þÿDoxycycline as an antimalarial : Impact on trave
þÿand doxycycline resistance among various stool
Prospective study and literature review

Kantele, Anu

2022-09-01

Kantele , A , Mero , S & Lääveri , T 2022 , ' Doxycycline as an antimalarial : Impact on
þÿtravellers diarrhoea and doxycycline resistance among various stool b
study and literature review ' , Travel medicine and infectious disease , vol. 49 , 102403 . https://doi.org/10.1016/j.tma

http://hdl.handle.net/10138/347680
https://doi.org/10.1016/j.tmaid.2022.102403

Travel Medicine and Infectious Disease 49 (2022) 102403

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Doxycycline as an antimalarial: Impact on travellers' diarrhoea and doxycycline resistance among various stool bacteria – Prospective study and literature review

Anu Kantele [a,b,c,*], Sointu Mero [a,b,c], Tinja Lääveri [a,b,d]

[a] Meilahti Vaccine Research Center MeVac, Department of Infectious Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[b] Multidiciplinary Center of Excellence in Antimicrobial Resistance Research, University of Helsinki, Helsinki, Finland
[c] Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland
[d] Department of Computer Science, Aalto University, Espoo, Finland

ARTICLE INFO

Keywords:
ESBL
Extended-spectrum beta-lactamase
Travellers' diarrhoea
Antimicrobial resistance
AMR
Doxycycline

ABSTRACT

Background: Antibiotics predispose travellers to acquire multidrug-resistant bacteria, such as extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE). Although widely used in antimalarial prophylaxis, doxycycline has scarcely been studied in this respect.

Methods: We explored the impact of doxycycline on rates of traveller's diarrhoea (TD), ESBL-PE acquisition and, particularly, doxycycline co-resistance among travel-acquired ESBL-PE in a sample of 412 visitors to low- and middle-income countries.

We reviewed the literature on traveller studies of doxycycline/tetracycline resistance among stool pathogens and the impact of doxycycline on TD rates, ESBL-PE acquisition, and doxycycline/tetracycline resistance.

Results: The TD rates were similar for doxycycline users (32/46; 69.6%) and non-users (256/366; 69.9%). Of the 90 travel-acquired ESBL-PE isolates, 84.4% were co-resistant to doxycycline: 100% (11/11) among users and 82.3% (65/79) among non-users.

The literature on doxycycline's effect on TD was not conclusive nor did it support a recent decline in doxycycline resistance. Although doxycycline did not increase ESBL-PE acquisition, doxycycline-resistance among stool pathogens proved more frequent for users than non-users.

Conclusions: Our prospective data and the literature review together suggest the following: 1) doxycycline does not prevent TD; 2) doxycycline use favours acquisition of doxy/tetracycline-co-resistant intestinal bacteria; 3) although doxycycline does not predispose to travel-related ESBL-PE acquisition per se, it selects ESBL-PE strains co-resistant to doxycycline; 4) doxycycline resistance rates are high among stool bacteria in general with no evidence of any tendency to decrease.

1. Introduction

The need for a less liberal use of antibiotics when visiting (sub) tropical low- and middle-income countries (LMICs) has become well recognised. Research amply supports the general recommendations highlighting prudent antibiotic use, particularly studies demonstrating that antibiotics predispose travellers to multidrug-resistant (MDR) bacteria [1–5]: up to 80% of visitors to LMICs acquire extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales (ESBL-PE) [1–4]. The use of antibiotics is expected to give a selective advantage to bacteria resistant to the drug taken, as has been shown among travellers using fluoroquinolones [3,6,7]: in our recent study [7], 96% of the ESBL-PE acquired by fluoroquinolone-users were found fluoroquinolone co-resistant, whereas the rate for non-users was 36%. While the risk of acquiring MDR bacteria has proved to be particularly high when using fluoroquinolones [5] and beta-lactams [2], scant attention has been paid to doxycycline which also ranks among the three most widely used

* Corresponding author. Meilahti Infectious Diseases and Vaccine Research Center, MeiVac, University of Helsinki and Helsinki University Hospital, Biomedicum 1, FI-00029, HUS, Finland.
E-mail address: anu.kantele@hus.fi (A. Kantele).

https://doi.org/10.1016/j.tmaid.2022.102403
Received 26 March 2022; Received in revised form 18 June 2022; Accepted 17 July 2022
Available online 21 July 2022

Abbreviations

AB – antibiotic
CLSI – Clinical and Laboratory Standards Institute
DEC – diarrhoeagenic *Escherichia coli*
EAEC – enteroaggregative *Escherichia coli*
ESBL – extended-spectrum beta-lactamase
ESBL-PE – extended-spectrum beta-lactamase-producing Enterobacterales
ETEC – enterotoxigenic *Escherichia coli*
EUCAST – European Committee on Antimicrobial Susceptibility Testing
DOX – doxycycline
LMIC – low- and middle-income country
MDR – multidrug-resistant
MDRE – multidrug-resistant Enterobacterales
RCT – randomised controlled trial
RR – relative risk
TD – travellers' diarrhoea

antimalarial prophylactic agents.

In addition to antimalarial chemoprophylaxis [8], doxycycline was also commonly prescribed for treatment and prophylaxis of TD in the 1960s and '70s [9]. However, due to its large-scale use among locals in LMICs in the 1980s and subsequent increase in resistance, doxycycline became gradually replaced by co-trimoxazole as a therapy for TD and later by fluoroquinolones, rifaximin, and azithromycin [10,11]. While previous studies demonstrated that doxycycline had lost its efficacy in preventing TD [12–14], a recent non-randomised study suggested that when used as an antimalarial agent doxycycline also prevents TD; the authors speculated that general resistance rates may have decreased [9].

As the impact of doxycycline taken during travel remains unclear in the literature, we sought to compile key data available on doxycycline employing two approaches: 1) analysis of doxycycline co-resistance among our ESBL-PE isolates in our previous prospective traveller study in relation to doxycycline use, and scrutiny of the occurrence of TD among doxycycline-users and non-users, and 2) review of relevant literature with respect to the impact of doxycycline use on TD and resistance rates among various intestinal bacteria followed by scrutiny of the trends in the levels of doxycycline/tetracycline resistance among stool pathogens.

2. Materials and methods

This research comprised two parts (Fig. 1): the first one a prospective study and the latter a literature review, both with a focus on the impact of doxycycline use on TD rates and resistance to doxycycline/tetracycline. The review also compiled studies on the general levels of doxycycline/tetracycline resistance among stool bacteria.

