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Original article

Fluoroquinolone antibiotic users select fluoroquinolone-resistant ESBL-producing *Enterobacteriaceae* (ESBL-PE) – Data of a prospective traveller study

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ABSTRACT

Background: One third of travellers to the poor regions of the (sub)tropics become colonized by extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE). Co-resistance to non-beta-lactam antibiotics complicates the treatment of potential ESBL-PE infections.
Methods: We analysed co-resistance to non-beta-lactams among travel-acquired ESBL-PE isolates of 90 visitors to the (sub)tropics with respect to major risk factors of colonization: destination, age, travellers' diarrhoea (TD) and antibiotic (AB) use.
Results: Of the ESBL-PE isolates, 53%, 52%, 73%, and 2% proved co-resistant to ciprofloxacin, tobramycin, co-trimoxazole, and nitrofurantoin, respectively. The rates were similar among those with (TD+) or without (TD-) travellers' diarrhoea. Among fluoroquinolone-users vs. AB non-users, the co-resistance rates for ciprofloxacin were 95% versus 37% ($p = 0.001$), for tobramycin 85% versus 43% ($p = 0.005$), co-trimoxazole 85% versus 68% ($p = 0.146$), and nitrofurantoin 5% versus 2% ($p = 0.147$). In multivariable analysis co-resistance to ciprofloxacin was associated with increasing age, fluoroquinolone use, and tobramycin resistance.
Conlusions: While TD predisposes to ESBL-PE non-selectively, antimicrobial use favours strains resistant to drug taken and, simultaneously, any drug with resistance genetically linked to the drug used. Antibiotics taken during travel predispose to ESBL-PE with a high co-resistance rate.

1. Introduction

International travel is one of the central means by which resistant intestinal bacteria spread across the globe [1–5]: a substantial proportion of travellers get colonized by multiresistant intestinal bacteria, especially extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) [6–15]. The risk is particularly high in regions with poor hygiene and weakly implemented antimicrobial policy, i.e. South Asia [6–9,11–15] followed by Southeast Asia, Africa and Latin America. The same regions are associated with increased risk of travellers' diarrhoea (TD) [16,17]. A number of studies have shown both TD [8,11–15] and antibiotic use during travel [11–15] to be independent risk factors for colonization by ESBL-PE. Besides ESBL-PE, antibiotic users also tend to acquire ciprofloxacin-resistant intestinal bacteria [14]. Likewise, in placebo-controlled studies of travellers taking mecillinam [18] or trimethoprim/co-trimoxazole [19], antibiotic users have shown increased rates of resistant *E. coli*.

Antimicrobials disrupt the ecological balance of an individual's own microbiota, thus breaking colonization resistance [20,21]. The presence of the antibiotic favours growth and colonization by drug-resistant organisms [20–22] and, occasionally, such bacteria succeed in causing a clinical infection. As the spread of antimicrobial resistance across the globe may to a large extent be ascribed to travellers, understanding the sequelae of their antibiotic use is especially valuable.

Several studies have shown that ESBL-PE carried by travellers

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List of abbreviations

AB	antimicrobial
ESBL	extended-spectrum beta-lactamase
ESBL-PE	extended-spectrum beta-lactamase-producing *Enterobacteriaceae*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FQ	fluoroquinolone
MIC	Minimum Inhibitory Concentration
PCR	polymerase chain reaction
SIR	**S**usceptible, **I**ntermediate, **R**esistant
TD	travellers' diarrhoea

may be co-resistant to non-beta-lactam antibiotics such as fluoroquinolones (FQs) [6,8–10]. Surprisingly, although co-resistance further complicates treatment of severe ESBL-PE infections, and adds to the toll of resistance at hospitals, no studies have thus far addressed factors determining whether travellers select ESBL-PE with high or low co-resistance rate. We examined whether the four major risk factors predisposing to ESBL-PE acquisition (destination, age, TD and antimicrobial use [6–15]) also contribute to selecting such co-resistant strains. In addition, we conducted a multivariable analysis to identify factors associated with/predisposing to decreased susceptibility to ciprofloxacin, searching tools for avoiding acquisition of ESBL-PE strains co-resistant to FQ, our most effective peroral antibiotics for severe ESBL-PE infections.

2. Materials and Methods

2.1. Study design, volunteers and samples

To focus on the risk of acquiring ESBL-PE with co-resistance to non-beta-lactams, we selected among the 430 travellers in our previous study [11] all the 90 who had contracted ESBL-PE while abroad. Volunteers had been recruited for the earlier investigation prospectively over a period of 12 months in 2009–2010; all our 90 ESBL-PE carriers had visited (sub) tropical regions. Each of them had provided pre- and post-travel stool samples and filled in two sets of forms: pre-travel questionnaire covering personal information, medical history, and itinerary, and post-travel questionnaire covering symptoms, medications etc. abroad.

