Original Article

Extended-spectrum beta-lactamase-producing strains among diarrhoeagenic Escherichia coli—prospective traveller study with literature review

Anu Kantele MD PhD Professor1,2, and Tinja Lääveri MD PhD2

1Meilahti Vaccine Research Center MeVac, University of Helsinki and Helsinki University Hospital, Helsinki, Finland and 2Department of Infectious Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

*To whom correspondence should be addressed. Professor Anu Kantele, Meilahti Vaccine Research Center MeVac, University of Helsinki and Helsinki University Hospital, Biomedicum 1, Haartmaninkatu 8, FI-00029 HUS, Finland; Tel: +358-50-309-7640; Email: anu.kantele@hus.fi

Submitted 3 January 2020; Revised 27 January 2020; Editorial Decision 29 January 2020; Accepted 29 January 2020

Abstract

Background: Antibiotics are no longer the primary approach for treating all travellers’ diarrhoea (TD): most cases resolve without antibiotics and using them predisposes to colonization by multidrug-resistant bacteria. Data are accumulating on increasing resistance among TD pathogens, yet research into the most common agents, diarrhoeagenic Escherichia coli (DEC), remains limited.

Methods: A total of 413 travellers to the (sub)tropics were analyzed for travel-acquired diarrhoeal pathogens and ESBL-PE. To identify ESBL-producing DEC, ESBL-producing E. coli (ESBL-EC) isolates were subjected to multiplex qPCR for various DEC pathotypes: enteroaggregative (EAEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC) E. coli. For a literature review, we screened studies among travellers and locals in low- and middle-income countries (LMICs) on the frequency of ESBL-EC, and among travellers, also DEC with resistance to ciprofloxacin, azithromycin, and rifamycin derivatives.

Results: Our rate of ESBL-EC among all DEC findings was 2.7% (13/475); among EAEC 5.7% (10/175), EPEC 1.1% (2/180), ETEC 1.3% (1/80) and EHEC (0/35) or EIEC 0% (0/5). The literature search yielded three studies reporting ESBL-EC frequency and thirteen exploring resistance to TD antibiotics among travel-acquired DEC. For EAEC and ETEC, the ESBL-EC rates were 10–13% and 14–15%, resistance to fluoroquinolones 0–42% and 0–40%, azithromycin 0–29% and 0–61%, and rifaximin 0% and 0–20%. The highest rates were from the most recent collections. Proportions of ESBL-producing DEC also appear to be increasing among locals in LMICs and even carbapenemase-producing DEC were reported.

Conclusion: ESBL producers are no longer rare among DEC, and the overall resistance to various antibiotics is increasing. The data predict decreasing efficacy of antibiotic treatment, threatening its benefits, for disadvantages still prevail when efficacy is lost.

Key words: Antimicrobial resistance, multidrug resistance, rifamixin, azithromycin, fluoroquinolone, travelers’diarrhea, ESBL

Introduction

Uncontrolled use of antibiotics is a major driver of the ongoing antimicrobial resistance (AMR) pandemic, which threatens global health.1 Increasing fastest in the tropics,1 AMR is being transported worldwide by international travellers: 20–70% of visitors to low- and middle-income countries (LMICs) carry multidrug-resistant bacteria (MDR), particularly extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE), to their home country2–7 and may spread them further.2,6 During the past decade, avoiding unnecessary antibiotic use while abroad has emerged as a means to combat travel-related global spread of AMR. In addition to the general pressure...
to avoid unnecessary antibiotics, this policy is particularly encouraged by findings that antibiotic use predisposes travellers to acquisition of multidrug-resistant intestinal bacteria—and thus contributes to the global spread of AMR, colonized travellers acting as intercontinental transporters.

Special attention has been paid to treatment of travellers’ diarrhoea (TD), which ranks as the most common indication for travellers’ antibiotic use: 5–45% of those with TD take these drugs to alleviate their symptoms. As described in the literature, stand-by antibiotics for TD are prescribed at pre-travel consultations for 7–20% of European and practically all US travellers. Recently, the rates have also decreased somewhat in the USA. While antibiotics certainly retain their place in treating the most severe TD cases, their use for moderate TD has recently become topical. Although compared to placebo, antibiotics shorten the disease duration by 0.7–1.5 days, in most TD cases, the drugs are not necessary, since the disease usually resolves spontaneously. Anti-diarrhoeals such as loperamide offer an alternative with no impact on AMR since the disease usually resolves spontaneously. Anti-diarrhoeal drugs are not necessary, since the disease usually resolves spontaneously. Anti-diarrhoeals such as loperamide offer an alternative with no impact on AMR.

In discussions concerning antibiotics for TD, limited attention has been given to resistance among diarrhoeagenic Escherichia coli (DEC), the most common TD pathogens; studies have mainly examined Salmonella, Campylobacter and Shigella. DEC include several pathotypes: enterohaemorrhagic (EAEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohaemorrhagic (EHEC) or shigatoxin-producing (STEC) E. coli. The paucity of resistance studies can be explained by the challenges in detecting the various DEC: as they in culture resemble any other E. coli, identifying a specific DEC type requires additional screening by PCR or other methods.

Resistance has been reported among DEC in LMICs against the antibiotics currently recommended for TD treatment, but for travel-acquired DEC, the rates are only provided by a few studies. Ouyang-Latimer et al. showed already 2011 a substantial increase in MIC values for ciprofloxacin and azithromycin against these pathogens. Emergence of MDR strains among DEC is not unexpected—a similar development has been reported for other stool bacteria such as Salmonella. Scarcity of research into travel-acquired ESBL-DEC prompted us to revisit our data on 413 Finnish travellers to investigate the frequency of ESBL-producing strains among various DEC. Since our samples were collected ten years ago and the global AMR situation is constantly deteriorating, to get a more accurate picture, we also screened the literature for investigations into ESBL-DEC producers among locals in LMICs. Research into the resistance of TD pathogens provides fundamental information for guidance on antibiotic treatment of TD.