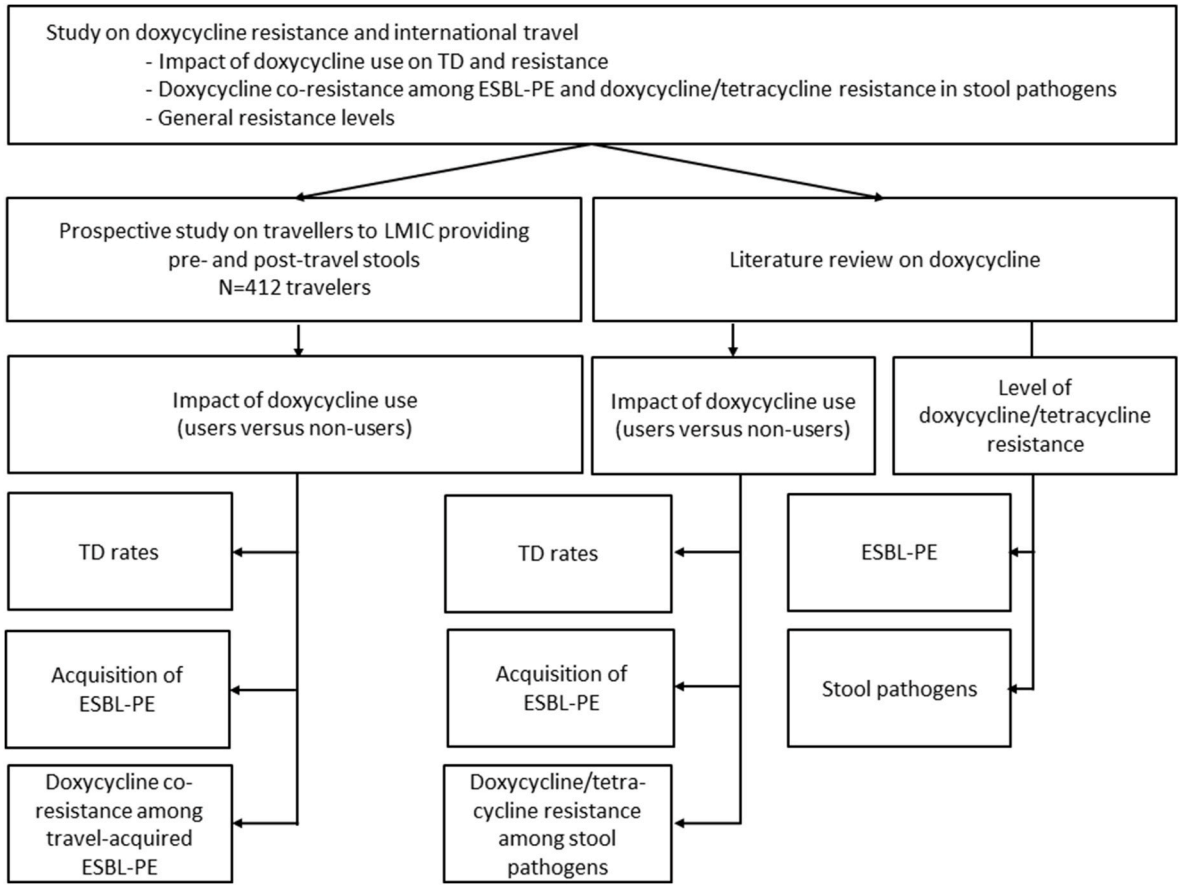

Fig. 1. Flowchart of study conduct.

2.1. Part 1: Prospective study

To explore the impact of doxycycline on TD rates and co-resistance among travel-acquired ESBL-PE, we revisited the data from our previous study exploring ESBL-PE acquisition among Finnish travellers in 2009–10 [1]. In this study, all volunteers had provided stool samples and questionnaires before and after their trip. Symptoms of TD and the use of medications, such as an antimalarial prophylaxis and antibiotics were included in the post-travel questionnaires. The countries visited were grouped as described earlier [1] (Table 1). The handling of stool specimens and the identification of ESBL-PE are detailed in our previous report [1]. In brief, ESBL-producing strains were identified by an automated VITEK GN system (bioMérieux, Marcy l'Etoile, France) and the ESBL phenotype by double disc synergy tests (Oxoid, Thermo Fisher Scientific, Cambridge, UK).

The doxycycline susceptibility test was performed according to the criteria of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org) using Muller Hinton agar (Oxoid, Thermo Fisher Scientific, Cambridge, UK) and Etests (bioMérieux, Marcy l'Etoile, France). As a breakpoint MIC for doxycycline resistance we used a value of >4. EUCAST does not define doxycycline's MIC for Enterobacterales but gives a MIC of >4 for tetracycline resistance among *Yersinia enterocolitica* [15]. The same value is given as a limit for susceptibility by CLSI [16].

TD was defined by the WHO criteria: passing three or more loose/liquid stools per 24 h, or more frequently than normal for the individual [17,18].

An ethics clearance was received from the Helsinki University Hospital ethics committee. All volunteers provided written informed consent.

For the present study, we selected 412 travellers who had visited LMICs and analysed the impact of doxycycline use on TD by comparing TD rates between doxycycline users and non-users.

As for the impact of doxycycline use on the acquisition of resistant strains, we analysed all 98 travel-acquired ESBL-PE strains contracted by 90 participants for doxycycline co-resistance and, for those with more than one ESBL-PE isolate differing by their susceptibility to doxycycline, we selected the more resistant isolate. Then, we compared the ESBL-PE isolates of doxycycline users versus non-users with respect to each isolate's co-resistance to doxycycline, ciprofloxacin, tobramycin cotrimoxazole, and nitrofurantoin; susceptibility for antibiotics other than doxycycline was retrieved from our previous study [7]. In addition to analysing co-resistance to doxycycline, we analysed factors associating with doxycycline co-resistance among ESBL-PE.

2.2. Part 2: Search for articles

We searched PubMed for "travel" and "doxycycline" or "tetracycline" or "ESBL", plus selected articles in our own collections. Articles were categorised into those assessing the impact of doxycycline use (either as TD prophylaxis or antimalarial) on (1) TD rates; (2) doxycycline/tetracycline (co-)resistance rates among various stool bacteria; and (3) ESBL-PE colonisation rates. Moreover, we searched for reports on (4) doxycycline/tetracycline resistance rates among various stool bacteria and grouped the findings by three common TD pathogens, ETEC, EAEC, and *Campylobacter*.

2.3. Statistical analyses

Pearson's chi-square test, Fisher's exact test or a binary logistic regression analysis were used to compare categorical variables, when applicable. Statistical significance was defined as $p < 0.05$. The statistical analyses were carried out using the SPSS 22 software (IBM Corp, Armonk, NY).

3. Results

3.1. Part 1: Prospective study

3.1.1. Demographics

Of the 412 participants, 251 (60.9%) were female; their median age was 36.5 years. The destinations in the LMICs included South Asia (61; 14.8%), South-East Asia (101; 24.5%), Sub- Saharan Africa (193; 46.8%), and South and Central America and the Caribbean (40; 9.7%). A

Table 1
Analysis of co-resistance to doxycycline among travel-acquired ESBL-PE with respect to various factors.

	Total n (%)	Doxycycline susceptible n (%)	Doxycycline MIC >4 n (%)	p-value	OR (95% CI)
Travellers with ESBL-PE	90	15 (16.7)	75 (83.3)		
Antimalarial prophylaxis					
Doxycycline	11 (12.2)	0 (0)	11 (100.0)	0.267	0.2 (0.02–3.1)
Mefloquine	14 (15.6)	1 (7.1)	13 (92.9)	0.859	0.2 (0.03–2.5)
Atovaquone-proguanil	22 (24.4)	5 (22.7)	17 (77.3)	0.246	0.8 (0.04–13.9)
Hydroxychloroquine	8 (8.9)	2 (25.0)	6 (75.0)	0.381	0.4 (0.04–3.4)
No antimalarial prophylaxis	35 (38.9)	6 (17.1)	29 (82.9)	Ref.	Ref.
Use of other antibiotics (AB) +/−					
AB – DOX +	7 (7.8)	0 (0.0)	7 (100.0)	0.999	N/A
AB + DOX +	4 (4.4)	0 (0.0)	4 (100.0)	0.999	N/A
AB + DOX –	24 (26.7)	1 (4.2)	23 (95.8)	0.131	5.1 (0.6–42.2)
AB – DOX –	55 (61.1)	10 (18.2)	45 (81.8)	Ref.	Ref.
Fluoroquinolone	20 (22.2)	1 (5.0)	19 (95.0)	0.175	versus no FQ 4.8 (0.6–38.6)
Travellers' diarrhoea					
Yes	75 (83.3)	13 (17.3)	62 (82.7)	1.000	1.1 (0.2–5.8)
No	15 (16.7)	2 (13.3)	13 (86.7)	Ref.	Ref.
Travel destination					
South Asia	28 (31.1)	10 (35.7)	18 (64.3)	Ref.	Ref.
South East Asia	33 (36.7)	3 (9.1)	30 (90.9)	0.018	5.6 (1.2–22.9)
Sub-Saharan Africa	23 (25.6)	0 (0)	23 (100.0)	0.998	N/A
East Asia	2 (2.2)	0 (0)	2 (100)	0.999	N/A
North Africa and Middle East	4 (4.4)	0 (0)	4 (100)	0.585	0.6 (0.07–4.5)
Age (median; IQR)	36.5/28–58	28/25–31	42/28–59	0.021	1.1 (1.0–1.1)
Sex					
Female	49 (54.4)	5 (10.2)	44 (89.8)	0.523	1.5 (0.4–5.4)
Male	41 (45.6)	6 (14.6)	35 (85.5)	Ref.	

CI – confidence interval; DOX – doxycycline; FQ – fluoroquinolone; N/A – not applicable; OR – odds ratio.

Table 2
Co-resistance to various antimicrobials among travel-acquired extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) isolates as evaluated with respect to doxycycline use.