The outlines of the current study are presented in Fig. 1. We have earlier reported findings of the same cohort with respect to travel-related health problems [23], travel-acquired ESBL-PE [11], and various diarrhoeal pathogens [24].

Written informed consent was obtained from all subjects. The study protocol was approved by the Ethics Committee of the Department of Medicine in Helsinki University Hospital (406/13/03/01/08).

2.2. Identification of ESBL-PE and analysis of co-resistance to various antibiotics

The identification of ESBL-producing strains by phenotypical and antibiotic resistance is described in detail in our previous study. In brief, the strains were identified by the automated VITEK GN system (bioMérieux, Marcy l'Etoile, France), and the production of extended-spectrum beta-lactamase by double disk synergy tests (Oxoid, Thermo Fisher Scientific, Cambridge, UK). Further susceptibility testing was carried out for various antimicrobials according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org), using Muller Hinton agar (Oxoid, Thermo Fisher Scientific, Cambridge, UK) and Etests (bioMérieux, Marcy l'Etoile, France; Liofilchem, Roseto Degli Abruzzi, Italy). The susceptibility results were interpreted as Minimum Inhibitory Concentrations (MIC) breakpoints according to limits recommended by the EUCAST (v 6.0) [25].

For travellers who had contracted more than one ESBP-PE strain, the most resistant one was selected (as graded by degree of resistance to ciprofloxacin and tobramycin, in this order). Of 98 strains, 90 were eventually selected.

2.3. Analysis of co-resistance with respect to major risk factors

The co-resistance of the ESBL strains was analysed with respect to the four main risk factors categorized as follows:

Travel destination: (1) South Asia, (2) Southeast Asia, (3) East Asia, (4) North Africa and the Middle East, (5) Sub-Saharan Africa, and (6) South and Central America, and the Caribbean.

Age: 0–30, 30–50 and over 50 years of age.

Travellers' diarrhoea: TD+ and TD− groups according to occurrence of travellers' diarrhoea [7]; TD defined by WHO criteria as passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual.

Antibiotic use: AB+ and AB− groups according to antibiotic use abroad. AB + group further divided by AB type: (1) FQ users and (2) users of other antibiotics or regimen not known.

2.4. Analysis of factors associated with FQ resistance

Using a multivariable model, data obtained from questionnaires concerning travel-related specifics (itinerary, behaviour, symptoms and medication during travel etc.) were studied for association with ciprofloxacin resistance.

2.5. Statistics

Statistical analyses were carried out by SPSS software version 22 (IBM Corp, Armonk, NY, USA). The chi-square test, Fisher's exact test or binary logistic regression analysis were used to compare categorical variables when applicable, and binary logistic regression, Mann-Whitney *U* test, and Kruskall Wallis test were used with continuous variables when applicable.

Variables with a p-value <0.1 in the univariate analysis for ciprofloxacin resistance were included in multivariable analyses together with TD and geographic region, both of which are risk factors that several studies have found to be linked with ESBL-PE colonization. We included age and age^2 as continuous variables in the model together with age as a categorical variable for sensitivity analysis. The final model was built by using binary logistic regression analysis with a stepwise backward selection of variables by Akaike Information Criteria (AIC).

3. Results

3.1. Description of travellers and travels

The demographics of the 90 volunteers who had contracted ESBL-PE while travelling are presented in Table 1. Data on AB+, AB−, TD+ and TD− groups are provided separately.

3.2. Antibiotic susceptibility of ESBL-PE strains

According to MIC values, most of the ESBL-PE strains were found resistant or showed decreased sensitivity to ampicillin (100%),