### Materials and Methods

The first part of this two-faceted study explored the rates and geographic origin of ESBL-producing strains among DEC contracted by Finnish travellers to LMICs (Figure 1). The second part searched PubMed for original studies of DEC exploring proportions of ESBL producers (travellers and locals) and resistance to commonly used TD antibiotics (only travellers).

### Study design, volunteers, samples and travel destinations

We prospectively recruited 526 Finnish travellers attending pre-travel consultation at the Travel Clinic of Aava Medical Centre before their journey outside the Nordic countries for more than four nights. Of these, 413 met our inclusion criteria (provided pre- and post-travel stools, filled in pre- and post-travel questionnaires, travel destination in LMICs). The details of stool collection, questionnaires and categorization of travel destinations have been described in our previous study.

Post-travel ESBL-producing Enterobacteriaceae (ESBL-PE) were considered as travel acquired only if pre-travel samples had been negative for ESBL-PE.

The protocol was approved by the Helsinki University Hospital ethics committee. All subjects provided written informed consent.

### Collection of specimens

Briefly, faecal samples were collected before departure and from the first or second stools passed after returning home. For collection, we used swabs in Copan M40 Transystem tubes (Copan Diagnostics, Brescia, Italy). Once the samples arrived, total nucleic acids were extracted using the standard semiautomated protocol of easyMAG (bioMérieux, Marcy l’Etoile, France) and the stools were cultured (see below).

### Identification of ESBL-PE

As described earlier, ESBL-PE were isolated and characterized using established methods with culture on chromID ESBL (bioMérieux, Marcy-l’Etoile, France), followed by double-disc synergy (Oxoid, Thermo Fisher Scientific, Hampshire, UK) test for cefotaxime, ceftazidime and cefpodoxime (30 μg each), alone or with clavulanic acid (10 μg), and species identification by Vitek GN (BioMérieux). Susceptibility testing for ciprofloxacin, cotrimoxazole, nitrofurantoin, tobramycin, ertapenem, imipenem and meropenem was conducted with E-test (BioMérieux) according to criteria set by the European Committee on Antimicrobial Susceptibility Testing EUCAST 5.0 (2018; www.eucast.org). Finally, beta-lactamase genes (TEM, OXA, SHV, CTX-M) and plasmid-mediated AmpC beta-lactamase genes (DHA, CIT) were identified by multiplex PCR. The co-resistance rates, prevalence of beta-lactamase genes, and phylogroup characterization of the ESBL-PE strains have been reported in our previous papers.
Figure 1. Flow chart of prospective study of ESBL-EC (extended-spectrum beta-lactamase-producing *Escherichia coli*) rates among DEC (diarrhoeagenic *E. coli*) of various pathotypes. Abbreviations: EAEC—enteroaggregative *E. coli*, EPEC—enteropathogenic *E. coli*, ETEC—enterotoxigenic *E. coli*, EIEC—enteroinvasive *E. coli*, EHEC—enterohaemorrhagic *E. coli* or STEC—shiga-toxin-producing (STEC) *E. coli*.

**Analysis of DEC by qPCR**

To explore the proportion of ESBL producers among various DEC (Figure 1), we first explored the total rates of stool samples positive for DEC by a multiplex qPCR assay, which identifies nine bacterial pathogens: *Salmonella*, *Yersinia*, *Campylobacter*, Vibrio cholerae, *Shigella*/EIEC, EHEC, ETEC, EAEC and EPEC. Second, to identify ESBL-DEC in the same samples, we subjected the ESBL-EC isolates to the multiplex qPCR for DEC.

**Search for articles in PubMed**

We searched PubMed for ‘ESBL’ or ‘extended-spectrum beta-lactamase’ or ‘CTX’ combined with ‘diarrhoeagenic’, ‘enteroaggregative’, ‘enteropathogenic’, ‘enterotoxigenic’, ‘enteroinvasive’, ‘enteroh(a)emorrhagic’, ‘shiga-toxin-producing’ or ‘verocytoxigenic’, ‘DEC’, ‘ETEC’, ‘EAEC’, ‘EPEC’, ‘EIEC’, ‘EHEC’, ‘STEC’ or ‘VTEC’ and ‘est’, ‘elt’, ‘eae’, ‘aggl’, ‘bfpA’, ‘ipaH’ and ‘stx’, plus selected articles in our own collections that reported ESBL-production among the various DEC in human samples. Although *Shigella* and EIEC often cannot be distinguished by qPCR, we did not collect resistance data from studies reporting the ESBL-producing strains as *Shigella*.

**Results**

**Participants**

Demographics of the 13 with travel-acquired ESBL-DEC are provided in Table 1. Of them, 12/13 (92%) had TD and 2/12 (17%) took antibiotics for it. The entire study cohort’s demographics have been published earlier; 67% had TD, 12% took antibiotics for it and 21% (90/430) were colonized by travel-acquired ESBL-PE (none of the travellers had ESBL-DEC in their pre-travel stools).

Eight of the 13 participants with ESBL-DEC (61.5%) had travelled to South Asia, and three (23.1%) to the Southeast Asia. None of the visitors to sub-Saharan Africa or Latin America had ESBL-DEC.