	Total n (%)	Doxycycline users (+) n (%)	Doxycycline non-users (−) n (%)	p-value
Travellers with ESBL-PE	90	11 (12.2)	79 (87.8)	
Doxycycline co-resistance (MIC >4)	76 (84.4)	11 (100)	65 (82.3)	0.129
Ciprofloxacin co-resistance	48 (53.3)	8 (72.7)	40 (50.6)	0.169
Tobramycin co-resistance	47 (52.2)	6 (65.5)	41 (51.9)	0.869
Nitrofurantoin co-resistance	2 (2.2)	0 (0)	2 (2.5)	0.594
Co-trimoxazole co-resistance	66 (73.3)	9 (81.8)	57 (72.2)	0.497

Table 3
Studies describing impact of doxycycline use on TD rates in our literature review.

First author, publication year	Year(s) of sample collection	Study setting	Impact of doxycycline use on TD/diarrhoea: proportion (%) of participants with diarrhoea
Lago, 2020 [9]	2010–18	3227 US military travellers; 20% with DOX as malaria prophylaxis	DOX: 10% Non-users: 19% (RR 0.45 (0.35–0.58), p < 0.01)
Terrell, 2015 [28]	2012–13	US military in Kenya, non-randomized, 867 mefloquine users, 685 DOX users	DOX: 16% Mefloquine: 7% (p-value not reported)
Kantele, 2015 [1]	2009–10	430 Finnish travellers; 19% with DOX as malaria prophylaxis	DOX: 70% Mefloquine: 76% Atovaquone/proguanil: 70% (p = 0.58)
Lee, 2013 [27]	2009	590 Australian military on overseas deployment; 30% with DOX	DOX did not prevent gastroenteritis in multivariable analysis; new-onset IBS was associated with doxycycline use
Haus-Cheymol, 2012 [26]	2010	14 French young voluntary workers (none with DOX as antimalarial) and 72 policemen (91% with doxycycline) during the cholera outbreak in Haiti	DOX: 14.9% No DOX: 71.4% (RR: 0.2; 95% CI: 0.1–0.4).
Arthur, 1990 [12]	1988	253 US military in Thailand; RCT DOX versus mefloquine	DOX: 49% Mefloquine: 48% (p-value not reported)
Sack, 1984 [25]	1980	44 US military in Honduras; RCT DOX versus placebo	DOX: 32% Placebo: 100% (p < 0.001)
Echeverria, 1984 [24]	1980	63 US military in Thailand; RCT DOX versus placebo	DOX: 10% Placebo: 24% (p = 0.12)
Freeman,L.D. 1983 [23]	1981	145 US travellers in Mexico; RCT DOX versus placebo	DOX: 4% Placebo: 21% (p = 0.002)
Santosham,M. 1981 [22]	1978	46 US military in Honduras; RCT DOX versus placebo	DOX: 33% Placebo: 45% (p = non-significant)
Sack 1979 [21]	1977	51 US military in Morocco; RCT DOX versus placebo	DOX: 7% Placebo: 46% (p < 0.01)
Sack 1978 [20]	1976	39 US military in Kenya; RCT DOX versus placebo	DOX: 6% Placebo: 43% (p = 0.012)

CI – confidence interval; DOX – doxycycline; IBS – irritable bowel syndrome; RCT – randomized controlled trial; RR – relative risk; TD – travellers' diarrhoea.

total of 260 participants (63.1%) took an antimalarial prophylaxis: 46 (11.2% of all) took doxycycline, 125 (30.3%) atovaquone + proguanil, 67 (16.3%) mefloquine, and 22 (5.3%) hydroxychloroquine.

On return, 90 (21.9%) travellers were colonised by ESBL-PE. All of the ESBL-PE isolates selected for the present study were *E. coli*. Among these, 13 were diarrhoeagenic *E. coli* (DEC), of which 90% (9/10) of the enteroaggregative (EAEC) and 50% (1/2) of the enteropathogenic (EPEC) and the enterotoxigenic (ETEC) *E. coli* were doxycycline resistant.

Among the volunteers colonised by ESBL-PE, 49 (54.4%) were female, median age 28 years with travel destinations in South Asia (28; 31.1%), South-East Asia (33; 36.7%), Sub- Saharan Africa (23; 25.6%) as reported earlier [19]; none had travelled in South and Central America or the Caribbean. A total of 35 (38.9%) had not taken any antimalarials, 11 (12.2%) took doxycycline, 8 (8.9%) hydroxychloroquine, 22 (24.4%) atovaquone-proguanil, and 14 (15.6%) mefloquine (Table 1). The median duration of travel was 29 (IQR 16–40.5) days among doxycycline users and 16 (12–25) days among nonusers; all users took doxycycline as an antimalarial prophylaxis according to the national recommendations (i.e. starting one week before departure abroad, taking 100 mg daily during travel and for four weeks after return).

3.1.2. Impact of doxycycline use on TD rate

Of the 412 travellers, TD was contracted by 288 (69.9%); TD rates proved identical among doxycycline users (32/46; 69.6%) and non-users (256/366; 69.9%; p = 0.958, OR 1.0, 95% CI 0.5–1.9). TD rates were similar for all antimalarial regimens: 69.6% for those taking doxycycline, 70.4% for atovaquone + proquanil, 76.1% for mefloquine, 72.7% for hydroxychloroquine, and 66.4% for those with no antimalarial prophylaxis (p = 0.700).

3.1.3. Impact of doxycycline use on ESBL-PE acquisition

We saw no significant differences in the ESBL-PE acquisition rates between doxycycline users versus non-users (11/46 (23.9%) versus 79/366 (21.6%); p = 0.719).

3.1.4. Impact of doxycycline use and other factors on doxycycline co-resistance rates among ESBL-PE

Eleven out of the ninety travellers (12.2%) with travel-acquired ESBL-PE took doxycycline as an antimalarial prophylaxis. When using ≤4 as the limit of sensitivity, 84.4% of all ESBL-PE strains proved co-resistant to doxycycline: for doxycycline users 100% (11/11) and for non-users 82.3% (65/79). As for users of the other antimalarials, the co-resistance rates were similar regardless of the regimen (Table 1).

TD was contracted by 75/90 (83.3%) of those having acquired ESBL-

PE; TD was not seen to have any effect on the doxycycline co-resistance rates (10/11; 90.9% among those with TD versus 65/79; 82.3% among those without). When compared with isolates obtained from visitors to South Asia, isolates from Southeast Asia appeared more resistant (64.3% versus 90.9%; p = 0.018; OR 5.6 95% CI 1.2–22.9). Doxycycline co-resistance was associated with older age (median 28 years for those with doxycycline-susceptible strains versus 48 for those with co-resistance; p = 0.021).

3.1.5. Impact of doxycycline use on co-resistance to other antimicrobials among ESBL-PE

The rates of co-resistance to ciprofloxacin, tobramycin, nitrofurantoin, and co-trimoxazole did not differ significantly between doxycycline users and non-users (Table 2).

3.2. Part 2: Literature review

3.2.1. Impact of doxycycline use on the risk of TD

Our literature search yielded twelve studies exploring the impact of doxycycline use on TD rates (see Table 3). Of these, seven randomised controlled trials (RCTs) had been conducted in the 1970s and '80s [12,20–25] and five non-randomised studies between 2009 and 2018 [1,9,26–28]. Nine [9,12,20,21,23–25,27,28] of these twelve were conducted among military, and only two [1,22] among regular travellers, and one among policemen and voluntary workers [26].

In studies conducted in 1976–81, six RCTs compared doxycycline as a TD prophylaxis with a placebo. A decreased TD risk was observed in four investigations: among US military in Kenya [20], Morocco [21] and Honduras [25], and regular US travellers in Mexico [23]. No difference was seen among US military in Honduras [22] and Thailand [24]. In an RCT among US military in Thailand in 1988, Arthur et al. found no difference in TD rates between doxycycline and mefloquine users [12].

In the 2000s, in non-randomised studies comparing doxycycline users with non-users, a decreased TD risk was reported for doxycycline users by Haus-Cheymol et al. among French policemen and voluntary workers in Haiti during the cholera outbreak [26], and by Lago et al. among US military travellers [9]. Two studies found no effect on TD rates: Kantele et al. reported data collected 2009–10 from 430 travellers, comparing doxycycline users to users of other antimalarials [1], and Lee et al. conducted a multivariable analysis in 2009 among 590 Australian

Table 4
Studies providing data on the impact of doxycycline use on the acquisition of ESBL-PE during international travel in our literature review.