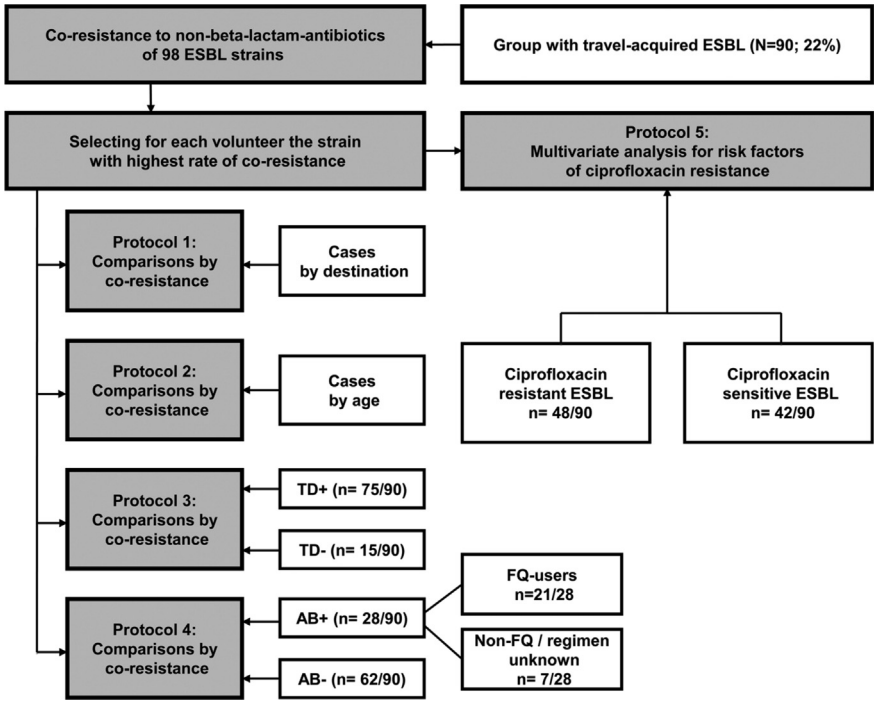

Fig. 1. Flow chart of study protocol. The ESBL-PE isolates were tested for antimicrobial susceptibility. Separate analyses were carried out to identify the influence of destination, age, TD (TD + versus TD− groups) and antibiotic use (AB− group, FQ users, those using other antibiotics/regimen not known) on co-resistance. Finally, multivariable analysis was conducted to find out factors associated with co-resistance to ciprofloxacin.

amoxicillin + clavulanic acid (59%), cefalexin (100%), cefuroxime (96%), ceftriaxone (96%), ceftatzidime (73%), cefepime (88%), ciprofloxacin (53%), levofloxacin (53%), tobramycin (52%), and co-trimoxazole (73%), whereas resistance proved uncommon to nitrofurantoin (2%), ertapenem (2%), meropenem (0%), imipenem (0%), piperacillin + tazobactam (4%), tigecyclin (0%), and colistin (1%).

Resistance to non-beta-lactams ciprofloxacin, tobramycin, co-trimoxazole and nitrofurantoin was subjected to further analyses. Table 2 shows medians of the MIC values and interpretation of co-resistance (intermediate or resistant) to these antibiotics.

3.3. Association of geographic region, age, TD, and antimicrobial use with co-resistance among travel-acquired ESBL-PE

In the destination-specific analysis, strains contracted in South Asia appeared more sensitive to tobramycin than those originating in other regions (Table 2). As for sensitivity to other non-beta-lactams covered, no differences were seen between the various regions.

Co-resistance of ESBL strains to ciprofloxacin and co-trimoxazole was associated with increasing age; no such differences were seen for tobramycin or nitrofurantoin (Table 2).

TD was reported by 75/90 (83%) of the subjects. Comparison between the TD+ and TD− groups revealed no significant differences in co-resistance rates to the antibiotics included in the analysis (Table 2).

The proportion of ESBL-PE strains resistant to ciprofloxacin was 37% among those who had refrained from taking antimicrobials, but 95% among those who had taken FQ while abroad (Table 2). The use of FQ also increased the proportion of ESBL-PE resistant to tobramycin (85% versus 32%, respectively). The indication for taking antibiotics was TD in 24/28 cases (86%).

Data on resistance to a given antimicrobial related to resistance to others are shown in Table 2.

3.4. Identification of factors associated with co-resistance to ciprofloxacin among travel-acquired ESBL-PE

The univariate analysis (Table 3) detected the following factors with $p < 0.1$: (1) tobramycin resistance; (2) co-trimoxazole resistance; (3) age and age group; (4) use of fluoroquinolones; (5) use of antibiotics other than fluoroquinolones/regimen not known. When all these were subjected to multivariable analysis together with geographic region and TD, the following were found to be independently associated with co-resistance to ciprofloxacin (Table 4): (1) tobramycin resistance; (2) age group; (3) use of fluoroquinolones; (4) use of antibiotics other than fluoroquinolones/regimen not known. Age explained the outcome better as categorical than continuous variable. The fit of the model appears rather good (Nagelkerke R square 0.543), suggesting that the model predicts outcome values quite well.

4. Discussion

Travellers visiting regions with uncontrolled antibiotic use and poor hygiene are known to acquire resistant bacteria and carry them from one geographic region to another [1–15,26]. While recent data show that destination [6–9,11–15], age [8,11], TD [7,8,11,13–15] and antibiotic use [11–15] all predispose to colonization by ESBL-PE, our data reveal that the menace posed by antimicrobials is even worse. Not only do antibiotic users contract higher rates of ESBL-PE but, indeed, the acquisition process is selective, favouring strains resistant to, besides many beta-lactams, also some clinically important non-beta-lactams.

4.1. Co-resistance to non-beta-lactam antibiotics among travel-acquired ESBL-PE

It is clinically noteworthy that many ESBL-PE strains are co-resistant to some non-beta-lactams [6–10]. Likewise, our strains

Table 1
Demographics of 90 travellers colonized by extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) during journey: data provided separately for those with TD (TD+) or asymptomatic (TD−) and those using (AB+) and not using (AB−) antibiotics.

