2.3 (0.3–19.7)
Findings by geographical region												
Asia (all)	184 (40)	130 (71)	69 (38)	83 (45)	74 (40)	35 (19)	10 (5)	78 (42)	12 (7)	25 (14)	7 (4)	2 (1)
South Asia	69 (15)	54 (78)	37 (54)	37 (54)	39 (57)	15 (22)	7 (10)	29 (42)	2 (3)	12 (17)	1 (1)	2 (3)
Southeast Asia	108 (24)	74 (68)	31 (29)	44 (41)	34 (31)	20 (19)	3 (3)	48 (44)	10 (9)	13 (12)	6 (6)	0 (0)
East Asia	7 (2)	2 (29)	1 (14)	2 (29)	1 (14)	0 (0)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)
Africa (all)	218 (47)	162 (74)	86 (39)	93 (43)	100 (46)	48 (22)	19 (9)	116 (53)	0 (0)	6 (3)	3 (1)	4 (2)
North Africa and Middle East	13 (3)	8 (62)	1 (8)	5 (39)	3 (23)	0 (0)	1 (8)	7 (54)	0 (0)	0 (0)	0 (0)	0 (0)
Southern Africa	24 (5)	14 (58)	5 (21)	7 (29)	8 (33)	2 (8)	2 (8)	11 (46)	0 (0)	0 (0)	0 (0)	0 (0)
West (and Middle) Africa	85 (19)	60 (71)	32 (38)	36 (42)	39 (46)	14 (16)	7 (8)	40 (47)	0 (0)	1 (1)	1 (1)	4 (5)
East Africa	96 (21)	80 (83)	48 (50)	45 (47)	50 (52)	32 (33)	9 (9)	58 (60)	0 (0)	5 (5)	2 (2)	0 (0)
Latin America (South and Central America and Caribbean)	40 (9)	31 (78)	11 (38)	17 (43)	15 (38)	4 (10)	7 (18)	26 (65)	0 (0)	0 (0)	1 (3)	0 (0)
Europe, Australia, North America	17 (4)	3 (18)	2 (12)	0 (0)	3 (18)	1 (6)	1 (6)	3 (18)	0 (0)	0 (0)	0 (0)	0 (0)

Data are presented as *n* (%) unless otherwise indicated. No *Yersinia* or *Vibrio cholerae* were detected.
CI, confidence interval; DEC, diarrhoeagenic *Escherichia coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; OR, odds ratio; TD, travellers' diarrhoea.
[a] Compared to no bacterial pathogens.