First author, publication year	Year(s) of sample collection	Study setting	Impact of doxycycline use on the risk of ESBL/MDR acquisition
Buchek, 2021 [34]	2015–17	99 US travellers; 8% ESBL-PE; 15% with DOX as malaria prophylaxis	DOX*: 12% (2/11) Non-users: 17% (9/53)
Tufic-Garutti, 2021 [35]	2015–19	210 Brazilian travellers, 22% ESBL-PE; 16% with DOX as malaria prophylaxis	DOX: 38% No antibiotic: 28% Other antibiotic: 78% (7/9)
Worby, 2020 [33]	not reported	608 US travellers; 38% MDR; 1% with DOX as malaria prophylaxis	DOX: 50% (3/6)
Dao, 2020 [32]	2017–19	382 French medical students abroad, 29% ESBL-PE; 18% with DOX as malaria prophylaxis	DOX: 29% Non-users: 29%
Maataoui, 2019 [31]	2012	189 French military; 38% ESBL-PE; 91% with DOX as malaria prophylaxis	DOX was not associated with increased ESBL-PE acquisition rates (exact numbers not given)
Flateau, 2018 [30]	2012–15	166 patients hospitalized in a military hospital in France with a travel history within 2 months; 24.7% ESBL-PE; 30% with DOX as malaria prophylaxis	DOX: 37% Non-users: 27% (p = 0.25)
Lääveri, 2018 [5]	2009–13	396 Finnish and Dutch travellers to various regions in Africa; 15.4% ESBL-PE; 8.6% with DOX as malaria prophylaxis	DOX: 14.7% Non-users: 15.5% (p = 0.906)
Ruppe, 2015 [2]	2012–13	574 French travellers, 51% MDRE, 13% with DOX as a malaria prophylaxis	DOX: 51%, Other antimalarials: 57%
Kantele, 2015 [1]	2009–10	430 Finnish travellers, 21% ESBL-PE; 19% with DOX as malaria prophylaxis	DOX: 24% Mefloquine: 21% Atovaquone/proguanil: 18%
Lübbert, 2015 [29]	2013–14	225 German travellers; 36% ESBL-PE; 8 (4%)with DOX as malaria prophylaxis	DOX: 25% (2/8)

DOX – doxycycline; ESBL-PE – extended-spectrum betalactamase producing Enterobacterales; MDRE – multidrug resistant Enterobacterales.

Table 5
Studies providing data on the impact of doxycycline use on doxycycline/tetracycline resistance rates in our literature review.

First author, publication year	Year(s) of sample collection	Study setting	Impact of doxycycline use on doxycycline/tetracycline resistance rates
Buchek, 2021 [34]	2015–17	99 US military travellers, 15% with DOX as malaria prophylaxis	No increased in DOX resistance rates (various *E. coli*) among 11 DOX users
Arthur, 1990 [12]	1988	253 US military in Thailand; RCT DOX versus mefloquine	ETEC: DOX: 77%, Mefloquine: 35% *Campylobacter*: DOX: 100%, Mefloquine 50%
Echeverria, 1984 [24]	1980	63 US military in Thailand; RCT DOX versus placebo	ETEC: DOX: 100%, Placebo: 82%
Sack, 1984 [25]	1980	44 US military in Honduras; RCT DOX versus placebo	ETEC: DOX: 91%, Placebo: 54%
Santosham, 1981 [22]	1978	46 US military in Honduras; RCT DOX versus placebo	ETEC DOX: 82%, Placebo: 66%
Sack, 1978 [20]	1976	39 US military in Kenya; RCT DOX versus placebo	Various *E. coli* DOX: 100%, Placebo: 25%

DOX – doxycycline; ETEC – enterotoxigenic *E. coli*; RCT – randomized controlled trial.

military personnel on overseas deployment and found an increased risk for irritable bowel syndrome (IBS) among doxycycline users, yet with no impact on the risk of TD [27]. Among US military in Kenya in 2012–13, Terrell et al. report an increased rate of diarrhoea among 685 doxycycline users as compared with 867 mefloquine users [28].

3.2.2. Impact of doxycycline use on ESBL risk

The ten non-randomised studies [1,2,5,29–35] suggested neither an increased nor a decreased risk of contracting ESBL-PE among doxycycline users versus non-users; we found no randomised trials on travellers comparing doxycycline users to those receiving a placebo or other antimalarials and reporting ESBL-PE rates (Table 4).

3.2.3. Impact of doxycycline use on doxycycline/tetracycline resistance rates among various stool bacteria

Four RCTs conducted between 1976 and 80 among US military compared doxycycline as a TD prophylaxis to a placebo; one study was carried out in Kenya [20], two in Honduras [22,25] and one in Thailand [24]; all report ETEC [22,24,25] or various *E. coli* [20] isolates with higher doxycycline/tetracycline resistance rates among doxycycline users than non-users. Later, in a study conducted in 1988 among US military in Thailand, Arthur et al. compared doxycycline users to mefloquine users and found 77% and 35% of all ETEC strains and 100% and 50% of all *Campylobacter* strains resistant to tetracycline. In a recent non-randomised study among US military travellers to various regions, Buchek et al. report no difference in the resistance rates between the 11 doxycycline users and 88 non-users; the authors do not provide exact rates or the number or type of strains analysed [34]. (Table 5).

Table 6
Studies reporting doxycycline/tetracycline co-resistance rates among ESBL/MDR strains obtained from travellers in our literature review.

First author, publication year	Year(s) of sample collection	Study setting	Doxycycline/tetracycline resistance among ESBL/MDR strains
Buchek, 2021 [34]	2015–17	99 US travelling military; 8 (8%) ESBL-PE (screening non-selective)	63% of 8 ESBL-PE strains resistant to DOX
Peng, 2021 [37]	2018–19	90 Hong Kong travellers, 49 ESBL-PE -negative before travel; 41% became colonized by 60 ESBL-PE strains	Of 60 ESBL-PE isolates, 80% resistant to tetracycline
Lubbert, 2015 [29]	2013–14	225 German travellers; ESBL-PE 36%	32% of 58 ESBL-PE strains resistant to DOX
Hopkins, 2014 [36]	2004–11	90 isolates each of *E. coli*, *Shigella*, non-typhoidal *Salmonella*, typhoidal *S. enterica* and *Campylobacter*, additional 60 CTX-M-producing diarrhoeal *E. coli* from travellers	73/90 (81%) of MDR*E. coli* isolates non-susceptible to DOX

DOX – doxycycline; ESBL-PE – extended-spectrum betalactamase producing Enterobacterales; MDR – multidrug resistant; MDRE – multidrug resistant Enterobacterales.

Table 7
Studies describing doxycycline/tetracycline resistance among ETEC, EAEC, and *Campylobacter* strains in our literature review.

First author, publication year	Year(s) of sample collection	Geographic region	Study setting	Tetracycline/doxycycline resistance among ETEC/EAEC/*Campylobacter* strains
ETEC				
Boxall, 2020 [54]	2015–17	various destinations	660 diarrhoeagenic *E. coli* isolates from travellers with TD	38% (23/61)
Guiral, 2019 [52]	2011–17	various destinations	39 EAEC and 43 ETEC clinical isolates from travellers with TD	40% (17/43)
Murphy, 2019 [53]	2012–14	South Asia	Travellers in Kathmandu, Nepal 433 TD, 209 non-diarrhoea controls	27% (16/60)
Margulieux, 2018 [51]	2001–16	South Asia	265 ETEC isolates from locals and travellers in Kathmandu, Nepal	28% (74/265)
Jennings, 2017 [49]	2003–10	Latin America	230 US language school students with TD in Cusco, Peru	41% (11/27)
Mason, 2017 [50]	2002–04	Southeast Asia	US marines in Thailand, 155 TD	37% (7/19)
Ouyang-Latimer, 2011 [48]	2006–08	Latin America and South Asia	456 enteropathogens isolated from travellers with TD in Mexico, Guatemala, and India, 2006 to 2008	India 49% (48/98) Mexico + Guatemala: 52% (140/270)
Porter, 2010 [47]	2002	North Africa and Middle East	202 US military with TD in Turkey, ETEC 41%	49% (41/83)
Mendez, 2009 [46]	1994–97, 2001–04	various destinations	134 EAEC and 190 ETEC clinical isolates from travellers with TD	1994–97: 57% (47/82) 2001–04 59% (64/108)
Shaheen, 2003 [45]	1996–1997	Sub-Saharan Africa	463 travellers with TD in Mombasa, Kenya, 164 ETEC	42% (66/157)
Vila, 2000 [44]	1994–97	various destinations	520 travellers with TD; 82 ETEC	57% (47/82)
Bandres,J.C. 1992 [42]	1980–89	Latin America	220 ETEC isolates from US student travellers with TD in Mexico	57% (only percentages given)
DuPont, 1992 [43]	1989–90	Latin America	189 US student travellers with TD in Mexico, RCT TD treatment study aztreonam versus placebo; 61 individuals had 71 ETEC strains	49% (only percentages given)
Haberberger, 1991 [40]	1987	North Africa and Middle East	4,500 US military in Cairo, Egypt; 183 TD; 33% had ETEC	10% (6/60)
Taylor, 1991 [41]	1989	North Africa and Middle East	104 US military Egypt, RCT TD treatment study ciprofloxacin + loperamide versus ciprofloxacin alone.	49% (only percentages given)
Arthur, 1990 [12]	1988	Southeast Asia	253 US military in Thailand; RCT DOX versusmefloquine; 122/253 TD	36% (5/14)
Taylor, 1988 [39]	1986	South Asia	328 US Peace Corps and traveller expatriates with TD in Kathmandu, Nepal	22% (17/79)