	ESBL(+) n (%)	TD+ n (%)	TD− n (%)	AB+ n (%)	**AB−** n (%)
Sex					
Male	51 (46)	34 (45)	7 (47)	12 (43)	29 (47)
Female	49 (54)	41 (55)	8 (53)	16 (57)	33 (53)
TD					
No	15 (17)	N/A	N/A	0 (0)	15 (24)
Yes	75 (83)	N/A	N/A	28 (100)	47 (76)
Geographic region					
South Asia	28 (31)	25 (33)	3 (20)	8 (29)	20 (32)
Southeast Asia	33 (37)	30 (40)	3 (20)	9 (32)	24 (39)
East Asia	2 (2)	2 (3)	0 (0)	2 (7)	0 (0)
North Africa and the Middle East	4 (4)	2 (3)	2 (14)	1 (4)	3 (5)
Sub Saharan Africa	23 (26)	16 (21)	7 (47)	8 (29)	15 (24)
South America, Central America and the Caribbean	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Europe, Australia, and North America	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Length of journey					
Days, median	16.5 (IQR 15.0)	19 (IQR 16.0)	13 (IQR 11.0)	21 (IQR 15.3)	16 (IQR 12.3)
Age					
Years, median	36.5 (IQR 30.0)	33 (IQR 23.0)	58 (IQR 20.0)	32 (IQR 31.0)	41 (IQR 29.5)
Total	**90 (100)**	**75 (83)**	**15 (17)**	**28 (31)**	**62 (69)**

proved co-resistant: 53%, 52% and 73% of them to ciprofloxacin, tobramycin and co-trimoxazole, respectively. The low degree of resistance to nitrofurantoin (2% in our study) is relevant to treatment, for this drug presents a peroral alternative in uncomplicated cystitis. Practically all strains were found sensitive to carbapenems, drugs commonly used as first-line intravenous alternatives in severe ESBL infections.

4.2. Effect of geographic region, age and TD on co-resistance among travel-acquired ESBL-PE strains

Our data did not reveal major differences in the antibiotic resistance profiles between the various destinations, which may reflect, besides high antibiotic pressure in all of them, also the liability of ESBL-PE clones to spread from one region to others, and, in some cases, even across the globe [1,2].

Our finding that older age is associated with risk of acquiring co-trimoxazole- and ciprofloxacin-resistant ESBL-PE was unexpected, and we can only speculate about the reasons. In previous reports, increasing age has been found to be a risk factor for contracting ESBL-PE [8,11].

The resistance profiles of the ESBL-PE strains did not differ between the TD+ and TD− groups. The underlying mechanisms in TD thus appear to be non-selective: disturbance in the microbiota [27] makes space for all new bacteria alike. The only difference to those with no TD is a higher load of newcomers, as suggested by the increased rate of ESBL-PE acquisition. Therefore the selection of ESBL-PE among these two groups can be presumed to reflect the general profile of the ESBL-PE to which travellers are exposed in their destinations (Fig. 2).

4.3. Effect of antimicrobial use on co-resistance among travel-acquired ESBL-PE strains

The main finding of the present study is that among antibiotic users the process of ESBL-PE acquisition is selective, favouring strains resistant to the drug taken (Fig. 2, Table 4). We discovered that while 37% of the ESBL-PE strains contracted by travellers who had not used antibiotics were ciprofloxacin-resistant, among FQ-users the rate was as high as 95%. This seems logical, for each antimicrobial is expected not only to kill bacteria sensitive to that drug but – at the same time – also open a niche in the intestinal microbiota for strains resistant to it [20,21,28].

Virtually all ESBL bacteria in the group using FQs were co-resistant to ciprofloxacin. This demonstrates the enormous impact of antibiotic pressure and selection in the intestine after the susceptible bacteria have been killed by the antibiotic. In fact, as reported also by a recent study by Reuland et al. [14], besides ESBLs, antibiotic users also acquire ciprofloxacin-resistant gram-negative rods. Likewise, in placebo-controlled studies of travellers taking mecillinam [18] or trimethoprim/co-trimoxazole [19], antibiotic users had increased rates of *E. coli* resistant to the drug taken; no such increase was seen when these drugs were taken by travellers to other than high risk regions [29,30]. Indeed, in our study, FQ use probably favoured any ciprofloxacin-resistant strains, yet, as only ESBL-PE was measured, we found an increase in ESBL-PE co-resistant to ciprofloxacin. It is noteworthy that, as ESBL bacteria have co-resistance to numerous other antimicrobials, using any of those will probably further increase the risk of contracting ESBL-PE.