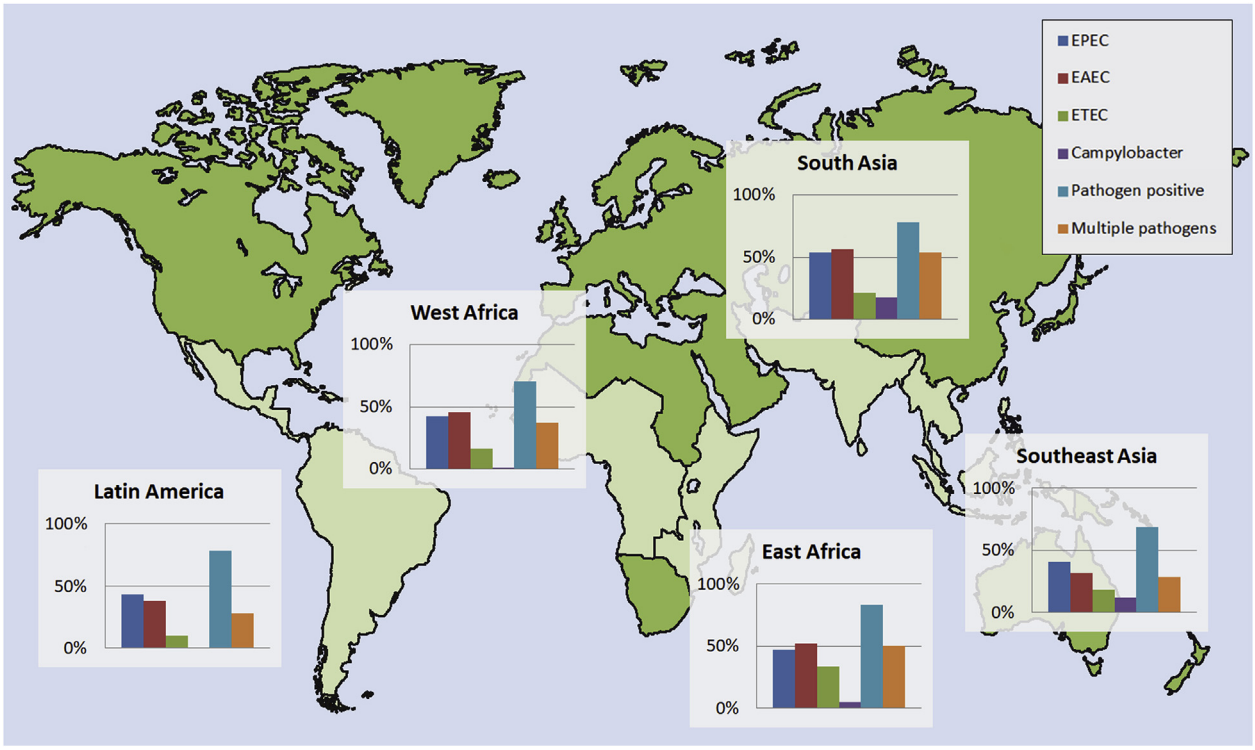

Fig. 1. Map showing proportions of findings for EPEC, EAEC, ETEC and *Campylobacter*, any pathogen or multiple pathogens among 459 Finnish travellers to South Asia, Southeast Asia, East Africa, West Africa and Latin America. EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*.

TD pathogens of travellers to South Asia

Bacterial pathogens were found in 82% (45/55) of stool samples from travellers to South Asia with TD. EAEC (33/55, 60%) and EPEC (31/55, 56%) were more common findings than ETEC (15/55, 27%) and *Campylobacter* (11/55, 20%); 60% (33/55) had two or more types of pathogens and 31% (14/55) had three or more. Of those whose symptoms had already resolved, *Campylobacter* was found in 30% (8/27). Of asymptomatic travellers, 64% (9/14) had a pathogen finding; 43% (6/14) had either EAEC or EPEC.

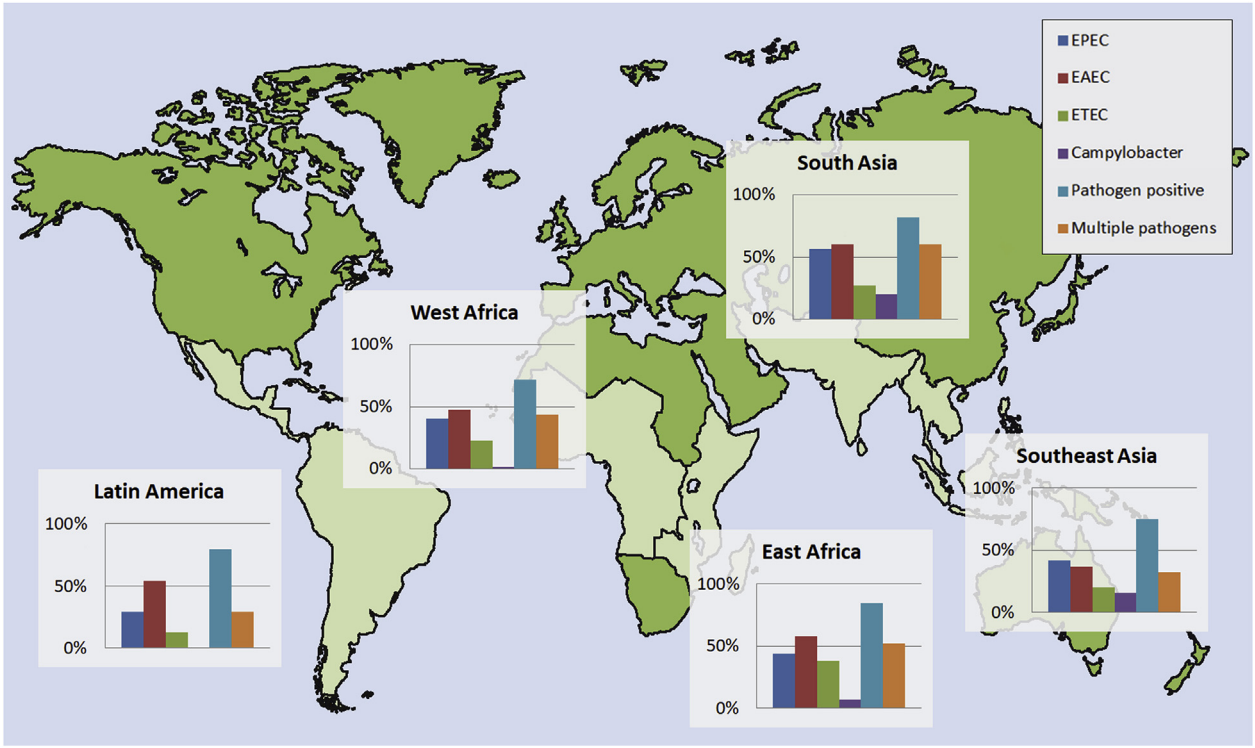

Fig. 2. Map showing proportions of findings for EPEC, EAEC, ETEC and *Campylobacter*, any pathogen or multiple pathogens among travellers with TD during travel. EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli;* TD, travellers' diarrhoea.