(*continued on next page*)

Table 7 (*continued*)

First author, publication year	Year(s) of sample collection	Geographic region	Study setting	Tetracycline/doxycycline resistance among ETEC/EAEC/*Campylobacter* strains
Sack 1984 [25]	1980	Latin America	44 US military Honduras; RCT DOX versus placebo; 29/44 TD	54% (7/13)
Echeverria 1981 [38]	1979	Southeast Asia	35 US military in Thailand, RCT DOX versus placebo; 20/35 TD,	0% (0/51)
Santosham,M. 1981 [22]	1978	Latin America	46 military in Honduras; RCT DOX versus placebo; 18/46 TD	62% 13/21)
Sack 1979 [21]	1977	North Africa and Middle East	51 US military in Morocco; RCT DOX versus placebo; 13/51 TD	36% (4/11)
Sack, 1978 [20]	1976	Sub-Saharan Africa	39 US military in Kenya, RCT DOX versus placebo: 22/39 TD	0% (0/15)
EAEC				
Boxall, 2020 [54]	2015–17	various destinations	660 diarrhoeagenic *E. coli* isolates from travellers with TD	32% (85/265)
Guiral, 2019 [52]	2011–17	various destinations	39 EAEC and 43 ETEC isolates from travellers with TD	51% (20/39)
Murphy, 2019 [53]	2012–14	South Asia	Travellers to Kathmandu, Nepal 433 TD, 209 non-diarrhoea controls	34% (71/208)
Jennings, 2017 [49]	2003–10	Latin America	230 US language school students with TD in Cusco, Peru	22% (2/9)
Mason, 2017 [50]	2002–04	Southeast Asia	155 US marines with TD in Thailand	100% (5/5)
Ouyang-Latimer, 2011 [48]	2006–08	Latin America and South Asia	456 enteropathogens isolated from travellers with TD in Mexico, Guatemala, and India, 2006 to 2008	India 0% (0/3) Mexico + Guatemala 60% (12/20)
Mendez, 2009 [46]	1994–97, 2001–04	various destinations	134 EAEC and 190 ETEC isolates from travellers with TD	1994–97: 64% (32/50) 2001–04: 76% (64/84)
Campylobacter				
Jennings, 2017 [49]	2003–10	Latin America	230 US language school students with TD in Cusco, Peru	0% (0/3)
Mason, 2017 [50]	2002–04	Southeast Asia	155 US marines with TD in Thailand	68% (39/57)
Post, 2017 [61]	2007–14	various destinations	261 *Campylobacter* isolates from travellers	Total 48.3% South-Eastern Asia 14/25 (56.0%) South Asia: 19/46 (56.0%) Latin America 10/19 (52.9%) Sub-Saharan Africa: 38/84 (45.2%) North Africa and Middle East:22/44 (50.0%)
Ouyang-Latimer, 2011 [48]	2006–08	Latin America and South Asia	456 enteropathogens isolated from travellers with TD in Mexico, Guatemala, and India, 2006 to 2008	India: 0% (0/17) Mexico and Guatemala 0% (0/6)
Serichantalergs,2010 [60]	1998–2003	Southeast Asia	312 *Campylobacter jejuni* isolates (46 from regular travellers and 266 US military)	82% (256/312)
Skjot-Rasmussen, 2009 [59]	1997–2007	various destinations	120 *Campylobacter* strains isolated from travellers in Denmark	35% (42/120)
Ruiz, 2007 [58]	1993–03	various destinations	92 *Campylobacter* strains isolated from travellers with TD	42% (39/92)
Ronner, 2004 [57]	2001–02	various destinations	112 *Campylobacter* strains from travellers	63% (70/112)
Hakanen, 2003 [56]	1995–2000	various destinations	354 nondomestic *Campylobacter* strains	46% (163/354)
Petruccelli, 1992 [55]	1990	not reported	142 US military with TD, RCT TD treatment study; ciprofloxacin + loperamide various lengths. All took DOX as an antimalarial	100% (54/54)
Arthur, 1990 [12]	1988	Southeast Asia	253 US military in Thailand; RCT DOX versus mefloquine; 122/253 TD	50% (2/4)

DOX – doxycycline; EAEC – enteroaggregative *E. coli*; ETEC – enterotoxigenic *E. coli*; RCT – randomized controlled trial; TD – travellers' diarrhoea.

Table 8
Studies describing doxycycline/tetracycline resistance rates among various *E. coli* (other than ETEC, EAEC)/Enterobacterales in our literature review.

First author, publication year	Year(s) of sample collection	Geographic regions	Study setting	Tetracycline/doxycycline resistance
Buchek, 2021 [34]	2015–17	various destinations	99 US travellers; 8% ESBL-PE; 15% took DOX as a malaria prophylaxis	*E. coli* 44% (11/25) ESBL-producing strains, 63% (5/8)
Blyth, 2016 [68]	2013	various destinations	58 US military, 3 with DOX; 9% ESBL	36 *E. coli* isolates; new resistance detected in 44%
Hopkins, 2014 [36]	2004–11	various destinations	90 isolates of each: *E. coli*, *Shigella*, non-typhoidal *Salmonella*, typhoidal *S. enterica* and *Campylobacter*, additional 60 CTX-M-producing diarrhoeagenic *E. coli* from travellers with TD	49.7% (179/360) of *Enterobacteriaceae* 81.1% (73/90) of MDR *E. coli*
Jiang, 2002; [67]	1996–98	various destinations	1079 international travellers to Kenya, India, and Jamaica	46%–57% of isolated enteropathogens
Huang, 2001 [66]	not reported	Latin America	39 US student travellers to Mexico; no TD	89% (16/18) of trimetoprim-resistant *E. coli* strains
Wiström, 1992 [6]	1990	Latin America	42 US travellers with TD to Mexico; RCT TD treatment study ciprofloxacin versus placebo	Ciprofloxacin users: 98% (22/23 *E. coli* strains) Placebo 62% (14/23) No TD 46% (46/100)

(*continued on next page*)

Table 8 (*continued*)

First author, publication year	Year(s) of sample collection	Geographic regions	Study setting	Tetracycline/doxycycline resistance
Rademaker, 1989 [65]	1988	North Africa	54 Dutch travellers to Sousse, Tunisia; RCT TD prevention study ciprofloxacin versus placebo	Various gram-negative Enterobacterales 47%
Wiström, 1989 [64]	1986–88	various destinations	447 Swedish travellers; RCT TD treatment study norfloxacin versus placebo	Various *E. coli* Norfloxacin users 74% (14/19) Placebo 86% (18/21) No TD 43% (9/21)
Wiström 1987 [63]	not reported	various destinations	62 Swedish travellers, RCT norfloxacin versus placebo TD prophylaxis	Various *E. coli* Norfloxacin users 40% Placebo 43%
Gaarslev, 1985 [62]	not reported	Latin America	33 Danish travellers to Mexico, RCT mecillinam versus placebo TD prophylaxis	Various *E.coli* Placebo: 33% (13/47 strains) Mecillinam: 52% (23/59 strains)
Echeverria, 1984 [24]	1980	Southeast Asia	63 US military in Thailand; RCT DOX versus placebo;	Various *E. coli* 82%

DOX – doxycycline; EAEC – enteroaggregative *E. coli*; ESBL-PE – extended-spectrum betalactamase producing Enterobacterales; ETEC –enterotoxigenic *E. coli*; MDR – multidrug resistant; RCT – randomized controlled trial; TD – travellers' diarrhoea.

