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Despite antibiotic treatment of travellers' diarrhoea, pathogens are found in stools from half of travellers at return☆

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ABSTRACT

Background: Among visitors to the (sub)tropics, 20–50% contract travellers' diarrhoea (TD) and 5–30% take antibiotics. While shortening the duration of illness, antimicrobials predispose to acquisition of multi-drug resistant bacteria. Therefore, liberal use is no longer advocated. Although antibiotics kill pathogens, no data support the view that they could prevent post-infectious sequelae. We investigated how antibiotic use for TD abroad impacts the pathogen findings at return.

Materials and methods: We revisited 456 travellers' clinical data and stool pathogens examined by qPCR for *Salmonella*, *Yersinia, Campylobacter, Shigella*, *Vibrio cholerae* and enteroaggregative (EAEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) and enteroinvasive (EIEC) *Escherichia coli*.

Results: Among travellers with TD, antibiotic users had pathogen-positive samples less frequently than non-users (50% versus 83%). The difference was significant for EPEC (23% versus 47%) and EAEC (27% versus 54%), but not ETEC (17% versus 26%) or the other pathogens. *Shigella*/EIEC was found more often among antibiotic users than non-users (4% versus 1%).

Conclusion: Despite antibiotic treatment of TD, half of the users still had stool pathogens at return, reflecting either antibiotic resistance of pathogens or recolonisation/reinfection while abroad. Treatment of TD with antibiotics during travel should not be interpreted to indicate eradication of pathogens.

1. Introduction

Of the 80–100 million contracting travellers' diarrhoea (TD) annually, 5–30% take antibiotics [1–11]. Antibiotic use for TD – a disease which generally remains mild or moderate and resolves spontaneously – has recently been debated, since ample studies show that antimicrobials used during travel double the risk of becoming colonised by intestinal multidrug-resistant bacteria, especially extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) [7,8,10–12]. Despite this risk, some guidelines [13] still recommend antibiotics as an alternative even for non-severe cases. Besides shortening the duration of TD, another justification for liberal use has been the presumed potential of antibiotics, by rapidly killing pathogens, to prevent post-infectious sequelae such as irritable bowel syndrome (IBS) [13].

TD is mostly caused by bacterial pathogens (50–80%) [13–16]. Numerous investigations suggest that although antibiotics are taken to kill pathogens, eradication rates do not correlate with cure [17–22]. Indeed, pathogens are still found, although in reduced numbers, in stool samples collected shortly after a course of antibiotics, completed while abroad [17–22]. Many studies looking at samples collected from returning travellers at home omit to specify whether antibiotics have been used during travel or not [14,15]; only few report separately the findings of those having taken antibiotics [23]. To obtain data on the impact of antibiotic use on post-travel pathogen findings, we revisited a

Abbreviations: AB, Antimicrobial/antibiotic; DEC, diarrhoeagenic *Escherichia coli*; EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; qPCR, quantitative PCR; TD, travellers' diarrhoea

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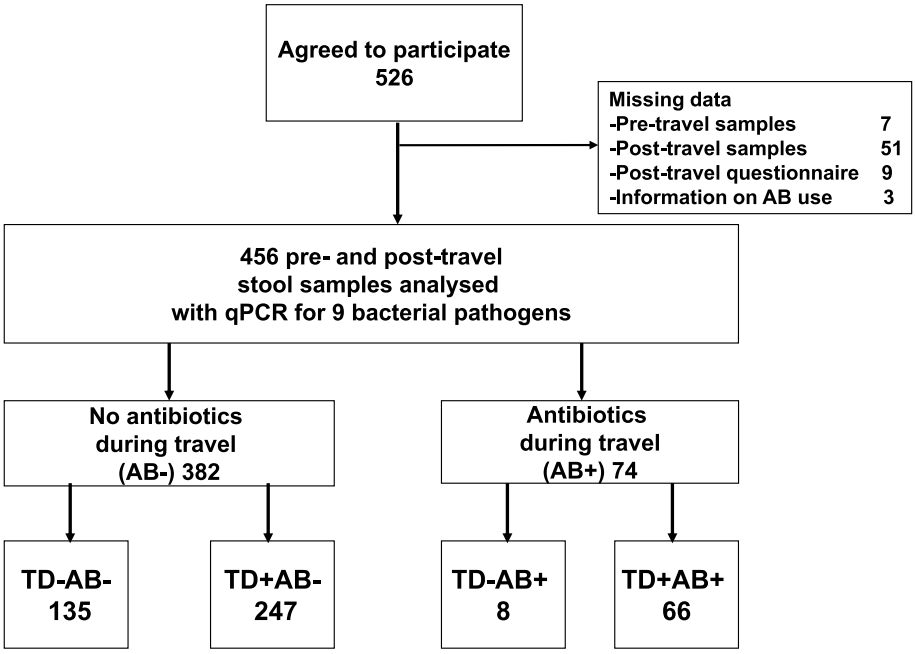