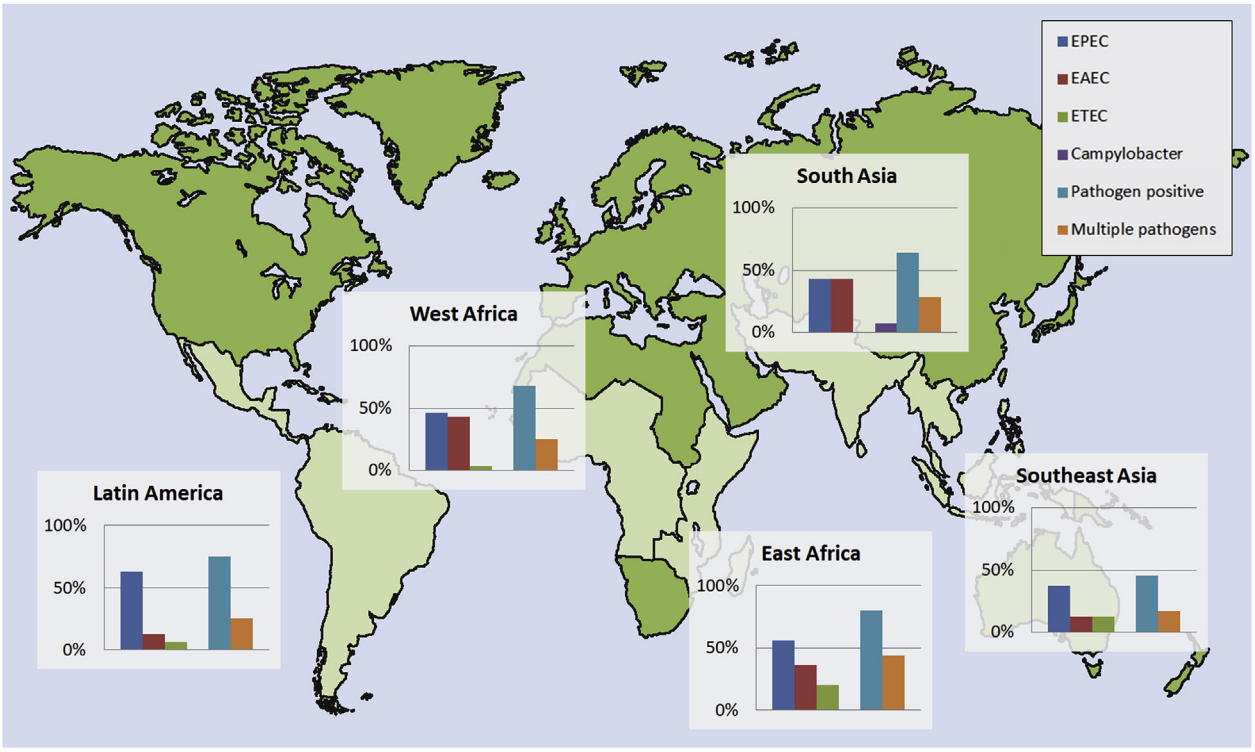

Fig. 3. Map showing proportions of findings for EPEC, EAEC, ETEC and *Campylobacter*, any pathogen or multiple pathogens among travellers who remained asymptomatic during travel. EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*.

TD pathogens of travellers to South Asia

Among the 108 travellers to Southeast Asia, pathogens were found in 75% (63/84) of those with TD. EPEC (35/84, 42%) and EAEC (31/84, 37%) were the most frequent findings, followed by ETEC (17/84, 20%) and *Campylobacter* (13/84, 15%). Among travellers with ongoing TD, multiple pathogens were detected in 50% (12/24), EPEC in 50% (12/24), EAEC in 38% (9/24), ETEC in 33% (8/24) and *Campylobacter* in 17% (4/24). Of those remaining asymptomatic, 46% (11/24) had a pathogen finding.

TD pathogens of travellers to West Africa

Among the 85 travellers to West Africa, pathogens were found in 72% (41/57) of those with TD. EAEC was detected in 47% (27/57) and EPEC in 40% (23/57), followed by ETEC in 23% (13/57) and *Campylobacter* in 2% (1/57). Two or more types of pathogens were found in 44% (25/57). In asymptomatic travellers, 68% (19/28) had pathogen findings.

TD pathogens of travellers to East Africa

Of the 96 travellers to East Africa, a pathogen was detected in the faecal samples of 85% (60/71) of those with TD. ETEC was almost as common (27/71, 38%) as EPEC (41/71, 58%) and EAEC (41/71, 44%). *Campylobacter* was detected in 7% (5/71). Two or more different types of pathogens were detected in 52% (37/71). These proportions were similar among travellers with ongoing TD. Of asymptomatic travellers, 80% (20/25) had pathogen findings.

TD pathogens of travellers to Latin America

Among 40 travellers to Latin America, pathogens were found in 79% (19/24) of those having experienced TD. EAEC (14/24, 54%) and EPEC (7/24, 30%) were found more commonly than ETEC (3/24, 13%); 30% (7/24) had two or more types of pathogens. *Campylobacter* was not detected. Among travellers with ongoing TD, EAEC, EPEC and ETEC were found in equal proportions (3/10, 30%). Of asymptomatic travellers, 75% (12/16) had pathogen findings.