3.2.4. Doxycycline/tetracycline co-resistance rates among ESBL-PE

We found four traveller studies of doxycycline/tetracycline co-resistance among ESBL-PE strains. They report rates in the range 32–81% (Table 6) [29,34,36,37]; none of the investigations provide co-resistance rates with respect to possible doxycycline use.

3.2.5. Doxycycline/tetracycline resistance rates among TD pathogens

As regards the ETEC strains isolated from travellers between 1976 and 2017, 0–62% were reported as resistant to doxycycline/tetracycline; no clear trends were seen over time [12,20–22,25,38–54]. Samples for EAEC were only collected 1994–2017, and 0–100% of all isolates proved resistant; no significant trends were observed here either [12,20–22,25,38–54]. Among *Campylobacter* strains collected in 1988–2014, resistance rates of 0–100% were reported; no increasing or decreasing trends were seen [12,48–50,55–61]. (Table 7).

3.2.6. Doxycycline/tetracycline resistance rates among other stool bacteria

Our search yielded ten traveller studies reporting doxycycline/tetracycline resistance rates among other stool bacteria (mostly non-DEC *E. coli*) [6,24,34,36,62–68]: the rates varied between 44% and 98% with no clear decreasing or increasing trends over time (Table 8).

4. Discussion

4.1. Data do not support use of doxycycline for prevention of TD

In the 1970s and '80s, doxycycline was used both to treat and prevent travellers' diarrhoea [9,13,14]. Indeed, the drug showed an efficacy of up to 90% as a prophylaxis against TD in most of the RCTs conducted in the 1970s and '80s [20–25]. An RCT comparing mefloquine and doxycycline that was carried out among US military in Thailand in 1987 found no protective effect against TD [12].

In non-randomised trials undertaken in the 2000s the data have been inconclusive. One study comparing doxycycline with other antimalarials found no protective effect against TD [1], another reports similar TD rates for doxycycline users and non-users but IBS more frequently for users [27], while a third investigation shows increased TD rates for doxycycline users [28]. Two studies, by contrast, report a reduced risk of TD for doxycycline users [9,26]. Our study accords with those not reporting protective efficacy: we found no difference in TD rates between doxycycline users and non-users. Taken together, the literature does not justify TD prevention as an indication for doxycycline.

4.2. Doxycycline resistance among intestinal bacteria

4.2.1. Doxycycline resistance rates among TD pathogens are generally high

In their recent article Lago et al. suggest, as an explanation for their finding of reduced TD rates for doxycycline users, that the general rates of doxycycline resistance could be decreasing [9]. Our literature review does not support this speculation. *Campylobacter* strains remained highly resistant between 1988 and 2014 (no earlier traveller studies were found) [12,48–50,55–61]. Likewise, no decrease in doxycycline resistance was seen for EAEC between 1994 and 2017 [46,48–50,52–54], or for ETEC between 1974 and 2017 [12,20–22,25,38–54]. Indeed, even though doxycycline has in the LMICs, as in most other countries, become partly replaced by other antibiotics, this change appears not to have translated into decreased resistance rates among TD pathogens.

Somewhat unexpectedly, the finding from our prospective study suggests higher doxycycline resistance rates for travellers to Sub-Saharan Africa and South-East Asia than those visiting South Asia. Future studies are needed to confirm this observation.

4.2.2. Doxycycline co-resistance is common among intestinal ESBL-PE

We considered 84.4% of the 90 ESBL-PE strains here co-resistant to doxycycline. Three out of four studies analysing co-resistance rates among ESBL-PE strains accord with our findings: Hopkins et al. report a rate of 81% for returning British travellers [36], Buchek et al. a rate of 63% for US military abroad [34], and Peng et al. a rate of 80% for among Hong Kong travellers [37]. Somewhat lower rates (36%) are reported by Lübbert et al. for German travellers [29].

4.3. Doxycycline use selects doxycycline-resistant strains

4.3.1. Impact on doxycycline resistance among TD pathogens and other stool bacteria

It is noteworthy that during doxycycline prophylaxis, TD pathogens appear to be mostly doxycycline-resistant. Although the number of RCTs comparing doxycycline with a placebo [20,22,24,25] or mefloquine [12] were limited, all of the studies report higher resistance rates for doxycycline users than for non-users.

4.3.2. Impact on doxycycline co-resistance among ESBL-PE isolates

To our knowledge, our study is the first to compare doxycycline co-resistance rates among ESBL-PE isolates between doxycycline users and non-users. The comparison posed a challenge because of the high general doxycycline co-resistance rate (84.4%) among the ESBL-PE isolates which was also reflected in the non-users' rates (65/79; 82.3%). Strikingly, however, every single ESBL-PE isolate of the doxycycline users (11/11; 100%) proved resistant to doxycycline. Thus, regardless of the

high overall resistance to doxycycline, its use appeared to select strains resistant to it, which accords with our respective findings on fluoroquinolones [7].

Interestingly, in our data, doxycycline co-resistance also appeared more common among fluoroquinolone users (19/20; 95.0%) than non-users (56/70; 80.0%), yet the difference did not reach statistical significance ($p = 0.112$). In the study by Wiström et al. [6] exploring the ecological short-term effects of ciprofloxacin treatment against TD, two days after travel 96% of fluoroquinolone users, but only 62% of those having taken a placebo, carried doxycycline-resistant strains.

4.4. Does doxycycline increase the risk of acquiring ESBL-PE?

Antibiotics [1–5], particularly beta-lactams [2] or fluoroquinolones [5], have been shown to predispose to ESBL-PE among travellers in LMICs, yet such an increased risk has not been reported for doxycycline [1,2,5,29–35]. As regards beta-lactam users, the explanation for the difference between the regimens is obvious: ESBL-PE are resistant to most beta-lactams and thus, beta-lactams specifically favour ESBL-PE over any beta-lactam-sensitive bacteria. As for fluoroquinolones, their use results in favouring fluoroquinolone-resistant ESBL-PE strains and indirectly increases the rate of ESBL-PE acquisition. These two phenomena have been demonstrated in our previous study: fluoroquinolone-users have higher ESBL-PE rates than non-users, and their ESBL-PE strains are more often co-resistant to fluoroquinolone [7]. Even though doxycycline co-resistance was common among our ESBL-PE isolates, doxycycline appeared not to increase ESBL-PE acquisition. This may at first sight appear illogical, but it may in fact be explained by the high background doxycycline resistance rate among ESBL-PE (over 80%): testing the significance for such a small difference would have required a considerably larger study population. It is noteworthy that even with our study population size, selection pressure shows in co-resistance rates: 100% of the doxycycline users and 82.3% of the non-users had doxycycline-resistant ESBL-PE strains.

Importantly, most of the studies above describe doxycycline resistance rates among TD pathogens or ESBL-PE. As the great majority of intestinal bacteria do not belong to these groups, the lack of effect on these specific populations should not be interpreted as proof of no impact on others, but these populations can rather be seen to serve here as sentinels of doxycycline resistance.

4.5. Should doxycycline be favoured as an antimalarial prophylaxis?

As we have discussed here, current data available on the efficacy of doxycycline against TD and the consequences of its long-term use remain inconsistent. Therefore, as long as new RCTs comparing doxycycline with other antimalarials have not been conducted, selection of antimalarial medications should not be based on a presumed protective effect of the drug against TD.

4.6. Limitations

Some limitations of the present study deserve to be discussed. As the number of ESBL-PE strains in our prospective data was 90, some of the statistics remained underpowered. Our strains were collected already in 2009–10. However, we only found one more recent study (comprising eleven doxycycline users) that assesses the impact of doxycycline on doxycycline/tetracycline resistance rates [34].

EUCAST has not established the breakpoint MICs for doxycycline. We selected >4, since it is used by CLSI [16] and several other studies [29,36,48,69]. Had we applied ≥16 as a breakpoint MIC value, the co-resistance rate would have been 83.3% (75/90), yielding the co-resistance rates of 90.9% for doxycycline users (10/11) and 82.3% for non-users (65/70).

5. Conclusion

Our analysis suggests that for travellers to LMICs the use of doxycycline does not reduce the risk of TD. High in all ESBL-PE isolates, doxycycline co-resistance proved highest among doxycycline users. In the literature comparing users and non-users, particularly high resistance figures were likewise reported for various stool bacteria from doxycycline users. On the basis of our own study data and the literature review, we conclude that the impact of doxycycline on AMR acquisition is limited but not negligible.