Fig. 1. Study protocol for investigating bacterial pathogen findings among Finnish travellers with respect to occurrence of TD and use of antibiotics.

data set collected on 456 Finnish travellers and compared individual results among those having taken versus not taken antimicrobials.

2. Methods

2.1. Study design and volunteers

The study was designed to assess how antibiotic use during travel impacts the bacterial pathogen findings in stool samples taken at return. We revisited our data on 526 Finnish travellers recruited 2009–2010 [16,24], selecting 456 who all had provided information on their use of antibiotics and provided both pre- and post-travel stool samples (Fig. 1).

The Ethics Committee of the Helsinki University Hospital approved the study protocol. All volunteers had given written informed consent. We have earlier reported stool pathogen findings among travellers visiting various regions of the world [24] and among those without antibiotic use [16,25] as well as the findings of resistant *Enterobacteriacae* [7,26,27], and travel-related health problems [28].

2.2. Definitions and categorisation of travel destinations

TD was defined according to the World Health Organisation (WHO) criteria for diarrhoea as loose/liquid stools three times per day or more frequently than usual for the individual, as previously described [16]. Severe TD was defined as six or more loose/liquid stools per day, TD accompanied by fever or overt blood in stools or TD requiring hospitalisation. Based on the presence or absence of symptoms at the time of the post-travel sampling, those with TD were further categorised into resolved TD and ongoing TD. Travel destinations were grouped into nine regions as modified from the United Nations classification, in line with our previous study [24] (Table 1).

Study participants were divided into four groups by the presence or absence of TD and antibiotic use (AB) during travel: 1) no TD and no antibiotic use (TD–AB–), 2) no TD but antibiotics taken during travel (TD–AB+), 3) TD during travel but no antibiotic use (TD+AB–), and 4) TD and antibiotic use during travel (TD+AB+). Since doxycycline as an antimalarial is taken throughout a journey, and is thus not expected to cause a sudden temporary impact on gut microbiota *while abroad*, we chose to include doxycycline users in the AB– group if no other antibiotics had been taken. The same approach has been used in studies assessing the impact of antibiotic use on risk of acquiring ESBL-PE [7,8].

2.3. Collection and laboratory analysis of stool samples

Collection and handling of the stool samples and the multiplex qPCR method have been described previously [29]. In short, after returning home, the travellers provided a post-travel sample from the first (or second) stools. The faecal samples were collected as swabs in Copan M40 Transystem tubes (Copan Diagnostics, Brescia, Italy) and mailed to the laboratory. Once arrived (within one to three days after sampling), total nucleid acids were extracted using the standard semi-automated protocol of easyMAG (bioMérieux, Marcy-l'Etoile, France). The analyses were carried out with a multiplex qPCR method that covers the following pathogens: diarrhoeagenic *E. coli* including enteroaggregative (EAEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) and enteroinvasive (EIEC) *E. coli* or *Shigella* as well as *Salmonella*, *Yersinia*, *Vibrio cholerae* and *Campylobacter coli/jejuni*.

2.4. Statistical analysis

Statistical analyses were carried out with SPSS 22 (IBM Corp, Armonk, NY).