Differences between regions by pathogens found in travellers with TD during travel

Geographical comparisons by pathogen of travellers with TD are presented in Table 2. The results with statistical significance are provided here.

EPEC

The region with the highest proportion was South Asia (56%); the comparison to Latin America was significant (29%, odds ratio (OR) 0.3, 95% CI 0.1–0.9).

EAEC

This pathogen was found more frequently in samples from travellers to South Asia than Southeast Asia (60% vs. 37%, OR 0.4, 95% CI 0.2–0.8).

ETEC

The highest proportion of this pathogen was detected among those visiting East Africa (38%); the comparison to Southeast Asia (20%, OR 0.4; 95% CI 0.2–0.9) and Latin America (13%, OR 0.2; 95% CI 0.1–0.9) was significant.

Campylobacter

This pathogen was most commonly detected among travellers to South Asia (20%); the proportions were significantly lower in West Africa (2%, OR 0.1, 95% CI 0.01–0.6) and East Africa (7%, OR 0.3, 95% CI 0.1–0.9). No cases with *Campylobacter* were detected in Latin America.

Table 2
Relationship among pathogen findings and travel destination among travellers with TD during travel

Pathogen and travel destination	All travellers			TD during travel			Ongoing TD			Asymptomatic		
	n (%) of travellers to region	p	OR (95% CI)	*n* (%) of travellers to region	p	OR (95% CI)	*n* (%) of travellers to region	p	OR (95% CI)	*n* (%) of travellers to region	p	OR (95% CI)
Any pathogen												
South Asia	54 (79)	0.412	0.7 (0.3–1.6)	45 (82)	0.688	0.8 (0.3–2.1)	24 (86)		1.0	9 (64)	0.286	0.5 (0.1–2.0)
Southeast Asia	74 (69)	0.015	0.4 (0.2–0.9)	63 (75)	0.148	0.6 (0.2–1.2)	18 (75)	0.334	0.5 (0.1–2.0)	11 (46)	0.016	0.2 (0.1–0.8)
West Africa	60 (71)	0.043	0.5 (0.2–1.0)	41 (72)	0.087	0.5 (0.2–1.1)	19 (73)	0.256	0.5 (0.1–1.8)	19 (68)	0.320	0.5 (0.2–1.9)
East Africa	80 (83)		1.0	60 (85)		1.0	33 (85)	0.901	0.9 (0.2–3.6)	20 (80)		1.0
Latin America	31 (78)	0.425	0.7 (0.3–1.7)	19 (79)	0.547	0.7 (0.2–2.3)	7 (70)	0.281	0.4 (0.1–2.2)	12 (75)	0.706	0.8 (0.2–3.4)
Multiple pathogens												
South Asia	37 (54)		1.0	33 (60)		1.0	18 (64)		1.0	4 (29)	0.346	0.5 (0.1–2.1)
Southeast Asia	31 (29)	0.001	0.3 (0.2–0.7)	27 (32)	0.001	0.3 (0.2–0.6)	12 (50)	0.300	0.6 (0.2–1.7)	4 (17)	0.044	0.3 (0.1–1.0)
West Africa	32 (38)	0.048	0.5 (0.3–1.0)	25 (44)	0.089	0.5 (0.2–1.1)	13 (50)	0.291	0.6 (0.2–1.7)	7 (25)	0.149	0.4 (0.1–1.4)
East Africa	48 (50)	0.646	0.9 (0.5–1.6)	37 (52)	0.141	0.7 (0.4–1.5)	18 (46)	0.145	0.5 (0.2–1.3)	11 (44)		1.0
Latin America	11 (28)	0.009	0.3 (0.1–0.8)	7 (29)	0.014	0.3 (0.1–0.8)	3 (30)	0.071	0.2 (0.1–1.1)	4 (25)	0.223	0.4 (0.1–1.7)
EPEC												
South Asia	37 (54)		1.0	31 (56)		1.0	17 (61)		1.0	6 (43)	0.285	0.5 (0.1–1.9)
Southeast Asia	44 (41)	0.094	0.6 (0.3–1.1)	35 (42)	0.091	0.6 (0.3–1.1)	12 (50)	0.439	0.6 (0.2–1.9)	9 (38)	0.125	0.4 (0.1–1.3)
West Africa	36 (42)	0.165	0.6 (0.3–1.2)	23 (40)	0.091	0.5 (0.2–1.1)	14 (54)	0.610	0.8 (0.3–2.2)	13 (46)	0.307	0.5 (0.1–1.8)
East Africa	45 (47)	0.393	0.8 (0.4–1.4)	31 (44)	0.158	0.6 (0.3–1.2)	18 (46)	0.241	0.6 (0.2–1.5)	14 (56)	0.681	0.8 (0.2–2.8)