Authors' contributions

Study concept and design AK, TL; acquisition of data AK, SM; literature review TL; statistical analysis TL; drafting the manuscript AK, TL; final approval of version published AK, SM, TL.

Funding

This work was supported by a Finnish government subsidy for health science research [grant numbers: TYH 2012141, TYH 2013218 and TYH 2014216], the Finnish Cultural Foundation, and the Sigrid Jusélius Foundation [grant number: 1726], Doctoral School in Health Sciences, University of Helsinki.

Declaration of competing interest

AK has received investigator-initiated grants from Valneva and Pfizer, neither of which are relevant for the current manuscript. SM and TL declare no conflicts of interest.

Acknowledgements

We express our gratitude to the late Dr Jukka Riutta and the nurses at the Travel Clinic of Aava Medical Centre for help in recruiting the volunteers.

References

[1] Kantele A, Lääveri T, Mero S, et al. Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing Enterobacteriaceae. Clin Infect Dis 2015;60:837–46.
[2] Ruppe E, Armand-Lefevre L, Estellat C, et al. High rate of acquisition but short duration of carriage of multidrug-resistant enterobacteriaceae after travel to the tropics. Clin Infect Dis 2015;61:593–600.
[3] Reuland EA, Sonder GJ, Stolte I, et al. Travel to Asia and traveller's diarrhoea with antibiotic treatment are independent risk factors for acquiring ciprofloxacin-resistant and extended spectrum beta-lactamase-producing Enterobacteriaceae-a prospective cohort study. Clin Microbiol Infect 2016;22:731.
[4] Arcilla MS, van Hattem JM, Haverkate MR, et al. Import and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis 2017;17:78–85.
[5] Lääveri T, Vlot JA, van Dam AP, et al. Extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) among travellers to Africa: destination-specific data pooled from three european prospective studies. BMC Infect Dis 2018;18:341.
[6] Wistrom J, Gentry LO, Palmgren AC, et al. Ecological effects of short-term ciprofloxacin treatment of travellers' diarrhoea. J Antimicrob Chemother 1992;30:693–706.
[7] Kantele A, Mero S, Kirveskari J, Lääveri T. Fluoroquinolone antibiotic users select fluoroquinolone-resistant ESBL-producing Enterobacteriaceae (ESBL-PE) - data of a prospective traveller study. Trav Med Infect Dis 2017;16:23–30.
[8] Yellow book, travel-related infectious diseases, malaria. Available at: https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/malaria. [Accessed 14 March 2022].
[9] Lago K, Telu K, Tribble D, et al. Doxycycline malaria prophylaxis impact on risk of travelers' diarrhea among international travelers. Am J Trop Med Hyg 2020;103:1864–70.
[10] Sack RB, Rahman M, Yunus M, Khan EH. Antimicrobial resistance in organisms causing diarrheal disease. Clin Infect Dis 1997;24(Suppl 1):S102–5.
[11] Steffen R, Hill DR, DuPont HL. Traveler's diarrhea: a clinical review. JAMA 2015;313:71–80.

[12] Arthur JD, Echeverria P, Shanks GD, Karwacki J, Bodhidatta L, Brown JE. A comparative study of gastrointestinal infections in United States soldiers receiving doxycycline or mefloquine for malaria prophylaxis. Am J Trop Med Hyg 1990;43:608–13.

[13] Taylor DN, Hamer DH, Shlim DR. Medications for the prevention and treatment of travellers' diarrhea. J Trav Med 2017;24:S17–22.

[14] Diptyanusa A, Ngamprasertchai T, Piyaphanee W. A review of antibiotic prophylaxis for traveler's diarrhea: past to present. Trop Dis Travel Med Vaccines 2018;4:14.

[15] The European committee on antimicrobial susceptibility testing. breakpoint tables for interpretation of MICs and zone diameters. version 6.0, 2016. http://www.eucast.org. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed 17 July, 2016.

[16] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial Susceptibility Testing; twenty-fourth informational supplement. CLSI document M100-S24; 2014 [electronic].

[17] World Health Organization. Health topics: diarrhoea. World health organization (WHO). Available at: https://www.who.int/health-topics/diarrhoea#tab=tab_1/. [Accessed 14 March 2022].

[18] Lääveri T, Pakkanen SH, Kirveskari J, Kantele A. Travellers' diarrhoea: impact of TD definition and control group design on study results. Travel Med Infect Dis 2018:37–43.

[19] Kantele A, Lääveri T, Mero S, et al. Despite predominance of Uropathogenic/Extraintestinal pathotypes among travel-acquired extended-spectrum beta-lactamase-producing Escherichia coli, the most commonly associated clinical manifestation is travelers' diarrhea. Clin Infect Dis 2019;70:210–8.

[20] Sack DA, Kaminsky DC, Sack RB, et al. Prophylactic doxycycline for travelers' diarrhea. results of a prospective double-blind study of peace corps volunteers in Kenya. N Engl J Med 1978;298:758–63.

[21] Sack RB, Froehlich JL, Zulich AW, et al. Prophylactic doxycycline for travelers' diarrhea: results of a prospective double-blind study of peace corps volunteers in Morocco. Gastroenterology 1979;76:1368–73.

[22] Santosham M, Sack RB, Froehlich J, et al. Biweekly prophylactic doxycycline for travelers' diarrhea. J Infect Dis 1981;143:598–602.

[23] Freeman LD, Hooper DR, Lathen DF, Nelson DP, Harrison WO, Anderson DS. Brief prophylaxis with doxycycline for the prevention of traveler's diarrhea. Gastroenterology 1983;84:276–80.

[24] Echeverria P, Sack RB, Blacklow NR, Bodhidatta P, Rowe B, McFarland A. Prophylactic doxycycline for travelers' diarrhea in Thailand. further supportive evidence of Aeromonas hydrophila as an enteric pathogen. Am J Epidemiol 1984;120:912–21.

[25] Sack RB, Santosham M, Froehlich JL, Medina C, Orskov F, Orskov I. Doxycycline prophylaxis of travelers' diarrhea in Honduras, an area where resistance to doxycycline is common among enterotoxigenic Escherichia coli. Am J Trop Med Hyg 1984;33:460–6.

[26] Haus-Cheymol R, Theodose R, Quilici ML, et al. A cluster of acute diarrhea suspected to be cholera in French travelers in Haiti, December 2010. J Trav Med 2012;19:189–91.

[27] Lee TW, Russell L, Deng M, Gibson PR. Association of doxycycline use with the development of gastroenteritis, irritable bowel syndrome and inflammatory bowel disease in Australians deployed abroad. Intern Med J 2013;43:919–26.

[28] Terrell AG, Forde ME, Firth R, Ross DA. Malaria chemoprophylaxis and self-reported impact on ability to work: mefloquine versus doxycycline. J Trav Med 2015;22:383–8.

[29] Lubbert C, Straube L, Stein C, et al. Colonization with extended-spectrum beta-lactamase-producing and carbapenemase-producing Enterobacteriaceae in international travelers returning to Germany. Int J Med Microbiol 2015;305:148–56.

[30] Flateau C, Duron-Martinaud S, Haus-Cheymol R, et al. Prevalence and risk factors for extended-spectrum beta-lactamase-producing- Enterobacteriaceae in French military and civilian travelers: a cross-sectional analysis. Trav Med Infect Dis 2018;23:44–8.

[31] Maataoui N, Mayet A, Duron S, et al. High acquisition rate of extended-spectrum beta-lactamase-producing Enterobacteriaceae among French military personnel on mission abroad, without evidence of inter-individual transmission. Clin Microbiol Infect 2019;25:631.

[32] Dao TL, Hoang VT, Magmoun A, et al. Acquisition of multidrug-resistant bacteria and colistin resistance genes in French medical students on internships abroad. Trav Med Infect Dis 2020;39:101940.

[33] Worby CJ, Earl AM, Turbett SE, et al. Acquisition and long-term carriage of multidrug-resistant organisms in US international travelers. Open Forum Infect Dis 2020;7:ofaa543.

[34] Buchek G, Mende K, Telu K, et al. Travel-associated multidrug-resistant organism acquisition and risk factors among US military personnel. J Trav Med 2021;28::taab028.

[35] Tufic-Garutti SDS, Ramalho JVAR, Longo LGA, et al. Acquisition of antimicrobial resistance determinants in Enterobacterales by international travelers from a large urban setting in Brazil. Trav Med Infect Dis 2021;41:102028.