The chi-square test or Fisher's exact test were used to compare categorical variables when applicable. Binomial regression model was used to obtain profile likelihood confidence intervals for the proportions of the various pathogens in geographical regions or, in cases where computation did not converge, asymptotic Wald confidence intervals were used. The possible differences in the effect of antibiotics on pathogen findings (those associated with TD in our previous study: any pathogen, EPEC, EAEC, ETEC or *Campylobacter* [15]) was compared in the five most popular regions with binomial logistic regression analysis (interaction between antibiotic and region). Statistical significance was determined as either 95% confidence intervals not overlapping, or ranging only either above or below 1.

Table 1
Characteristics of 456 Finnish travellers with or without TD (TD+ and TD–) and using or not using antibiotics (AB+ and AB–).[a]

	AB+/–TD+/–				Total
	AB–TD–N (%)	AB–TD+N (%)	AB+TD–N (%)	AB+TD+ N (%)	N (%)
Ongoing TD symptoms	0	115 (47)	0	28 (42)	143 (46)
Age (years) median	46 (IQR 30–60)	33 (IQR 27–50)	27.5 (IQR 23–60.25)	32.5 (IQR 28–56.75)	36 (IQR 27–54.25)
Male	54 (40)	95 (38)	4 (50)	21 (32)	174 (38)
Female	81 (60)	152 (62)	4 (50)	45 (68)	282 (62)
Duration of travel (days), median	15 (IQR 10–18)	17 (IQR 14–28.25)	34 (IQR 12.75–75)	26 (IQR 16–39.5)	16 (IQR 13–27)
South Asia	12 (9)	40 (16)	2 (25)	15 (23)	69 (15)
Southeast Asia	23 (17)	68 (28)	1 (13)	15 (23)	107 (23)
South and Central America and the Caribbean	16 (12)	20 (8)	0 (0)	4 (6)	40 (9)
Europe, Australia, North America	13 (10)	3 (1)	1 (13)	0 (0)	17 (4)
East Asia	3 (2)	2 (1)	0 (0)	2 (3)	7 (2)
Northern Africa and Middle East	7 (5)	4 (2)	0 (0)	1 (2)	12 (3)
Southern Africa	11 (8)	10 (4)	1 (13)	2 (3)	24 (5)
Western and Middle Africa	25 (19)	43 (17)	3 (38)	13 (20)	84 (18)
Eastern Africa	25 (19)	57 (23)	0 (0)	14 (21)	96 (21)
Total (% of all travellers)	135 (30)	247 (54)	8 (2)	66 (15)	456 (100)

[a] Doxycycline as continuing antimalarial included in AB– if no other antibiotic was used.

3. Results

3.1. Traveller characteristics and use of antibiotics

The background data for the 456 travellers are provided in Table 1. In total, 74 travellers (16%) reported antibiotic use during journey; 89% (66/74) of them had taken antibiotics for TD. Of these regimens, 50 (76%) were fluoroquinolones and eleven (17%) macrolides. The probability of taking antibiotics for TD was found similar across the various geographical regions ($p = 0.737$).

Of travellers with TD, 21% (66/313) took antibiotics, whereas among the asymptomatic, antibiotic use was reported by 6% (8/143). Symptoms were still ongoing at return among 47% (115/247) of those in group TD+AB- and 42% (28/66) in group TD+AB+ ($p = 0.549$).

3.2. Bacterial pathogen findings among travellers having used antibiotics

Bacterial pathogens were found in a total of 49% of the post-travel stool samples from antibiotic users (Table 2); 19% (n = 14) had two or more types of pathogens (14/36; 39% of those with a pathogen finding). EAEC (20; 27%), EPEC (17; 23%) and ETEC (11; 15%) were the most frequent findings, followed by *Campylobacter* (3; 4%), *Shigella*/EIEC (3; 4%), EHEC (2; 3%) and *Salmonella* (2; 3%). No *Vibrio cholerae* or *Yersinia* were detected.

In the whole group of 456 travellers, antibiotic use was associated with fewer findings of any pathogen, multiple pathogens, EPEC or EAEC, but more frequent cases of *Shigella*/EIEC. Doxycycline as an antimalarial had no impact on pathogen findings.