Latin America	17 (43)	0.264	0.6 (0.3–1.4)	7 (29)	0.029	0.3 (0.1–0.9)	3 (30)	0.105	0.3 (0.1–1.3)	10 (63)		1.0
EAEC												
South Asia	39 (57)		1.0	33 (60)		1.0	18 (64)		1.0	6 (43)		1.0
Southeast Asia	34 (31)	0.001	0.4 (0.2–0.7)	31 (37)	0.008	0.4 (0.2–0.8)	9 (38)	0.057	0.3 (0.1–1.0)	3 (13)	0.043	0.2 (0.04–1.0)
West Africa	39 (46)	0.190	0.7 (0.3–1.2)	27 (47)	0.181	0.6 (0.3–1.3)	12 (46)	0.183	0.5 (0.2–1.4)	12 (43)	1.000	1.0 (0.3–3.7)
East Africa	50 (52)	0.573	0.8 (0.4–1.6)	41 (56)	0.799	0.9 (0.4–1.9)	19 (49)	0.208	0.5 (0.2–1.4)	9 (36)	0.673	0.8 (0.2–2.9)
Latin America	15 (38)	0.057	0.5 (0.2–1.0)	13 (54)	0.629	0.8 (0.3–2.1)	3 (30)	0.071	0.2 (0.1–1.1)	2 (13)	0.074	0.2 (0.03–1.2)
ETEC												
South Asia	15 (22)	0.106	0.6 (0.3–1.1)	15 (27)	0.206	0.6 (0.3–1.3)	10 (36)	0.660	0.8 (0.3–2.2)	0 (0)	NA	
Southeast Asia	20 (19)	0.017	0.5 (0.2–0.9)	17 (20)	0.016	0.4 (0.2–0.8)	8 (33)	0.542	0.7 (0.2–2.1)	3 (13)	0.481	0.6 (0.1–2.7)
West Africa	14 (16)	0.011	0.4 (0.2–0.8)	13 (23)	0.067	0.5 (0.2–1.1)	7 (27)	0.247	(0.5 80.2–1.6)	1 (4)	0.092	0.1 (0.02–1.4)
East Africa	32 (33)		1.0	27 (38)		1.0	44 (35)		1.0	5 (20)		1.0
Latin America	4 (10)	0.008	0.2 (0.1–0.7)	3 (13)	0.028	0.2 (0.1–0.9)	3 (30)	0.526	0.6 (0.1–2.7)	1 (6)	0.249	0.3 (0.03–2.5)
Campylobacter												
South Asia	12 (17)		1.0	11 (20)		1.0	3 (11)	0.534	0.6 (0.1–3.0)	1 (7)	NA	NA
Southeast Asia	13 (12)	0.321	0.7 (0.3–1.5)	13 (15)	0.491	0.7 (0.3–1.8)	4 (17)		1.0	0 (0)	NA	NA
West Africa	1 (1)	0.006	0.1 (0.01–0.4)	1 (2)	0.013	0.1 (0.01–0.6)	0 (0)	0.998	NA	0 (0)	NA	NA
East Africa	5 (5)	0.016	0.3 (0.1–0.8)	5 (7)	0.037	0.3 (0.1–0.9)	3 (8)	0.282	0.4 (0.1–2.1)	0 (0)	NA	NA
Latin America	0 (0)	0.998	NA	0 (0)	0.998	NA	0 (0)	0.999	NA	0 (0)	NA	NA

CI, confidence interval; EAEC, enteroaggregative *E. coli;* EPEC, enteropathogenic *E. coli;* ETEC, enterotoxigenic *E. coli;* OR, odds ratio; TD, travellers' diarrhoea.

Multiple pathogen findings

Multiple pathogen findings were more frequent among travellers to South Asia (60%) than Southeast Asia (32%; OR 0.3, 95% CI 0.2–0.6) or Latin America (29%; OR 0.3, 95% CI 0.1–0.8).

Discussion

The perception of TD aetiology has been revolutionized by modern molecular methods offering high sensitivity and covering a wide range of pathogens. ETEC has long been considered the most common TD pathogen (apart from in Thailand) [5,6]. Our earlier data showed EPEC and EAEC to predominate [16], which is in accord with other recent findings [10–12,14]. The regional differences revealed by our analysis were mostly related to ETEC and *Campylobacter*. We next discuss our pathogen findings by region.

South Asia: multiple pathogen findings

Our data show multiple pathogens to be especially common among travellers to South Asia. Of those with ongoing TD, 64% had more than one pathogen in their stool samples. This corresponds with earlier investigations reporting rates of 27% to 60% for multiple pathogens [17,18]. We also detected pathogens in the stools of 74% of the asymptomatic travellers, 29% of whom were coinfected. Indeed, our data indicate that travellers visiting the region are very commonly exposed to stool pathogens.