[36] Hopkins KL, Mushtaq S, Richardson JF, et al. In vitro activity of rifaximin against clinical isolates of Escherichia coli and other enteropathogenic bacteria isolated from travellers returning to the UK. Int J Antimicrob Agents 2014;43:431–7.

[37] Peng Y, Liang S, Poonsuk K, et al. Role of gut microbiota in travel-related acquisition of extended spectrum beta-lactamase-producing Enterobacteriaceae. J Trav Med 2021;28::taab022.

[38] Echeverria P, Blacklow NR, Sanford LB, Cukor GG. Travelers' diarrhea among American peace corps volunteers in rural Thailand. J Infect Dis 1981;143:767–71.

[39] Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BL, Echeverria P. Etiology of diarrhea among travelers and foreign residents in Nepal. JAMA 1988;260:1245–8.

[40] Haberberger Jr RL, Mikhail IA, Burans JP, et al. Travelers' diarrhea among United States military personnel during joint American-Egyptian armed forces exercises in Cairo, Egypt. Mil Med 1991;156:27–30.

[41] Taylor DN, Sanchez JL, Candler W, Thornton S, McQueen C, Echeverria P. Treatment of travelers' diarrhea: ciprofloxacin plus loperamide compared with ciprofloxacin alone. A placebo-controlled, randomized trial. Ann Intern Med 1991;114:731–4.

[42] Bandres JC, Mathewson JJ, Ericsson CD, Dupont HL. Trimethoprim/sulfamethoxazole remains active against enterotoxigenic Escherichia coli and Shigella species in Guadalajara, Mexico. Am J Med Sci 1992;303:289–91.

[43] DuPont HL, Ericsson CD, Mathewson JJ, de la Cabada FJ, Conrad DA. Oral aztreonam, a poorly absorbed yet effective therapy for bacterial diarrhea in US travelers to Mexico. JAMA 1992;267:1932–5.

[44] Vila J, Vargas M, Ruiz J, Corachan M, Jimenez De Anta MT, Gascon J. Quinolone resistance in enterotoxigenic Escherichia coli causing diarrhea in travelers to India in comparison with other geographical areas. Antimicrob Agents Chemother 2000;44:1731–3.

[45] Shaheen HI, Kamal KA, Wasfy MO, et al. Phenotypic diversity of enterotoxigenic escherichia coli (ETEC) isolated from cases of travelers' diarrhea in Kenya. Int J Infect Dis 2003;7:35–8.

[46] Mendez Arancibia E, Pitart C, Ruiz J, Marco F, Gascon J, Vila J. Evolution of antimicrobial resistance in enteroaggregative Escherichia coli and enterotoxigenic Escherichia coli causing traveller's diarrhoea. J Antimicrob Chemother 2009;64:343–7.

[47] Porter CK, Riddle MS, Tribble DR, et al. The epidemiology of travelers' diarrhea in Incirlik, Turkey: a region with a predominance of heat-stabile toxin producing enterotoxigenic Escherichia coli. Diagn Microbiol Infect Dis 2010;66:241–7.

[48] Ouyang-Latimer J, Jafri S, VanTassel A, et al. In vitro antimicrobial susceptibility of bacterial enteropathogens isolated from international travelers to Mexico, Guatemala, and India from 2006 to 2008. Antimicrob Agents Chemother 2011;55:874–8.

[49] Jennings MC, Tilley DH, Ballard SB, et al. Case-case analysis using 7 years of travelers' diarrhea surveillance data: preventive and travel medicine applications in Cusco, Peru. Am J Trop Med Hyg 2017;96:1097–106.

[50] Mason CJ, Sornsakrin S, Seidman JC, et al. Antibiotic resistance in Campylobacter and other diarrheal pathogens isolated from US military personnel deployed to Thailand in 2002-2004: a case-control study. Trop Dis Travel Med Vac 2017;3:13.

[51] Margulieux KR, Srijan A, Ruekit S, et al. Extended-spectrum beta-lactamase prevalence and virulence factor characterization of enterotoxigenic Escherichia coli responsible for acute diarrhea in Nepal from 2001 to 2016. Antimicrob Resist Infect Control 2018;7:87.

[52] Guiral E, Goncalves Quiles M, Munoz L, et al. Emergence of resistance to quinolones and beta-lactam antibiotics in enteroaggregative and enterotoxigenic Escherichia coli causing traveler's diarrhea. Antimicrob Agents Chemother 2019;63::e01745–18.

[53] Murphy H, Bodhidatta L, Sornsakrin S, et al. Traveler's diarrhea in Nepal-changes in etiology and antimicrobial resistance. J Trav Med 2019;26::taz054.

[54] Boxall MD, Day MR, Greig DR, Jenkins C. Antimicrobial resistance profiles of diarrhoeagenic Escherichia coli isolated from travellers returning to the UK, 2015-2017. J Med Microbiol 2020;69:932–43.

[55] Petruccelli BP, Murphy GS, Sanchez JL, et al. Treatment of traveler's diarrhea with ciprofloxacin and loperamide. J Infect Dis 1992;165:557–60.

[56] Hakanen AJ, Lehtopolku M, Siitonen A, Huovinen P, Kotilainen P. Multidrug resistance in Campylobacter jejuni strains collected from Finnish patients during 1995-2000. J Antimicrob Chemother 2003;52:1035–9.

[57] Ronner AC, Engvall EO, Andersson L, Kaijser B. Species identification by genotyping and determination of antibiotic resistance in Campylobacter jejuni and Campylobacter coli from humans and chickens in Sweden. Int J Food Microbiol 2004;96:173–9.

[58] Ruiz J, Marco F, Oliveira I, Vila J, Gascon J. Trends in antimicrobial resistance in Campylobacter spp. causing traveler's diarrhea. APMIS 2007;115:218–24.

[59] Skjot-Rasmussen L, Ethelberg S, Emborg HD, et al. Trends in occurrence of antimicrobial resistance in campylobacter jejuni isolates from broiler chickens, broiler chicken meat, and human domestically acquired cases and travel associated cases in Denmark. Int J Food Microbiol 2009;131:277–9.

[60] Serichantalergs O, Pootong P, Dalsgaard A, et al. PFGE, lior serotype, and antimicrobial resistance patterns among campylobacter jejuni isolated from travelers and US military personnel with acute diarrhea in Thailand, 1998-2003. Gut Pathog 2010;2:15.

[61] Post A, Martiny D, van Waterschoot N, et al. Antibiotic susceptibility profiles among campylobacter isolates obtained from international travelers between 2007 and 2014. Eur J Clin Microbiol Infect Dis 2017;36:2101–7.

[62] Gaarslev K, Stenderup J. Changes during travel in the composition and antibiotic resistance pattern of the intestinal enterobacteriaceae flora: results from a study of mecillinam prophylaxis against travellers' diarrhoea. Curr Med Res Opin 1985;9:384–7.

[63] Wiström J, Norrby SR, Burman LG, Lundholm R, Jellheden B, Englund G. Norfloxacin versus placebo for prophylaxis against travellers' diarrhoea. J Antimicrob Chemother 1987;20:563–74.

[64] Wiström J, Jertborn M, Hedstrom SA, et al. Short-term self-treatment of travellers' diarrhoea with norfloxacin: a placebo-controlled study. J Antimicrob Chemother 1989;23:905–13.

[65] Rademaker CM, Hoepelman IM, Wolfhagen MJ, Beumer H, Rozenberg-Arska M, Verhoef J. Results of a double-blind placebo-controlled study using ciprofloxacin for prevention of travelers' diarrhea. Eur J Clin Microbiol Infect Dis 1989;8:690–4.
[66] Huang DB, Jiang ZD, Ericsson CD, Adachi J, Dupont HL. Emergence of trimethoprim-resistant escherichia coli in healthy persons in the absence of prophylactic or therapeutic antibiotics during travel to guadalajara, Mexico. Scand J Infect Dis 2001;33:812–4.
[67] Jiang ZD, Lowe B, Verenkar MP, 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.
[68] Blyth DM, Mende K, Maranich AM, et al. Antimicrobial resistance acquisition after international travel in U.S. travelers. Trop Dis Travel Med Vaccines 2016;2:4.
[69] Lai CC, Chen CC, Huang HL, Chuang YC, Tang HJ. The role of doxycycline in the therapy of multidrug-resistant E. coli - an in vitro study. Sci Rep 2016;6:31964.