**ESBL producers among DEC**

The rate of ESBL-EC was 2.7% (13/475) among all DEC strains; 5.7% (10/175) among EAEC, 1.1% (2/180) among EPEC,
Table 1. Demographics of 13 prospectively recruited travellers who contracted extended-spectrum beta-lactamase-producing diarrhoeagenic Escherichia coli (ESBL-DEC) during visits to low- and middle-income countries (LMICs)

| Age (years) | Gender | Type of ESBL-DEC | Concomitant other ESBL-PE | AB use | TD | Travel destination(s) | Length of travel (days) | Non-ESBL co-pathogens |
|------------|--------|------------------|--------------------------|--------|----|----------------------|------------------------|-----------------------|
| 23         | Male   | EAEC             | No                       | Yes    | Yes | Laos, Cambodia, Vietnam | 22                     | None                  |
| 31         | Female | EPEC             | No                       | Yes    | Yes | India                | 11                     | EAEC, Campylobacter    |
| 61         | Female | EPEC             | FQ                       | Yes    | Yes | China                | 12                     | ETEC                  |
| 56         | Female | EAEC             | No                       | Yes    | Yes | India                | 7                      | ETEC                  |
| 67         | Male   | EAEC             | No                       | No     | No  | Egypt, Jordan Thailand, Cambodia, Vietnam | 7                     | None                  |
| 24         | Female | EAEC             | No                       | Yes    | Yes | Thailand, Cambodia, Vietnam | 110                   | EPEC, Campylobacter    |
| 46         | Female | EAEC             | Non-DEC E. coli          | No     | Yes | Cambodia             | 19                     | EPEC                  |
| 47         | Male   | EAEC             | No                       | Yes    | Yes | India                | 16                     | EHEC                  |
| 22         | Female | ETEC             | No                       | Yes    | Yes | India                | 14                     | EPEC, EAE, Salmonella, Campylobacter |
| 20         | Male   | EAEC             | Klebsiella pneumoniae    | FQ     | Yes | India                | 16                     | EPEC                  |
| 31         | Male   | EAEC             | No                       | Yes    | Yes | India                | 27                     | EPEC                  |
| 25         | Male   | EAEC             | E. bermannii             | No     | Yes | India                | 32                     | EPEC                  |
| 59         | Male   | EAEC             | No                       | Yes    | Yes | India                | 13                     | EPEC                  |

Data are provided for concomitant other ESBL-producing Enterobacteriaceae (ESBL-PE), antibiotic (AB) use, travellers’ diarrhoea (TD), destination, length of travel and non-ESBL-PE co-pathogens.

1.3% (1/80) among ETEC and 0% among EHEC (0/35) or Shigella/EIEC (0/5) strains (Table 2). EIEC and Shigella are indistinguishable in the qPCR assay, but as the same samples proved negative in Shigella culture, the isolates were considered as EIEC.

Among strains originating in South Asia, 8.3% (1/12) of ETEC and 3.3% (1/30) of EPEC produced ESBL. The highest frequencies of ESBL-EAEC were seen for South Asia (6/33; 18.2%), the Southeast Asia (3/33; 9.1%) and North Africa and the Middle East (1/3; 33.3%).

Two volunteers had taken antibiotics (ciprofloxacin) for TD; both had an ESBL-DEC co-resistant to ciprofloxacin and tobramycin, whereas among those without antibiotic use, only one strain (1/11; 9.1%) was co-resistant to ciprofloxacin (Supplementary Table 1).

ESBL genes
A total of 8/13 (61.5%) of the ESBL-DEC had blaCTX-M-15. The genes characterized for the nine ESBL-EAEC strains were blaCTX-M-1 (5/9), blaCTX-M-9 (3/9), blaTEM (4/9), and blasIV (1/9); for the two ESBL-EPEC strains blartEM (2/2) and blaCTX-M-1 (2/2); and the only ESBL-ETEC blaCTX-M-1 (1/1) (Supplementary Table 2). Six of nine ESBL-DEC harboured genes of two types.

Literature on resistance among DEC, special focus on rates of ESBL-DEC
In our literature search for studies of ESBL-DEC, we omitted those not reporting total number of DEC or strain-specific travel data; these reports prove existence of ESBL-DEC, though. Instead, we selected, in accord with our initial aim, papers providing prevalence data on resistance among travel-acquired DEC or rates of ESBL-DEC among DEC originating in LMICs. Due to meagre search results especially among travellers, we also reviewed our own files on TD studies.

Our search only yielded 24 original studies of ESBL-DEC rates among one or more types of DEC, three traveller studies (Table 3), and 21 looking at locals in LMICs (Table 4). As for travellers, we found four other investigations into resistance rates to 3GC.

Resistance among EAEC strains
Eight traveller studies describe resistance among EAEC strains (Table 3). Guiral et al. report for Spanish travellers with TD ESBL-EAEC rates of 10% (among 51 EAEC isolates in 2005–06) and 13% (39 EAEC in 2011–17),

Among samples from language school students in Peru (2003–10), 11% of the EAEC isolates proved resistant to 3GC,

Foreign Assistance Act (FAA)
for travellers to Mexico/Guatemala and India the figures were 20 and 0%, respectively (2006–08), and for the US military in Thailand 0% (2013–17). Among travel-acquired EAEC, resistance rates of 0–42% have been reported to fluoroquinolones (eight articles); 0–61% to azithromycin (six articles) and 0% to rifaximin (three articles).

The seven LMICs investigations show rates of 11–85% for ESBL-EAEC among EAEC (Table 4). Among locals the six studies reported rates of 11–80% for ESBL-EPEC (Table 4).

Resistance among EHEC/STEC strains
None of the traveller studies reviewed provided rates of antibiotic resistance for EHEC/STEC isolates.

Amaya et al. did not find any ESBL-EC among eight EHEC strains from Nicaraguan children with diarrhoea (Table 4).

Resistance among EIEC strains
Our search yielded two traveller studies of resistance looking at EIEC isolates: among samples from US military in Thailand 2013–17 no resistance was detected but in Nepal 2012–14, 10% of the EIEC strains proved resistant to ciprofloxacin and 30% to azithromycin.

In LMICs, studies among local children with diarrhoea have found the few EIEC strains to be mostly ESBL producers.