3.3. Comparisons of findings among travellers with TD and with and without use of antibiotics

Among those with TD, the proportion of pathogen-positive stool samples was found lower among antibiotic users than non-users (33/66; 50% versus 205/247; 83%) (Table 2.). The difference was statistically significant also for EPEC (15/66; 23% versus 117/247; 47%) and EAEC (18/66; 27% versus 133/247; 54%), but not ETEC (11/66; 17% versus 64/247; 26%), *Campylobacter* (3/66; 5% versus 25/247; 10%) or other pathogens.

Of the 50 travellers reporting use of fluoroquinolones, *Campylobacter* was detected in the stools from three (6%); the proportion was practically the same (7%) among those having not taken antibiotics. Among those having used macrolides (n = 11), no *Campylobacter* was found.

3.4. Differences in impact of antibiotics on pathogen findings across geographical regions

There was no significant interaction between antibiotic use and region with regard to any of the pathogens (Table 3.).

4. Discussion

Despite antimicrobial medications earlier being the mainstay of TD treatment, few are the studies that report post-travel findings related to taking them. Our results show antibiotic use to be associated with a lower proportion of pathogen-positive stool samples – AB users 50% versus non-users 83% – yet even among the users bacterial pathogens were seen in half of the samples after return.

4.1. High rates of pathogens after antibiotic use

Pathogen findings despite antibiotic treatment may reflect not only acquisition during continuing exposure afterwards but also the pathogens not being affected by the drugs taken, due to either initial resistance or one developed over the course. In fact, new resistant pathogens can even be favoured during antibiotic use because of selection pressure and decreasing colonisation resistance. Indeed, we have recently showed that fluoroquinolone users are prone to contract ESBL-PE strains resistant to fluoroquinolone antibiotics [27].

4.2. Antibiotic use reduced findings with EAEC and EPEC but not with ETEC

As expected, among those with TD, the proportion of pathogen-positive stool samples proved lower for antibiotic users than non-users (50% vs. 83%). This finding contrasts the results of Gascon et al. [23], who explored returning travellers with TD: prior antibiotic use did not influence the proportion of pathogens in this group. The study does not report findings with individual pathogens [23]. In our study, antibiotic use reduced the findings with EAEC (23% vs. 47%) and EPEC (27% vs. 54%), but not other individual pathogens (Table 2.). The results accord with those reported by Schultz et al. [1] who found EAEC and diffusely adherent *E. coli* but not ETEC in reduced proportions, if returning travellers had taken antibiotics while abroad. The resistance rates for ETEC, and EAEC have increased over the years [30,31]; research into the resistance for EPEC is scarce. We believe that the smaller impact of antibiotic use on ETEC rates rather reflects this pathogens' relatively fast disappearance from the stools. In our previous report on travellers not having used antimicrobials [16] ETEC was identified at lower rates among travellers with resolved (22/132; 17%) than ongoing TD

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Table 2
Pathogen findings in the stool samples of 456 Finnish travellers with or without TD (TD+ and TD–), with or without antibiotic use (AB+ and AB–) (users of doxycycline as continuous antimalarial prophylaxis were included in the AB– group if no other antibiotic was used), and with and without use of doxycycline as an antimalarial.[a]