4.4. Impact of genetic linkage between resistance to various antibiotics

Another interesting aspect is the genetic linkage between certain resistance genes: the plasmid harbouring the bla-CTX-M gene, for example, may carry also FQ resistance genes, and genes encoding AmpC beta-lactamases (plasmid blaAmpC) and carbapenemases or enzymes inactivating aminoglycosides [31]. Due to the co-selection process, the presence of any of these antibiotics would give advantage to blaCTX-M [31]. In addition to common topoisomerase gene point-mutations and active efflux pumps, norfloxacin and ciprofloxacin resistance may be linked with aminoglycoside resistance owing to the plasmid-mediated resistance gene aac(6′)-*Ib-cr* which confers resistance to both antibiotic groups [32]. Depending on sequence type and plasmid profile, strains may also confer resistance to tetracycline, nalidixic acid, chloramphenicol, trimethoprim, and sulfonamides [33,34]. In accord with the genetic link between FQ and tobramycin resistance, tobramycin co-resistance was more frequent among ESBL-PE contracted by those who had taken ciprofloxacin during travel than

Table 2
Relation between co-resistance among travel-acquired ESBL-PE and geographic region visited, age group, TD, use of antimicrobials (AB) and simultaneous co-resistance to ciprofloxacin, tobramycin, co-trimoxazole and nitrofurantoin. For antimicrobial use, data are shown separately for those not having used antibiotics (AB−), those having taken fluoroquinolones, and those having used other antibiotics/whose regimen was not known. MICs are given as medians of values. Each strain was interpreted as resistant (R) or intermediate (I) according to MICs using EUCAST clinical breakpoints. All strains were *Escherichia coli*, apart from one *Klebsiella pneumoniae* and one *E. hermannii*.

	Total	Ciprofloxacin				Tobramycin				Co-trimoxazole				Nitrofurantoin			
	n (%)	MIC (mg/l)	(R + I) n (%)	OR (95% CI)	p-value	MIC (mg/l)	(R + I) n (%)	OR (95% CI)	p-value	MIC (mg/l)	(R + I) n (%)	OR (95% CI)	p-value	MIC (mg/l)	(R + I) n (%)	OR (95% CI)	p-value
Geographic region																	
South Asia	28 (31)	0.44	13 (46)	1.0		<1	7 (25)	1.0		>32	18 (64)	1.0		12	0 (0)	1.0	
South East Asia	33 (37)	2	17 (52)	1.2 (0.4–3.3)	0.692	3	22 (67)	6.0 (2.0–18.4)	0.002	>32	26 (79)	2.1 (0.7–6.4)	0.212	8	3 (9)	0.8 (0.1–14.1)	0.906
East Asia	2 (2)	>32	2 (100)	N/A	N/A	5	2 (100)	N/A	N/A	>32	2 (100)	N/A	0.999	16	0 (0)	N/A	0.999
North Africa and Middle East	4 (4)	0.014	1 (25)	0.4 (0.0–4.2)	0.432	<1	1 (25)	1.0 (0.1–11.2)	1.000	16	2 (50)	0.6 (0.1–4.6)	0.585	12	0 (0)	N/A	0.999
Sub–Saharan Africa	23 (26)	>32	15 (65)	2.2 (0.7–6.7)	0.183	6	15 (65)	5.6 (1.7–18.9)	0.005	>32	18 (78)	2.0 (0.6–7.0)	0.280	8	1 (4)	N/A	0.998
p-value for MIC (geographic region)		0.172				0.118				0.414				0.196			
Age group																	
0–30	29 (32)	0.25	10 (34)	1.0		2	14 (48)	1.0		>32	23 (55)	1.0		12	2 (7)	1.0	
31–50	33 (37)	>32	18 (55)	2.3 (0.8–6.4)	0.116	3	18 (55)	1.3 (0.5–3.5)	0.622	>32	27 (82)	3.7 (1.2–11.5)	0.027	8	0 (0)	N/A	0.998
over 50	28 (31)	>32	20 (71)	4.8 (1.5–14.6)	0.006	3	15 (54)	1.2 (0.2–3.5)	0.689	>32	23 (82)	3.7 (1.1–12.6)	0.033	12	0 (0)	N/A	0.998
p-value for MIC (age group)		0.059				0.778				0.056				0.291			
TD																	
No TD	15 (17)	0.38	7 (47)	1.0		1	6 (43)	1.0		>32	9 (60)	1.0		12	0 (0)	1.0	
TD	75 (83)	32	41 (55)	1.4 (0.5–4.2)	0.572	3	41 (55)	1.6 (0.5–5.1)	0.416	>32	57 (76)	2.1 (0.7–6.7)	0.207	12	2 (3)	1.0 (1.0–1.1)	0.522
p-value for MIC (TD)		0.198				0.332				0.195				0.830			
Use of antimicrobials																	
No use of antimicrobials	60 (69)	0.38	23 (37)	1.0		1	26 (43)	1.0		>32	42 (68)	1.0		12	1 (2)	1.0	
Use of fluoroquinolones[a]	21 (23)	>32	19 (95)	32.2 (4.0–256.8)	0.001	7	17 (85)	5.5 (1.7–18.3)	0.005	>32	17 (85)	2.7 (0.7–10.3)	0.146	8	1 (5)	3.2 (0.2–53.8)	0.417
Use of antimicrobial other than FQ/regimen not known[b]	7 (8)	>32	6 (75)	5.1 (0.9–27.3)	0.058	3	4 (50)	1.0 (0.2–4.7)	0.972	>32	7 (88)	3.3 (0.4–29.0)	0.275	N/A	0 (0)	N/A	N/A
p-value for MIC (use of AB)		<0.001				0.002				0.184				0.011			
Co-resistance to other antimicrobials																	
Ciprofloxacin	48 (53)	–	–	–	–	6	35 (73)	6.7 (2.7–17.0)	<0.001	>32	42 (88)	5.3 (1.8–15.0)	0.002	8	1 (2)	0.9 (0.1–14.4)	1.000
Tobramycin	47 (52)	32	35 (75)	6.7 (2.7–17.0)	<0.001	–	–	–	–	>32	41 (87)	4.9 (1.7–14.1)	0.002	8	2 (4)	N/A	0.495
Co-trimoxazole	66 (73)	32	42 (64)	5.3 (1.8–15.0)	0.002	3	41 (62)	4.9 (1.7–14.1)	0.002	–	–	–	–		1 (2)	0.4 (0.0–5.9)	0.464
Nitrofurantoin	2 (2)	N/A	1 (50)	0.9 (0.1–14.4)	1.000	N/A	2 (100)	N/A	0.495	N/A	1 (2)	0.4 (0.0–5.9)	0.464	–	–	–	–
Total	**90**	**6**	**48 (53)**			**3**	**47 (52)**			**>32**	**66 (73)**			**12**	**4 (4)**		