Discussion
Despite the vast discussion around antibiotic use for treating TD, paradoxically scant attention has been paid to resistance among the most common TD pathogens, DEC. The handful of reports published mostly do not focus on travellers. Apart from resistance to individual antibiotics, multidrug resistance is increasingly common among intestinal bacteria in clinical samples worldwide, ESBL-PE ranking as the most prevalent MDR type. Our data together with those from a literature search for studies among travellers and locals in LMICs destinations show an emergence of ESBL producers among DEC.

Rates of ESBL producers among DEC
Our rate, 3–7% of ESBL producers among the various DEC strains collected 2009–10, appears consistent with the three other traveller studies of ESBL-DEC: among Spanish travellers, the rates of ESBL-EAEC were 10% in 2005–6, and 12.8% in 2011–17. Among residents and travellers with acute diarrhoea in Kathmandu an increase from 1.5 to 35% was observed between 2001 and 2012.
| First author     | Year(s) of stool sampling | Population, number of isolates | ESBL-EC | Cipro-floxacin resistance | Azithromycin resistance | Rifaximin resistance |
|------------------|---------------------------|--------------------------------|---------|---------------------------|------------------------|---------------------|
| Lurchachaiwong   | 2013–17                  | US military, Thailand          | ETEC 3  | ETEC 0%                   | NT                     | NT                  |
|                  |                           | EAE 3  | EPEC 13                   | EPEC 8% EIEC 0%         |                        |                     |
|                  |                           | EIEC 1 |                          |                        |                        |                     |
| Murphy           | 2012–14                  | Travellers in Nepal            | NT      | ETEC 23%                  | ETEC 22%               | NT                  |
|                  |                           | ETEC 60 |                         | EAE 15% EPEC 23% EIEC 10% |                        |                     |
|                  |                           | EAE 208 |                        |                         |                        |                     |
|                  |                           | EPEC 65 |                        |                         |                        |                     |
|                  |                           | EIEC 10 |                        |                         |                        |                     |
| Guiral           | 2011–17                  | TD Spain                       | ETEC 14%| ETEC 33% EAE 13% EAE 42% | ETEC 29%            | ETEC 0% |
|                  |                           | ETEC 43 |                         |                         |                        |                     |
|                  |                           | EAE 39 |                         |                         |                        |                     |
| Margulieux       | 2001–16                  | Locals and travellers,         | ETEC 15%| ETEC 6% NT                |                        | NT                  |
|                  |                           | Kathmandu, Nepal               |         |                          |                        |                     |
|                  |                           | ETEC 265 |                        |                         |                        |                     |
| Mason            | 2002–04                  | US military, Thailand          | NT      | ETEC 0% EAE 0% EPEC 0% EIEC 0% | ETEC 0% | ETEC 0% |
|                  |                           | ETEC 29 |                         |                         |                        |                     |
|                  |                           | EAE 5 |                         |                         |                        |                     |
|                  |                           | EPEC 16 |                         |                         |                        |                     |
| Jennings         | 2003–10                  | Language school travellers,    | ETEC 0% | ETEC 0% EAE 0% EAE 7% ETEC 22% | ETEC 11% | ETS 33% |
|                  |                           | Cuzco, Peru                     |         |                          |                        |                     |
|                  |                           | ETEC 27 |                         |                         |                        |                     |
|                  |                           | EAE 9 |                         |                         |                        |                     |
| Pandey           | 2001–03                  | Travellers and expatriates,    | NT      | ETEC 0% EPEC 10% EPEC 37% | ETEC 16% | EPEC 37% |
|                  |                           | Nepal                          |         |                          |                        |                     |
|                  |                           | ETEC 50 |                         |                         |                        |                     |
|                  |                           | EPEC 38 |                         |                         |                        |                     |
| Guiral           | 2005–06                  | Spanish travellers to India    | EAEC 10%| Not reported  | Not reported | Not reported |
|                  |                           | with TD                        |         |                          |                        |                     |
|                  |                           | ETEC 51 |                         |                         |                        |                     |
| Ouyang-Latimer   | 2006–08                  | TD among travellers to         | Resistance to | India 28% | India 25% | India 20% |
|                  |                           | Mexico, Guatemala, India       | ceftriaxone         | EAE 0% Mexico,   | EAE 0% Mexico,   | EAE 0% Mexico,   |
|                  |                           | ETEC 365 |                        | Guatemala           | Guatemala           | Guatemala           |
|                  |                           | EAE 26 |                        |                         |                        |                     |
|                  |                           | India                          | ETEC 6% EAE 0%      |                         |                        |                     |
|                  |                           | ETEC 98 |                        |                         |                        |                     |
|                  |                           | EAE 3 |                        |                         |                        |                     |
|                  |                           | Mexico, Guatemala              |                         |                         |                        |                     |
|                  |                           | ETEC 5 |                        |                         |                        |                     |
|                  |                           | EAE 20 |                        |                         |                        |                     |
| Porter           | 2002                     | US military, Turkey            | NT      | ETEC 5%                  | Not reported | NT |
|                  |                           | ETEC 82 |                         |                         |                        |                     |
| Mendez           | 1994–97 and 2001–04      | Spanish travellers              | NT      | 1994–97 ETEC 1%          | NT                     | NT |
|                  |                           | 1994–97                        |         | EAE 2% 2001–04 ETEC 8%  |                        |                     |
|                  |                           | ETEC 82 |                         |                         |                        |                     |
|                  |                           | EAE 50 |                         |                         |                        |                     |
|                  |                           | ETEC 108 |                        |                         |                        |                     |
| Gomi             | 1997                     | Travellers to India, Mexico,   | a       | ETEC 3/61 (4.9%)         | a                      | a |
|                  |                           | Jamaica, Kenya                 |         | EAE 4/44 (9.1%)          |                        |                     |
|                  |                           | ETEC 97 |                         |                         |                        |                     |
|                  |                           | EAE 75 |                         |                         |                        |                     |
| Vila             | 1994–97                  | Spanish travellers             | NT      | ETEC 1%                  | NT                     | NT |
|                  |                           | ETEC 82 |                         |                         |                        |                     |