	Total	Any bacterial pathogen	Two or more pathogens	DEC	EPEC	EAEC	ETEC	EHEC	Non-DEC	Campylobacter	Salmonella	Shigella/EIEC
	n (% of all travellers)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total	456	323 (71)	152 (40)	311 (68)	191 (42)	191 (42)	87 (19)	36 (8)	43 (9)	29 (6)	11 (2)	6 (1)
No antibiotics during travel (AB–)												
AB – all	382 (84)	287 (75)	152 (40)	277 (73)	174 (46)	171 (45)	76 (20)	34 (9)	36 (9)	26 (7)	9 (2)	3 (1)
AB– TD–	135 (30)	82 (61)	34 (25)	81 (60)	57 (42)	38 (28)	12 (9)	10 (7)	4 (3)	1 (1)	3 (2)	0 (0)
AB– TD+	247 (54)	205 (83)	118 (48)	196 (79)	117 (47)	133 (54)	64 (26)	24 (10)	32 (13)	25 (10)	6 (2)	3 (1)
Antibiotics during travel (AB+)												
AB + all	74 (16)	36 (49)	14 (19)	34 (46)	17 (23)	20 (27)	11 (15)	2 (3)	7 (9)	3 (4)	2 (3)	3 (4)
AB + TD–	8 (2)	3 (38)	1 (13)	3 (38)	2 (25)	2 (25)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	1 (13)
AB+TD+ all (ongoing + resolved)	66 (15)	33 (50)	13 (20)	31 (47)	15 (23)	18 (27)	11 (17)	2 (3)	6 (9)	3 (5)	2 (3)	2 (3)
AB+TD resolved	38 (51)	20 (53)	8 (21)	18 (47)	10 (26)	10 (26)	8 (21)	1 (3)	3 (8)	2 (5)	1 (3)	0
AB+TD ongoing	28 (38)	13 (46)	5 (18)	13 (46)	5 (18)	8 (29)	3 (11)	1 (4)	3 (11)	1 (4)	1 (4)	2 (7)
Doxycycline as an antimalarial (information missing = 62)												
No	345 (88)	257 (74)	133 (39)	250 (72)	157 (46)	148 (43)	68 (20)	31 (9)	32 (9)	23 (7)	8 (2)	3 (1)
Yes	49 (12)	36 (74)	20 (41)	32 (65)	19 (39)	26 (53)	9 (18)	3 (6)	6 (12)	3 (6)	2 (4)	1 (2)
Univariate statistics												
AB+ all versus AB– all	p-value	< 0.001	0.001	< 0.001	< 0.001	0.005	0.313	0.070	0.567	0.277	0.556	0.024
	OR (95% CI)	0.3 (0.2–0.5)	0.4 (0.2–0.7)	0.3 (0.2–0.5)	0.4 (0.2–0.6)	0.5 (0.3–0.8)	0.7 (0.4–1.4)	0.3 (0.1–1.2)	1.0 (0.4–2.4)	0.6 (0.2–2.0)	1.2 (0.2–5.4)	5.3 (1.1–27.0)
AB+TD+ (resolved + ongoing) versus AB–TD+	p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.118	0.080	0.393	0.159	0.783	0.285
	OR (95% CI)	0.2 (0.1–0.4)	0.3 (0.1–0.5)	0.2 (0.1–0.4)	0.3 (0.2–0.6)	0.3 (0.2–0.6)	0.6 (0.4–1.4)	0.3 (0.1–1.3)	0.7 (0.3–1.6)	0.4 (0.1–1.9)	1.3 (0.3–6.4)	2.5 (0.4–15.5)
Doxycycline vs. no doxycycline (as antimalarial)	p-value	0.878	0.761	0.299	0.375	0.180	0.824	0.785	0.448	1.000	0.359	0.413
	OR (95% CI)	0.9 (0.5–1.9)	1.1 (0.6–2.0)	0.7 (0.4–1.3)	0.8 (0.4–1.4)	1.5 (0.8–2.7)	0.9 (0.4–2.0)	0.7 (0.2–2.2)	1.4 (0.5–3.5)	0.9 (0.3–3.2)	1.8 (0.4–8.7)	2.4 (0.2–23.3)

[a] DEC = Diarrhoeagenic *Escherichia coli*; EPEC = Enteropathogenic *E. coli*; EAEC = Enteroaggregative *E. coli*; ETEC = Enterotoxigenic *E. coli*; EHEC = Enterohaemorrhagic *E. coli;* EIEC = Enteroinvasive *E. coli*. No *Vibrio cholerae* or *Yersinia* were detected.

Table 3
Findings with any pathogen, EPEC, EAEC, ETEC and *Campylobacter* in relation to the use of antibiotics (AB) in five geographical subregions: South Asia, Southeast Asia, West and Central Africa, East Africa, and South and Central America, and the Caribbean.