[a] In addition to FQ, one subject took also ceftriaxone, one amoxixillin-clavulanic acid, and one nitroimidazole plus roxithromycin.
[b] Three took azithromycin, one amoxicillin-clavulanic acid; in three cases the regimen was unknown.

those with no antibiotic use (85% versus 43%). By contrast, nitrofurantoin and co-trimoxazole resistance rates were not significantly influenced by antimicrobial use, presumably because of their resistance mechanism not being linked to ciprofloxacin resistance genes for instance in the same success clones with typical mobile elements. In principle one could, therefore, to a certain degree predict the types of potential resistance problems by the antimicrobial taken.

4.5. Scenery of impact of antibiotic use

Taken together, antibiotics appear to play three vicious tricks on travellers. Firstly, they disrupt intestinal colonization resistance and make space for newcomers; secondly, they only favour colonization by bacteria resistant to the antibiotic taken; and last but not least, resistant bacteria tending to be co-resistant to other antibiotics, users end up selecting the most resistant bacteria from the surroundings (Fig. 2). Besides adding to the vast body of research reporting an increased risk for travellers to contract ESBL-PE [6–15,26], the present data reveal that the strains acquired are not just any ESBL-PE but those with the highest co-resistance rates: in FQ users the selection favours co-resistance to FQs and tobramycin, both drugs used for severe infections and thus of great clinical importance.

Table 3
Factors associated with colonization by ciprofloxacin-resistant ESBL-PE strains: results of univariate analysis.