* Resistance rates for ETEC and EAEC only provided together; cases with both reported as ‘highly sensitive’. Some studies were conducted among both travellers and locals in LMICs. Table combines results from analyses of ESBL-DEC and resistance to TD antibiotics, fluoroquinolones, azithromycin, and rifaximin. Three studies only report resistance rates to third-generation cephalosporins but not ESBL-DEC (NT = not tested).
Table 4. Results of literature search for studies exploring rates of ESBL producers among various DEC isolated from stools of locals in various regions in LMICs

| First author year | Year(s) of stool sampling | Population, number of isolates | ESBL-EC | Carbapenem resistance | Ciprofloxacin resistance | Azithromycin resistance | Rifaximin resistance |
|-------------------|---------------------------|--------------------------------|---------|-----------------------|------------------------|------------------------|---------------------|
| South Asia        |                           |                                 |         |                       |                        |                        |                     |
| Moharana          | 2012–17                   | Indian children with diarrhoea  | 4%      | 3%                    | 74%                    | NT                     | NT                  |
|                   |                           | DEC 77                          |         |                       |                        |                        |                     |
| Mandal            | 2017                      | not reported ("during two        | All DEC 38% | 0%                      | DEC 50% resistant to    | NT                     | NT                  |
|                   |                           | consecutive years")             | ETEC 18%|                        | levofoxacin            |                        |                     |
|                   |                           | Indian children with diarrhoea  |         |                       |                        |                        |                     |
|                   |                           | DEC 191                         |         |                       |                        |                        |                     |
| Khalil            | 2010–11                   | Pakistani children with diarrhoea| 34%     | NT                    | 69%                    | NT                     | NT                  |
|                   |                           | EAEC 35                         |         |                       |                        |                        |                     |
| Younas            | 2010–12                   | Pakistani children EPEC 46      | 59%     | NT                    | 39%                    | NT                     | NT                  |
| Malvi             | 2012–13                   | Indian children with/without     | 25%     | 30%                   | 25%                    | 14%                    | NT                  |
|                   |                           | diarrhoea EPEC 59                |         |                       |                        |                        |                     |
| Southeast Asia    |                           |                                 |         |                       |                        |                        |                     |
| Our search yielded |                           | no studies conducted in the     |         |                       |                        |                        |                     |
| East Asia         |                           | Southeast Asia                  |         |                       |                        |                        |                     |
| Xu                | 2006–15                   | Chinese patients with diarrhoea  | 25%     | 0%                    | 5%                     | NT                     | NT                  |
|                   |                           | aEPEC 151                       |         |                       |                        |                        |                     |
| Zhou              | 2015–16                   | Chinese children with diarrhoea  | 52%     | 6%                    | 50%                    | NT                     | NT                  |
|                   |                           | DEC 54                          |         |                       |                        |                        |                     |
| Wang              | 2015                      | Chinese healthy elderly (> 65   | 56%     | NT                    | NT                     | NT                     | NT                  |
|                   |                           | years) EAEC 96                   |         |                       |                        |                        |                     |
| North Africa and  |                           |                                 |         |                       |                        |                        |                     |
| Middle East       |                           |                                 |         |                       |                        |                        |                     |
| Farajzadeh-       | 2016–17                   | Iranian children EIEC 13 (5.1% | EIEC 69%| EIEC 15%               | 0%                     | NT                     | NT                  |
| Sheikh            |                           | of all DEC strains) ; other DEC|         |                       |                        |                        |                     |
|                   |                           | not specified                   |         |                       |                        |                        |                     |
| Eltai             | 2017–18                   | Quatarian children EAEC 20      | EAEC; 20%| EAEC; 10%         | 0%                     | NT                     | NT                  |
|                   |                           | EPEC 23                         |         |                       |                        |                        |                     |
|                   |                           | EPEC 56                         |         |                       |                        |                        |                     |
| Taghadosi         | 2014–15                   | Iranian children ETEC 13        | ETEC 54%| 0%                    | ETEC 46%               | NT                     | NT                  |
|                   |                           | EPEC 26                         |         |                       | EPEC 19%               |                        |                     |
| First author year | Year(s) of stool sampling | Population, number of isolates | ESBL-EC | Carbapenem resistance | Ciprofloxacin resistance | Azithromycin resistance | Rifaximin resistance |
|-------------------|--------------------------|--------------------------------|---------|-----------------------|------------------------|------------------------|---------------------|
| Mahdavi 2018<sup>82</sup> | 2015–16 | Iranian children with diarrhoea | ETEC 100% | ETEC 17% | NT | NT |
|                   |               | ETEC 6 | EAEC 74% | EAEC 20% | | | |
|                   |               | EAEC 35 | EPEC 90% | EPEC 40% | | | |
|                   |               | EPEC 10 | EIEC 83% | EIEC 0% | | | |
|                   |               | EIEC 6 | | | | | |
| Amin 2018<sup>84</sup> | 2015–16 | Iranian children with diarrhoea | 28% | 19% | 78% | NT |
|                   |               | EAEC 32 | | | | | |
|                   |               | | | | | | |
| Aminshahidi 2017<sup>77</sup> | 2014–15 | Iranian children | DEC 67% | DEC 31% | NT | NT |
|                   |               | DEC 48 | ETEC 75% | ETEC 25% | | | |
|                   |               | EAEC 85% | EPEC 85% | EAEC 27% | | | |
|                   |               | EPEC 33% | EIEC 50% | EPEC 33% | | | |
| Karami 2017<sup>80</sup> | Not reported | Iranian children with/without diarrhoea | 80% | 0% | 21% | NT |
|                   |               | EPEC 192 | | | | | |
| Memariani 2015<sup>52</sup> | 2011–13 | Iranian children with diarrhoea | 21% | NT | 17% | NT |
|                   |               | EPEC 42 | | | | | |
| Ghorbani-Dalini 2015<sup>51</sup> | 2010 | Iranian adults with diarrhoea | 13% | 6% resistant to imipenem | 8% | NT |
|                   |               | DEC 54; DEC types not specified | | 4% resistant to imipenem | | | |
| Khoshvacght 2014<sup>49</sup> | 2011–12 | Iranian children with diarrhoea | 53% | NT | NT | NT |
|                   |               | EAE 36 | | | | | |
| Sonnevend 2006<sup>48</sup> | 2003–04 | children and adults with and without diarrhoea, United Arab Emirates | 11% | NT | NT | NT |
|                   |               | EAEC 44 | | | | | |
| Sub-Saharan Africa Konate 2017<sup>79</sup> | 2013–15 | children with diarrhoea, Burkina Faso | 68% | 16% resistant to imipenem | 0% | NT |
|                   |               | DEC 31 | | | | | |
| Downloaded from https://academic.oup.com/jtm/advance-article/doi/10.1093/jtm/taab042/6217594 by guest on 01 May 2021 | | | | | | |
Table 4. Continued