	All	Any pathogen		EPEC		EAEC		ETEC		*Campylobacter*	
	n (%)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	N	% (95% CI)
South Asia											
Total	69 (15)	54	78 (69–88)*	37	54 (42–66)	39	57 (45–69)	15	22 (12–32)	12	17 (8–27)
AB−	52 (75)	45	87 (77–96)*	29	56 (42–69)	32	62 (48–74)	12	23 (13–36)	10	19 (10–31)
AB+	17 (25)	9	53 (29–77)*	8	47 (25–70)	7	41 (20–65)	3	18 (5–40)	2	12 (2–32)
Southeast Asia											
Total	108 (26)	74	69 (60–77)*	44	41 (31–50)	34	31 (23–41)	20	19 (11–26)	13	12 (6–18)
AB−	91 (84)	65	71 (62–80)	42	46 (36–56)	30	33 (24–43)	18	20 (13–29)	11	12 (6–20)
AB+	16 (16)	8	50 (27–73)	2	13 (2–34)	3	19 (5–42)	1	6 (0–25)	1	6 (0–25)
West and Central Africa											
Total	85 (19)	60	71 (61–80)*	36	42 (32–53)	39	46 (35–57)	14	16 (8–25)	1	1 (0–3)*
AB−	68 (80)	51	75 (64–84)	31	46 (34–57)	34	50 (38–62)	11	16 (9–26)	0	0
AB+	16 (20)	8	50 (27–73)	4	25 (9–49)	5	31 (13–56)	3	19 (5–42)	0	0
East Africa											
Total	96 (21)	80	83 (76–91)*	45	47 (37–57)	50	52 (42–62)	32	33 (24–43)	5	5 (1–10)
AB−	82 (85)	71	87 (79–94)*	44	54 (43–64)	45	55 (44–65)	28	34 (25–45)	5	6 (2–13)
AB+	14 (15)	9	64 (39–89)*	1	7 (0–28)	5	36 (15–62)	4	29 (10–54)	0	0
South and Central America and Caribbean											
Total	40	31	78 (65–90)*	17	43 (26–59)	15	38 (22–53)	4	10 (0–20)	0	0
AB−	36 (90)	30	83 (69–93)	20	56 (39–72)*	15	42 (27–58)	4	11 (4–24)	0	0
AB+	4 (10)	1	25 (0–67)*	3	75 (33–100)*	0	0	0	0	0	0
Statistical analysis on difference between regions (interaction AB*region)											
P-value for difference in effect of AB		0.664		0.290		1.000		0.854		1.000	

95% confidence intervals are profile likelihood confidence intervals except when computation did not converge and confidence intervals were calculated using asymptotic Wald formula (marked with *).

symptoms (42/115; 37%; OR:2.9:95% CI:1.6–5.2); the same did not appear to apply to EAEC.

Interestingly, the impact of antibiotic treatment on pathogen detection rates did not differ between the various geographical regions (Table 3), suggesting that differences in resistance do not account for the findings after antibiotic use, at least not entirely.

4.3. *Shigella*/EIEC more common after antibiotic use

It is noteworthy that during travel *Shigella*/EIEC was seen more frequently among antibiotic users than non-users. Even though the proportion of travellers carrying *Shigella*/EIEC remained small, this finding could reflect antibiotics disrupting colonisation resistance, thus facilitating colonisation and infection with pathogenic bacteria. Indeed, antimicrobial treatment has been found to be associated with increased risk of contracting *Salmonella* [32,33] and any bacteria resistant to the antibiotic administered [27]; we are not aware of any similar reports concerning *Shigella*/EIEC.

4.4. *Campylobacter* not found after macrolide use

The finding that *Campylobacter* was detected among travellers having used fluoroquinolones but not among those who had taken macrolides accords with the commonness of fluoroquinolone resistance among *Campylobacter* strains particularly in South and Southeast Asia [34], where the majority of our cases with *Campylobacter* had visited [24]; strains from these geographical regions are still mostly sensitive to macrolides [34]. In line with our results, prior treatment with FQ but not macrolide antibiotics has been shown to be connected with *Campylobacter* findings [35]. However, *Campylobacter* eradication rates have not been associated with clinical cure [18,21]. Interestingly, among our travellers, the clinical picture of TD was similar for macrolide (4/9; 44% severe TD) and fluoroquinolone (21/48; 44% severe TD) users.