Risk factors	All (n = 90) n (%)	Ciprofloxacin I + R n (%)	Univariate OR (95% CI)	p in univariate analysis
Decreased susceptibility or resistance to non-beta-lactams				
Nitrofurantoin I + R	2 (2)	1(50)	0.9 (0.1–14.4)	1.000
Tobramycin I + R[a]	47 (52)	35 (75)	6.7 (2.7–17.0)	<0.001
Co-trimoxazole I + R[a]	66 (73)	42 (64)	5.3 (1.8–15.0)	0.002
Sex				
Male	41 (46)	18 (44)	1.0	N/A
Female	49 (54)	30 (61)	2.0 (0.9–4.7)	0.101
Age[a], years, median, IQR	36.5 (IQR 30) (range 11–77)	47 (IQR 29.5) (range 24–77)	1.0 (1.0–1.1)	0.007
Age group[a]				0.020
0–30	29 (32)	10 (34)	1.0	N/A
31–50	33 (37)	18 (55)	2.3 (0.8–6.4)	0.116
Over 50	28 (31)	20 (71)	4.8 (1.5–14.6)	0.006
Geographic region[a]				0.295
South Asia	28 (31)	13 (46)	1.0	N/A
Southeast Asia	33 (37)	17 (52)	1.2 (0.4–3.3)	0.692
East Asia	2 (2)	2 (100)	N/A	N/A
North Africa and the Middle East	4 (4)	1 (25)	0.4 (0.0–4.2)	0.432
Sub Saharan Africa	23 (26)	15 (65)	2.2 (0.7–6.7)	0.183
South America, Central America and the Caribbean	0 (0)	0	N/A	N/A
North America, Europe, Australia	0 (0)	0	N/A	N/A
Length of journey				
Days, median	16.5 (IQR 15) (range 5–133)	17.5 (IQR 15.5) (range 5–133)	1.0 (1.0–1.0)	0.679
Group				0.177
2 weeks or less	33 (37)	15 (46)	1.0	N/A
2 weeks–1 month	42 (47)	26 (62)	2.0 (0.8–4.9)	0.157
Over 1 month	15 (47)	7 (47)	0.9 (0.3–3.5)	0.938
TD[a]				
No TD	15 (17)	7 (47)	1.0	N/A
TD	75 (83)	41 (55)	1.4 (0.5–4.2)	0.572
Use of loperamide	28 (31)	13 (46)	0.7 (0.3–1.6)	0.378
Use of antimicrobial medications[a]				
None	62 (69)	23 (37)	1.0	
FQ (vs noAB)	20 (22)	19 (95)	32.2 (4.0–256.8)	0.001
other/regimen unknown (vs no AB)	8 (9)	6 (75)	5.1 (0.9–27.3)	0.058
Other factors				
Use of alcohol: 3 or more vs. 0–2 units/day (information missing 8)	16 (20)	10 (63)	1.6 (0.5–4.8)	0.429
Meals with locals (information missing 3)	7 (8)	3 (43)	0.6 (0.1–2.9)	0.536
Site of meals: restaurant>50% vs. mainly at own household (information missing 1)	82 (92)	45 (55)	1.6 (0.3–7.7)	0.540
Contact with local healthcare	15 (17)	10 (67)	1.9 (0.6–6.2)	0.257
Accommodation hotel/guesthouse vs. with locals/own household (information missing 3)	80 (92)	43 (54)	1.6 (0.3–7.4)	0.580
Use of probiotics	51 (57)	29 (57)	1.4 (0.6–3.2)	0.443
Use of bottled water	86 (96)	46 (54)	1.2 (0.2–8.5)	0.891
Uncooked meat or fish	12 (13)	6 (50)	0.8 (0.2–2.9)	0.731
Neglecting hand-washing (information missing 1)	10 (11)	5 (50)	0.9 (0.2–3.3)	0.850
Eating salads (information missing 5)	64 (75)	36 (56)	1.4 (0.5–3.8)	0.491
Chronic illness	22 (24)	13 (59)	1.4 (0.5–3.6)	0.533
Antimalarial chemoprophylaxis	48 (53)	25 (52)	0.9 (0.4–2.1)	0.799

[a] Included in multivariable analysis (see Table 4).

Table 4
Factors associated with colonization by fluoroquinolone-resistant ESBL-PE strains in final multivariable model after backward selection of factors by AIC. Backward selection eliminated the following factors: decreased sensitivity to co-trimoxazole, TD, age as a continuous variable and geographic region. Values are presented for proportions with a given risk factor, adjusted odds ratios (AOR), and p-values in multivariable analysis.

Risk factors	All (n = 90) n (%)	Ciprofloxacin I + R	OR (95% CI)	p in univariate analysis	AOR (95% CI)	p in multivariable analysis
Decreased susceptibility or resistance to non-beta-lactams						
Tobramycin R + I	47 (52)	35 (75)	6.7 (2.7–17.0)	<0.001	5.4 (1.7–17.0)	0.004
Age group						
0–30	29 (32)	10 (34)	1.0	N/A	1.0	N/A
31–50	33 (37)	18 (55)	2.3 (0.8–6.4)	0.116	4.9 (1.1–23.1)	0.430
Over 50	28 (31)	20 (71)	4.8 (1.5–14.6)	0.006	14.0 (2.7–71.5)	0.002
Use of antimicrobial medications						
None	62 (69)	23 (37)	1.0	N/A	1.0	N/A
FQ (vs noAB)	20 (22)	19 (95)	32.2 (4.0–256.8)	0.001	41.8 (4.0–436.3)	0.002
Other/regimen unknown (vs no AB)	8 (9)	6 (75)	5.1 (0.9–27.3)	0.058	8.4 (1.2–59.1)	0.032