| First author year | Year(s) of stool sampling | Population, number of isolates | ESBL-EC diarroha: | Carbapenem resistance | Ciprofloxacin resistance | Azithromycin resistance | Rifaximin resistance |
|-------------------|---------------------------|--------------------------------|------------------|----------------------|------------------------|-----------------------|---------------------|
| South and Central America and the Caribbean | Amaya 2011 | 2005-06 Nicaraguan children DEC 332 | ETEC: 5/64 (8%) | EAE: 23/134 (17%) | EPEC: 3/38 (9%) | EHEC: 0/8 (0%) | no diarrhoea: |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

From the same papers, resistance rates are given also for carbapenems, fluoroquinolones, azithromycin and rifaximin, if tested (NT = not tested).

2008 and 2016. These data suggest increasing rates of ESBL producers among DEC. We found more investigations into the ESBL production of DEC among locals in LMICs than among travellers, with rates of positive findings varying by pathotype, time and destination. It should be noted that none of the analyses focused on the main tourist destinations in Southeast Asia, Africa, or South and Central America, and the Caribbean. In 18 of the 21 studies, the data were from local children, without diarrhoea, highlighting the clinical concern related to resistance. Likewise, among locals, the highest rates were recorded over the most recent years, according with the steady global increase in the rates of ESBL-producing strains among all E. coli in clinical samples.

Our search did not focus on carbapenemase-producing DEC, but we found 16 studies from LMICs reporting resistance rates of 0–50% to carbapenems among DEC. Our samples showed no carbapenemase-producing genes.

ESBL producers among various DEC

In our data, the ESBL-EC rates appeared higher among EAE than EPEC and ETEC (5.7% versus 1.1% versus 1.3%). This accords with other traveller studies reporting ESBL-EAEC rates of 10% and 12.8% among travellers reporting diarrhoea in Iran and China, respectively, and 25% in England. Likewise, substantial rates (53 and 57%) of ESBL-EAEC have been reported among clinical EAEC isolates in England; yet they do not report which of the strains were travel-acquired nor their countries of origin.

For ESBL-EPEC, our rate 1.3% (Table 2) was much lower than that found among Spanish traveller (14%) or in Nepal (1.5%). The top rates (15%) for non-traveller EPEC were recorded in Iran (25% of all EPEC) and in China (11%).

As for EPEC, we only identified two ESBL-EPEC strains (1.1%). None of the traveller studies surveyed covered ESBL-EPEC, but among locals rates as high as 80% have been reported in Iran, and 59% in Pakistan.

We detected no ESBL-EC among EIEC and EHEC, neither did we find in the literature any other studies exploring ESBL-EC of these pathotypes; only few investigations among travel-acquired ESBL-DEC rates of 1% in 2010–10 and 5.7% in 2017–18 have been shown for ESBL-DEC.

Geographic distribution of ESBL-DEC

Most of our ESBL-DEC originated in South Asia, which also proved to have the highest rates of ESBL-DEC among DEC. Indeed, South Asia has exceptionally high-resistance rates among gram-negative bacteria in clinical samples, and top ESBL-PE colonization rates among visitors from South or Southeast Asia (23.7, 44.2) than those from Africa (6.3%, 17.6, and Latin America (0.6, 0.1, 0.5).

Our data agree with previous data showing higher resistance rates among AEAE isolates from South or Southeast Asia (33.3%; 4/12) than those from Africa (10.5%, 1/10) and Latin America (0.5%, 1/200).

As for ESBL-PE, our rate 1.3% (Table 2) was much lower than that found among Spanish traveller (14%) or in Nepal (1.5%). The top rates (15%) for non-traveller EPEC were recorded in Iran (25% of all EPEC) and in China (11%).

As for EPEC, we only identified two ESBL-EPEC strains (1.1%). None of the traveller studies surveyed covered ESBL-EPEC, but among locals rates as high as 80% have been reported in Iran, and 59% in Pakistan.

We detected no ESBL-EC among EIEC and EHEC, neither did we find in the literature any other studies exploring ESBL-EC of these pathotypes; only few investigations among travel-acquired ESBL-DEC rates of 1% in 2010–10 and 5.7% in 2017–18 have been shown for ESBL-DEC.
Resistance to commonly used TD antibiotics

While our own results centre around ESBL-DEC, we also reviewed the literature for data on resistance among travel-acquired DEC to commonly used TD antibiotics (fluoroquinolones, azithromycin and rifaximin). Recent traveller studies have present alarming data: for EAEC strains resistance rates of 15–42%, 33–61% and 0% to fluoroquinolones, azithromycin and rifaximin, and for ETEC 23–33%, 22–29% and 0%, respectively.