Some reports suggest that *Campylobacter* may be contracted in South Asia as frequently as in Southeast Asia [18–20]. Correspondingly, we found *Campylobacter* among 20% and 15% of travellers with TD visiting South Asia and Southeast Asia, respectively. However, even in South Asia, EAEC (60%) and EPEC (56%) were the most common pathogens, followed by ETEC (27%). Although consistent with some other studies [10,17], this observation is contradicted by Jiang et al. [18], who reported only 5% to be infected with EAEC, but 76% with ETEC and 20% with *Campylobacter*. The varying detection rates for EAEC may be explained by methodologic differences. In our data, DEC strains were seen in all but two stool samples with a pathogen finding.

Southeast Asia: DEC more common than Campylobacter

Review articles looking at travellers to Southeast Asia report *Campylobacter* to be the most common TD pathogen (25–35%), with low proportions of DEC infections [5,6]. Our findings in this geographical region contradict previous reports. We detected both EPEC and EAEC (42% and 37% of travellers with TD, respectively) more frequently than *Campylobacter* (15%); ETEC was seen in 20%. One reason for overestimating the role of *Campylobacter* may be the limited coverage of the various DEC in earlier investigations among travellers to Southeast Asia; we are not aware of any studies covering all four pathogens—EAEC, EPEC, ETEC and *Campylobacter*—in a single report. Moreover, it should be noted that most research into TD in Thailand has been conducted by studying military personnel instead of ordinary tourists [7–9]. The high rate of DEC (87% of our pathogen findings) in our data shows a greater prevalence of these pathogens in Southeast Asia than previously reported.

West Africa: DEC predominate

The few fairly recent studies of TD aetiology in Western or Middle Africa [10,12,14] have presented results concurring with ours showing EAEC and EPEC (47% and 40% of those with TD during travel) to be at least as common as ETEC (23%) among travellers to West Africa. These data dispute earlier investigations finding ETEC predominant and other DEC uncommon [21–23]. Together with the very low rates of *Campylobacter* (2%), our data indicate that DEC also predominates in this geographical area.

East Africa: ETEC especially common

Interestingly, in East Africa, coinfections were almost as common as in South Asia; DEC were identified in all stool samples with a positive pathogen finding. We are aware of only two studies published over the past 30 years analysing TD among travellers to East Africa. Among 464 travellers, Jiang et al. [24] identified ETEC in the specimens of 35% visiting Kenya (5% had *Campylobacter;* EPEC or EAEC were not screened for), and Paschke et al. [10] reported that of 12 travellers to East Africa, six had EAEC, four had heat-labile ETEC and four heat-stable ETEC; EPEC was not analysed. In our data, East Africa was the region with the highest rate of ETEC, which corresponds with the rates in earlier reports [10,24], yet here also EPEC and EAEC were equally frequent. Our five *Campylobacter* cases (7% of those 71 with TD) in East Africa and one case (1/57, 2%) in West Africa support earlier observations [19] of a tenfold difference in the risk between East and West Africa. The cause of the great regional variations in *Campylobacter* findings among travellers has remained unclear.

Latin America: other DEC as common as ETEC

Our data accord with recent studies [11,13,17,25] indicating considerable frequency of EAEC in Latin America [18,26], but contradict others reporting EAEC in only 3% to 13% of TD cases [18,26]. To our knowledge, only one study has included EPEC in the analyses of TD pathogens in Latin America [11]; our results showing 29% of travellers with TD to have EPEC concur with these findings. In our data, EPEC and EAEC were as commonly found as ETEC. Consistent with earlier investigations, we found no cases of *Campylobacter* [5,6,27]. It should be noted that only seven of our 40 volunteers visited Mexico or Guatemala, where the earlier aetiologic studies have mostly been conducted. Nevertheless, EPEC and EAEC should evidently also be included in future analyses of the aetiology of TD in Latin America.

Study limitations

Multiplex PCR methods have been criticized as having too high a sensitivity, as they may identify pathogens in too scanty a number to cause clinical symptoms. At the same time, it has been recognized that besides the quantity of pathogens, the development of symptoms also depends on a number of other factors, such as the infective dose and the host's preexisting immunity. Furthermore, multiplex PCR assays, by covering a wide selection of pathogens, reveal the multibacterial nature of TD diseases, thus suggesting that older studies with narrower coverage of pathogens may have misinterpreted the role and prevalence of individual findings. The role of each pathogen needs to be studied further. As a limitation, our multiplex qPCR did not cover any viral or parasitic pathogens.

Because the stool samples were taken only after travel, the prevalence of some pathogens may have been underestimated. EPEC and ETEC were more likely to be found in the specimens of travellers with ongoing symptoms than those recovered from the disease, whereas equal rates of *Campylobacter* and EAEC were detected in both groups [16]. Indeed, at least ETEC has been suggested to disappear rapidly from stools [25]. Such characteristics of these pathogens may lead to underestimating the rates of ETEC and EPEC, thereby overemphasizing the role of *Campylobacter* and EAEC. Moreover, despite being more common among travellers with TD than those asymptomatic in our earlier report [16], the role of EPEC in TD remains disputable.