4.5. Doxycycline as antimalarial has no impact on pathogen findings

When taken as an antimalarial during the journey, no effect was seen of doxycycline on pathogen findings. This was expected, since doxycycline is no longer considered a valid drug for intestinal pathogens [13,30] nor in prevention of TD [13,36]. Moreover, in one study [36], doxycycline as an antimalarial did not influence the pathogen findings among travellers with TD; those remaining asymptomatic were not included in the analyses.

4.6. Considerations of antibiotic use for TD

Liberal use of antibiotics for TD has been justified by the disruption to travel plans caused by TD [13]. In addition, theoretic preventive effect on post-TD sequelae such as IBS has been suggested [13]. However, the relationship between common TD pathogens and IBS is not clear: in the study by Nair et al. only LT-ETEC and *Providencia spp.* were associated with post-travel functional abdominal symptoms; none of the pathogens analysed related to IBS [37]; they did not report possible antibiotic use. Moreover, in two large prospective traveller studies [5,9], and in one randomised trial [38], antimicrobials had no effect on the risk of post-travel IBS. Contrariwise, some investigations have shown that taking them for TD or infectious diarrhoea rather increases the risk of IBS [39,40]. Pathogens found in stools after antibiotic treatment, as seen in our research, may partly explain why antibiotics used to treat TD have not reduced the risk of IBS.

The proportion of travellers with symptoms still ongoing at return was similar between TD + AB– (47%) and TD + AB + (42%) groups. The lack of difference may be explained by 32% of the volunteers (data not shown) having taken, instead of antibiotics, loperamide, a drug effective for TD [41] which does not predispose to acquisition of multidrug-resistant bacteria [26]. Actually, in our recent review, we found no studies comparing the efficacy of loperamide to current antibiotics taken singly for TD, but it is evident that both are efficacious in treating the disease [41].

4.7. Implications for further research

When assessing the role of various aetiological agents in TD, a number of investigations have overlooked the possible use of antibiotics

earlier during the journey [14,15], while some others have excluded from their analyses participants who had taken antibiotics three to seven days prior to stool sampling [17,19,21,22,42]. This interval appears too short, since intestinal microbiota have been shown to be affected by antimicrobials for up to twelve months [43]. If stools are analysed shortly after antibiotic treatment from samples collected while abroad [17–22,44], the results fail to represent the 'final' pathogen findings on travellers' return to better hygienic conditions at home. Our data show that the specimens of those having used antimicrobials should be examined separately, but not omitted from studies analysing the prevalence of various pathogens.

4.8. Limitations

The principal limitation of our study was not having available the exact start or end dates of antibiotic treatment, which made it impossible to assess the duration of the effect of antibiotics on pathogen findings and the time the travellers were exposed to new pathogens during/after the course; we had only recorded the presence/absence of symptoms at return. Changes in the intestinal microbiota have, however, been described to persist for even a year [43]. Molecular diagnostic methods have been criticized for oversensitivity in detecting pathogens [45,46], yet also studies applying culture-based methods have detected bacterial pathogens in up to 33% of stools after use of antibiotics [19,21,22]. Moreover, it is unlikely that free nucleic acids could resist active DNAses in the intestinal environment and qPCR methods would thus measure non-viable bacteria [47,48]. Since diarrhoeagenic *E. coli* were not cultured, we have no data on the susceptibility profiles.

5. Conclusion

Bacterial pathogens were found in stools from half of travellers using antibiotics for diarrhoea. It appears that either the pathogens have initially been resistant to the drug taken or the individuals have contracted other, potentially resistant bacteria during or soon after the intake abroad, while still under exposure. Treatment of TD with antibiotics while abroad should not be interpreted to indicate pathogen eradication.

Authors' contributions

Study concept and design JK, AK; acquisition of data TL, KV, SHP, JK, AK; analysis and interpretation of results TL, KV, SHP, JK, AK; drafting of manuscript TL, AK; statistical analysis TL; final approval of version published TL, KV, SHP, JK, AK.

Potential conflicts of interest

TL, KV, SHP, and JK declare no conflicts of interest. AK has received honorary for lectures (Pfizer, MSD, Valneva, Immuron) and an investigator-initiated grant (Pfizer), and has on two occasions consulted an advisory board (Valneva), none of these relevant to the current manuscript.

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