Resistance genes among DEC

Our data include thirteen ESBL-DEC isolates, with bla_{CTX-M-1} as the most common finding in genetic analyses, followed by bla_{TEM}. Only a small proportion of our strains carried the bla_{CTX-M-15} gene despite the worldwide spread of E. coli clone of sequence type 131 (ST131) carrying the CTX-M-15 ESBL both in clinical and non-clinical settings. In contrast, a previous traveller study reports a total of 11 ESBL-DEC strains, all harbouring either of the two genes bla_{CTX-M-15} or bla_{CTX-M-27}. Likewise, from the samples of residents and travellers in Nepal, \( \text{bla}_{\text{CTX-M-15}} \) was detected in 80% of the ESBL-ETEC strains.

Clinical implications

While ESBL-EC are considered resistant to 3GC (e.g. ceftriaxone), the resistance profile as such does not cover the most commonly used TD regimens, i.e. fluoroquinolones, azithromycin and rifaximin. Unfortunately, however, ESBL-producing strains often harbour co-resistance to other antibiotics, especially fluoroquinolones. Of our ESBL-DEC strains, 3/13 (23.1%) were co-resistant to fluoroquinolones, yet higher co-resistance rates have been reported among travel-acquired ESBL-PE in general, particularly for the South Asia and related to fluoroquinolone intake abroad. Indeed, ESBL-producing strains are of special concern, since in cases severe enough to require hospitalization empiric treatment often relies on either 3GC or fluoroquinolones.

Interpreting the efficacy of various antibiotics is somewhat complicated, for faecal antibiotic levels tend to exceed the minimum inhibitory concentration (MIC). Furthermore, presence of antibiotics in stools, while indicating an antibiotic pressure to other intestinal bacteria, may also drive transfer of resistance genes to other Enterobacteriaceae, some of which are potential pathogens.

An ineffective drug does not offer benefits, and yet retains its disadvantages. Although the adverse effects rate appears to be low, recently, for example, the US Food and Drug Administration has warned about some serious adverse effects of fluoroquinolones (e.g. tendinitis and prolonged QT interval) and azithromycin (e.g. prolonged QT interval), the most popular TD antibiotics. Furthermore, data are lacking on the suggested smaller impact of one-day antibiotic treatment on acquisition of MDR bacteria abroad. The adverse effect profile would favour rifamycins such as rifaximin. However, the drug is non-absorbable and should not be used in cases with fever and invasive disease—i.e. it does not meet the most important indications for antibiotics. We only found a few studies exploring resistance rates to rifaximin among DEC; Ouyang-Latimer et al. reported 16–25% resistance rates among ETEC already in 2011.

Limitations of our data

Firstly, collected 2009–10, our strains do not fully represent the current situation. Unfortunately, though, the same applies to the other traveller studies found in our search, only three of which provide data from a later time period. The increase in resistance recorded among locals suggests growing pressure also for travellers. Our data may thus present a slight underestimation, calling for updated surveillance.

Secondly, qPCR of stools cannot distinguish whether the samples contain one DEC strain or several of similar type. Likewise, in culturing ESBL-EC strains, those which appear phenotypically different are picked, and therefore strains may be missed that are similar or of only a slightly different phenotype, but genetically unlike. Fortunately, these sources of error may at least partly overcome one another.

Thirdly, in the various studies reviewed there are methodological differences (assessment of the various DEC, pre-analytical handling of the specimens, etc.); therefore, the data may not be fully comparable.

Conclusions

ESBL-producing DEC are no longer rare, particularly in Asia. Among travel-acquired DEC, their rates appear fairly low as yet, but in many regions, increase is already seen among DEC isolated from locals with acute diarrhoea, also portending increase among travel-acquired DEC, many strains even to be carried by travellers to their countries. While antibiotics certainly retain their place in the treatment of the most severe TD cases, data showing increasing resistance among stool pathogens further encourage cutting back on use of antibiotics for TD, and opting for non-antibiotic alternatives for mild and moderate cases. After all, an ineffective drug, while obviously useless, retains all its disadvantages.

Authors’ contributions

Study concept and design by A.K. and T.L.; acquisition of data by A.K.; literature review by T.L.; statistical analysis by T.L.; drafting the manuscript by A.K. and T.L. and final approval of version published by A.K. and T.L.

Supplementary data

Supplementary data are available at JTM online.