Practical considerations

Our data indicate that in all the geographical regions we selected for study, DEC are found in the majority of TD cases, with EPEC and EAEC as predominating findings. However, it should be pointed out that the clinical picture of the disease caused by different pathogens may vary. *Campylobacter* has been associated with more severe TD including fever and abdominal pain [28,29] and ETEC with acute watery diarrhoea [29]. One study suggests similar clinical pictures for EPEC and ETEC [28]. Although we failed in our previous report to find differences in the severity of TD caused by the various TD pathogens, EPEC and EAEC were also commonly detected in the stool samples of asymptomatic travellers, while ETEC and *Campylobacter* were almost always associated with symptomatic disease [16]. Therefore, the prevalence rate of a pathogen should not be equated to its clinical pertinence.

Conclusions

When using modern molecular methods with enhanced coverage of bacterial pathogens, fewer stools remain without any findings (in our data 26% of those with TD during travel). DEC were revealed to be the most prevalent pathogens in all regions encompassed by the study. Indeed, while the proportions of ETEC and *Campylobacter* were similar or slightly lower than in earlier reports, the main benefit of modern techniques lies in revealing the concomitant high prevalence of EPEC and EAEC. Regional differences were mostly seen in the prevalence of ETEC and *Campylobacter*, yet the multibacterial nature of the findings attests to the need to redefine the clinical picture and prevalence of each pathogen.

Acknowledgements

We express our gratitude to the late J. Riutta for recruiting the patients. We also thank the nurses at the travel clinic of Aava Medical Centre for help in recruiting the volunteers, the personnel of Helsinki University Hospital Laboratory for processing the stool specimens and J. Ollgren for expert advice regarding statistical analyses.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2017.10.034.

Transparency Declaration

This work was supported by a Finnish government subsidy for health science research (grants TYH2012141, TYH 2013218 and TYH 2014216), the Scandinavian Society for Antimicrobial Chemotherapy Foundation (grant SLS-504141) and the Paulo Foundation. JK is an employee of Mobidiag Ltd. outside the submitted work and has patent EP13809410.7 pending. AK reports funding for an ETEC vaccine study from Scandinavian Biopharma during conduct of the study; honoraria from Novartis, Crucell and Valneva, and grant and personal fees from Crucell outside the submitted work. The other authors report no conflicts of interest relevant to this article.