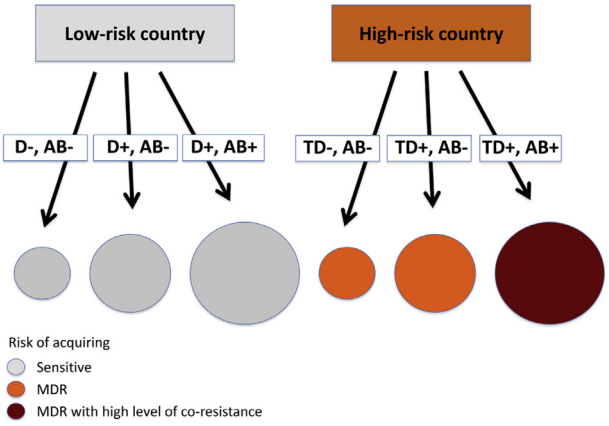

Fig. 2. Hypothetical model of the influence of destination, diarrhoea (D)/travellers' diarrhoea (TD) and antibiotic (AB) use on acquisition of multidrug-resistant (MDR) intestinal bacteria. Size of circle corresponds to relative risk of acquisition. Interpretation: MDR bacteria can only be contracted in an environment where they are prevalent (low-versus high-risk regions). Diarrhoea predisposes to bacterial acquisition *unselectively*: higher loads are contracted by those *with* than those without D/TD, yet the MDR proportion equals that in the surroundings. ABs predispose to bacterial acquisition *selectively*: (1) users not only contract MDR in greater proportions than present in the surroundings, but (2) they select those MDR which are co-resistant to the AB they used – and possibly even resistance to ABs genetically linked to that particular drug. AB users thus end up selecting MDR bacteria with the highest co-resistance rates.

4.6. Factors associated with ciprofloxacin resistance of ESBL-PE

To explore further the clinically relevant FQ resistance, we searched for factors associated with colonization by ciprofloxacin-resistant strains among ESBL-PE. A recent study identified two independent risk factors predisposing to ciprofloxacin-resistant strains: travel to Asia, and combination of TD and antibiotic use [14]. Our analysis showed FQ use, use of other antimicrobials, tobramycin resistance, and age to be linked with co-resistance to ciprofloxacin among ESBL-PE. Interestingly, TD was not a significant risk factor for ciprofloxacin co-resistance. This finding accords with the discussion above, where TD is considered to predispose travellers to resistant intestinal bacteria in the surroundings non-selectively, without favouring FQ-resistant strains.

4.7. Limitations

Although our statistical analyses showed that the use of "other antibiotics/regimen not known" predisposes to selecting ciprofloxacin-resistant ESBL-PE, we do not regard this result as quite reliable, with the number of cases being only seven and the antibiotic regimen not known in three of them, meaning that it could have been a FQ.

It would have been of interest also to examine the resistance profiles of some other intestinal bacteria, but we only had the ESBL-PE available. On the other hand, it appears reasonable to consider ESBL-PE here as a model, and our findings thus applicable to other intestinal gram-negative bacteria.

4.8. Practical points

We recently concluded that to prevent ESBL-PE colonization, travellers should be advised to prevention of TD and avoiding unnecessary antimicrobials [11,35]. The current results highlight the very same point: to prevent travellers from contracting the most resistant ESBL, i.e. those with co-resistance to clinically important non-beta-lactams, when giving pre-travel advice, particular effort should be put into educating travellers to caution in treating TD with antibiotics. Since mild and moderate TD are typically characterized by spontaneous recovery [17], antibiotics should be reserved for severe disease only. In mild/moderate cases where medication is needed, antisecretory/antimotility regimens can be used: our recent systematic review on loperamide found no proof of it being less effective than antibiotic treatment [36]. Furthermore, loperamide is safe [36], and when taken singly, does not increase the risk of colonization by ESBL-PE [37].

If hospitalized at home, returning travellers having treated TD with antimicrobials while abroad should be considered risk patients: co-resistance to non-beta-lactams among ESBL strains leads to increased use of carbapenems, further adding to selection pressure at hospitals. Practically all our FQ users had ESBL-PE co-resistant to ciprofloxacin, which indicates that fluoroquinolones should not be used as empiric treatment (in urinary tract infection, for example) for returning travellers who have used these drugs when visiting high-risk regions.

4.9. Conclusions

Researching into the effect of TD and antibiotic use on the acquisition of ESBL-PE while abroad, we found TD to predispose travellers unselectively to any ESBL-PE, while FQ proved to predispose both to strains co-resistant to the antibiotic used and those genetically linked to FQ-resistance. Thus, use of FQs predisposes not only to contracting ESBL-PE strains but, indeed, also to selecting ESBL-PE strains co-resistant to certain clinically important non-beta-lactam antibiotics. The data reveal that the perils of antibiotic use during travel are even greater than hitherto recognized.

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Author contributions

Study concept and design: AK, JK; acquisition of data AK, SM, JK; analysis and interpretation of data AK, TL; statistical analysis TL; drafting of manuscript AK, TL; critical comments on manuscript SM, JK; final approval of version submitted AK, SM, JK, TL.

Conflicts of interest

AK has received honorary for lectures (Pfizer, MSD, Valneva) and membership in advisory board (Valneva), and an investigator-initiated grant (Pfizer), none of these relevant to the current manuscript. SM, JK, TL declare no conflicts of interest.

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