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.
34. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev 1998; 11:142–201.
35. Vila J. New molecular diagnostic tools in traveller’s diarrhea. J Travel Med 2017; 24:523–8. doi: 10.1093/jtm/taab071.
36. Ouyang-Latimer J, Jafari S, Van Tassel 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.
37. Guiral E, Mendez-Arancibia E, Soto SM et al. CTX-M-15-producing enteroaggregative Escherichia coli as cause of travellers’ diarrhea. Emerg Infect Dis 2011; 17:1950–3.
38. Margulieux KR, Srijan A, Ruckt 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.
39. Guiral E, Goncalves Quiles M, Munoz L et al. Emergence of resistance to quinolones and beta-lactam antibiotics in enterotoxigenic and enterotoxigenic Escherichia coli causing Traveller’s Diarrhea. Antimicrob Agents Chemother 2019; 63:e01745–18.
40. Ryan ET. Treatment and prevention of enteric (typhoid and paratyphoid) fever. In: UpToDate. 2020; https://www.uptodate.com/contents/treatment-and-prevention-of-enteric-typhoid-and-paratyphoid-fever
41. Dallenne C, Da Costa A, Decre D et al. Comparison of phenotypic and genotypic characterization of diarrheagenic Escherichia coli isolates from travellers returning to the UK, 2015-2017. J Med Microbiol 2020; 69:932–43.
42. Do Nascimento V, Day MR, Dowmith M et al. Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of enterogroupaggregative Escherichia coli isolated from cases of diarrheal disease in England, 2015-16. J Antimicrob Chemother 2017; 72:3288–97.
43. Boxall MD, Day MR, Greig DR, Jenkins C. Antibacterial resistance profiles of diarrhoeagenic Escherichia coli isolated from travellers returning to the UK, 2015-2017. J Med Microbiol 2020; 69:932–43.
44. Sonnevend A, Al Dhaheri K, Mag T et al. CTX-M-15-producing multidrug-resistant enterogroupaggregative Escherichia coli in the United Arab Emirates. Clin Microbiol Infect 2006; 12:582–5.
45. Ambroziak M, Marzec M, Szewczyk K et al. Antibiogram and resistance patterns of gut bacteria in children with traveler’s diarrhea. Clin Microbiol Infect 2014; 20:319–23.
46. Khoshvaght H, Haghi F, Zeighami H. Extended spectrum beta-lactamase producing Enterogroupaggregative Escherichia coli from young children in Iran. Gastroenterol Hepatol Bed Bench 2014; 7:131–6.
47. Ghorbani-Dalini S, Kargar M, Doosti A et al. Molecular epidemiology of ESBL genes and multi-drug resistance in Diarrheagenic Escherichia coli strains isolated from adults in Iran. Iran J Pharm Res 2015; 14:1257–62.
48. Mominian M, Karami P, Moghadam H et al. Characteristics of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli isolates from travellers returning to Iran. Iran J Pharm Res 2018; 14:1090–4.
49. Taghadosi R, Shokouhi-Mehr M, Hosseini-Nave H. Antibiotic resistance, ESBL genes, integrons, phylogenetic groups and MIVA profiles of Escherichia coli pathotypes isolated from patients with diarrheaea and farm animals in south-east of Iran. Comp Immunol Microbiol Infect Dis 2019; 63:117–26.
67. Eltai NO, Al Thani AA, Al Hadidi SH et al. Antibiotic resistance and virulence patterns of pathogenic Escherichia coli strains associated with acute gastroenteritis among children in Qatar. BMC Microbiol 2020; 20:54-020–01732-8.

68. Farajzadeh-Sheikh A, Savari M, Hosseini Nave H et al. Frequency and molecular epidemiology of class a ESBLs producing enteroinvasive Escherichia coli (EIEC) isolates among patients with diarrhea. Gastroenterol Hepatol Bed Bench 2020; 13: 77–85.

69. Gomi H, Jiang ZD, Adachi JA et al. European Centre for Disease Prevention and Control Surveillance of gastroenteritis with acute gastroenteritis among children in Qatar. Virulence 2016; 7:252–66.

70. Jennings MC, Tilley DH, Ballard SB et al. Case-case analysis using 7 years of Traveler’s Diarrhea surveillance data: preventive and travel medicine applications in Cusco, Peru. Am J Trop Med Hyg 2017; 96:1097–106.

71. Lurchachaiwong W, Serichantalergs O, Lertsethtakarn P et al. Enteric etiological surveillance in acute diarrhea stool of United States military personnel on deployment in Thailand, 2013-2017. Gut Pathog 2020; 12:17.

72. Vila J, Vargas M, Ruiz J et al. 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.

73. Mendez Arancibia E, Pitart C, Ruiz J et al. Evolution of antimicrobial resistance in enteraggregative Escherichia coli and enterotoxigenic Escherichia coli causing traveller’s diarrhea. J Antimicrob Chemother 2009; 64:343–7.

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

75. Pandey P, Bodhidatta L, Lewis M et al. Travelers’ diarrhea in Nepal: an update on the pathogens and antibiotic resistance. J Travel Med 2011; 18:102–8.

76. Murphy H, Bodhidatta L, Sornsakrin S et al. Traveler’s Diarrhea in Nepal - changes in etiology and antimicrobial resistance. J Travel Med 2019; 26:taz054.

77. Ruppe E, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in gram-negative bacilli. Ann Intensive Care 2015; 5:61.

78. European Centre for Disease Prevention and Control Surveillance of antimicrobial resistance in Europe 2018. Stockholm, 2019.

79. CDC. Antibiotic Resistance Threats in the United States. Atlanta, GA: U.S. Department of Health and Human Services, CDC, 2019.

80. Akova M. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence 2016; 7:252–66.

81. Chong Y, Shimoda S, Shimono N. Current epidemiology, genetic evolution and clinical impact of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infect Genet Evol 2018; 61:185–8.

82. Kuenzli E, Jaeger VK, Frei R et al. High colonization rates of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli in Swiss travellers to South Asia - a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. BMC Infect Dis 2014; 14:528-2334–14-528.

83. 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.

84. Nicolas-Chanoine MH, Bertrand X, Madec JY. Escherichia coli ST131, an intriguing clonal group. Clin Microbiol Rev 2014; 27:543–7.

85. Carattoli A. Plasmids in gram negatives: molecular typing of resistance plasmids. Int J Med Microbiol 2011; 301:654–8.

86. Canton R, Gonzalez-Alba JM, Galan JC. CTX-M enzymes: origin and diffusion. Front Microbiol 2012; 3:110.

87. Martinez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev 2002; 15:647–79.

88. DuPont HL, Steffen R. Use of antimicrobial agents for treatment and prevention of travellers’ diarrhoea in the face of enhanced risk of transient fecal carriage of multi-drug resistant Enterobacteriaceae: setting the stage for consensus recommendations. J Travel Med 2017; 24:S57–62.

89. US Food and Drug Administration. Fluoroquinolone Antimicrobial Drugs Information, 2018. https://www.fda.gov/drugs/information-drug-class/fluoroquinolone-antimicrobial-drugs-information#:~:text=Fluoroquinolones%20are%20drugs%20approved%20for%20such%20uses%20as%20colds%20or%20flu%20(Accessed%203%20April%2C%202021).

90. US Food and Drug Administration. Azithromycin (marketed as Zithromax or Zmax) Information, 2018. https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/azithromycin-marketed-zithromax-or-zmax-information#:~:text=Azithromycin%20is%20an%20FDA%2Dapproved,by%20many%20different%20drug%20companies%20(Accessed%203%20April%2C%202021).