References

[1] Antikainen J, Kantele A, Pakkanen SH, Lääveri T, Riutta J, Vaara M, et al. A quantitative polymerase chain reaction assay for rapid detection of 9 pathogens directly from stools of travelers with diarrhea. Clin Gastroenterol Hepatol 2013;11:1300–7.
[2] Hill DR. Health problems in a large cohort of Americans traveling to developing countries. J Travel Med 2000;7:259–66.
[3] Vilkman K, Pakkanen SH, Lääveri T, Siikamaki H, Kantele A. Travelers' health problems and behavior: prospective study with post-travel follow-up. BMC Infect Dis 2016;16:328.
[4] Herbinger KH, Alberer M, Berens-Riha N, Schunk M, Bretzel G, von Sonnenburg F, et al. Spectrum of imported infectious diseases: a comparative prevalence study of 16,817 German travelers and 977 immigrants from the tropics and subtropics. Am J Trop Med Hyg 2016;94:757–66.
[5] Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers' diarrhea: systematic review from 1973 to the present. Am J Trop Med Hyg 2009;80:609–14.
[6] Steffen R, Hill DR, DuPont HL. Traveler's diarrhea: a clinical review. JAMA 2015;313:71–80.
[7] Echeverria P, Jackson LR, Hoge CW, Arness MK, Dunnavant GR, Larsen RR. Diarrhea in US troops deployed to Thailand. J Clin Microbiol 1993;31:3351–2.
[8] Kuschner RA, Trofa AF, Thomas RJ, Hoge CW, Pitarangsi C, Amato S, et al. Use of azithromycin for the treatment of *Campylobacter* enteritis in travelers to Thailand, an area where ciprofloxacin resistance is prevalent. Clin Infect Dis 1995;21:536–41.
[9] Tribble DR, Sanders JW, Pang LW, Mason C, Pitarangsi C, Baqar S, et al. Traveler's diarrhea in Thailand: randomized, double-blind trial comparing single-dose and 3-day azithromycin-based regimens with a 3-day levofloxacin regimen. Clin Infect Dis 2007;44:338–46.
[10] Paschke C, Apelt N, Fleischmann E, Perona P, Walentiny C, Loscher T, et al. Controlled study on enteropathogens in travellers returning from the tropics with and without diarrhoea. Clin Microbiol Infect 2011;17:1194–200.
[11] Paredes-Paredes M, Okhuysen PC, Flores J, Mohamed JA, Padda RS, Gonzalez-Estrada A, et al. Seasonality of diarrheagenic *Escherichia coli* pathotypes in the US students acquiring diarrhea in Mexico. J Travel Med 2011;18:121–5.
[12] Lääveri T, Pakkanen SH, Antikainen J, Riutta J, Mero S, Kirveskari J, et al. High number of diarrhoeal co-infections in travellers to Benin, West Africa. BMC Infect Dis 2014;14:81.
[13] Grimes KA, Mohamed JA, Dupont HL, Padda RS, Jiang ZD, Flores J, et al. PCR-based assay using occult blood detection cards for detection of diarrheagenic *Escherichia coli* in specimens from US travelers to Mexico with acute diarrhea. J Clin Microbiol 2008;46:2227–30.
[14] Frickmann H, Warnke P, Frey C, Schmidt S, Janke C, Erkens K, et al. Surveillance of food- and smear-transmitted pathogens in european soldiers with diarrhea on deployment in the tropics: experience from the European Union Training Mission (EUTM) Mali. Biomed Res Int 2015;2015, 573904.
[15] Kantele A, Lääveri T, Mero S, Vilkman K, Pakkanen SH, Ollgren J, et al. Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing *Enterobacteriaceae*. Clin Infect Dis 2015;60:837–46.
[16] Lääveri T, Antikainen J, Pakkanen SH, Kirveskari J, Kantele A. Prospective study of pathogens in asymptomatic travellers and those with diarrhoea: aetiological agents revisited. Clin Microbiol Infect 2016;22:535–41.
[17] Adachi JA, Jiang ZD, Mathewson JJ, Verenkar MP, Thompson S, Martinez-Sandoval F, et al. Enteroaggregative *Escherichia coli* as a major etiologic agent in traveler's diarrhea in 3 regions of the world. Clin Infect Dis 2001;32:1706–9.
[18] Jiang ZD, Dupont HL, Brown EL, Nandy RK, Ramamurthy T, Sinha A, et al. Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic *Bacteroides fragilis* and *Arcobacter* species. J Clin Microbiol 2010;48:1417–9.
[19] Ekdahl K, Andersson Y. Regional risks and seasonality in travel-associated campylobacteriosis. BMC Infect Dis 2004;4:54.
[20] Hakanen A, Jousimies-Somer H, Siitonen A, Huovinen P, Kotilainen P. Fluoroquinolone resistance in *Campylobacter jejuni* isolates in travelers returning to Finland: association of ciprofloxacin resistance to travel destination. Emerg Infect Dis 2003;9:267–70.
[21] Steffen R, Mathewson JJ, Ericsson CD, DuPont HL, Helminger A, Balm TK, et al. Travelers' diarrhea in West Africa and Mexico: fecal transport systems and liquid bismuth subsalicylate for self-therapy. J Infect Dis 1988;157:1008–13.
[22] Gascon J, Vila J, Valls ME, Ruiz L, Vidal J, Corachan M, et al. Etiology of traveller's diarrhea in Spanish travellers to developing countries. Eur J Epidemiol 1993;9:217–23.
[23] Bourgeois AL, Gardiner CH, Thornton SA, Batchelor RA, Burr DH, Escamilla J, et al. Etiology of acute diarrhea among United States military personnel deployed to South America and West Africa. Am J Trop Med Hyg 1993;48:243–8.
[24] Jiang ZD, Lowe B, Verenkar MP, Ashley D, Steffen R, Tornieporth N, et al. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). J Infect Dis 2002;185:497–502.
[25] Adachi JA, Ericsson CD, Jiang ZD, DuPont MW, Pallegar SR, DuPont HL. Natural history of enteroaggregative and enterotoxigenic *Escherichia coli* infection among US travelers to Guadalajara, Mexico. J Infect Dis 2002;185:1681–3.
[26] DuPont HL, Petersen A, Zhao J, Mundt A, Jiang ZD, Miller S, et al. Targeting of rifamycin SV to the colon for treatment of travelers' diarrhea: a randomized, double-blind, placebo-controlled phase 3 study. J Travel Med 2014;21:369–76.
[27] Villa NA, Okhuysen PC, Flores-Figueroa J, Jiang ZD, Belkind-Gerson J, Paredes M, et al. *Campylobacter jejuni* is not an important pathogen as a cause of diarrhea in US travelers to Mexico. J Travel Med 2011;18:56–8.
[28] Sanders JW, Isenbarger DW, Walz SE, Pang LW, Scott DA, Tamminga C, et al. An observational clinic-based study of diarrheal illness in deployed United States military personnel in Thailand: presentation and outcome of *Campylobacter* infection. Am J Trop Med Hyg 2002;67:533–8.
[29] Kollaritsch H, Paulke-Korinek M, Wiedermann U. Traveler's diarrhea. Infect Dis Clin North Am 2012;26:691–706.