Colonization of Returning Travelers With CTX-M-Producing Escherichia coli

Gisele Peirano, PhD,† Kevin B. Laupland, MD,†§ Daniel B. Gregson, MD,*†† and Johann D.D. Pitout, MD*‡∥

*Division of Microbiology, Calgary Laboratory Services; †Department of Pathology and Laboratory Medicine; ‡Department of Medicine; §Department of Critical Care, and ∥Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada.

Background. We previously identified foreign travel as a risk factor for acquiring infections due to CTX-M (active on cefotaxime first isolated in Munich) producing Escherichia coli. The objective of this study was to assess the prevalence of extended-spectrum β-lactamase (ESBL)-producing E. coli among stool samples submitted from travelers as compared to non-travelers (a non-traveler had not been outside of Canada for at least 6 months before submitting a stool specimen).

Methods. Once a travel case was identified, the next stool from a non-traveler (not been outside of Canada for at least 6 months) was included and cultured on the chromID-ESBL selection media. Molecular characterization was done using polymerase chain reaction and sequencing for blaCTX-Ms, blaTEMs, blaSHVs, plasmid-mediated quinolone-resistant determinants, O25-ST131, phylogenetic groups, pulsed-field gel electrophoresis (PFGE), and multilocus sequencing typing.

Results. A total of 226 individuals were included; 195 (86%) were negative, and 31 (14%) were positive for ESBL-producing E. coli. Notably, travelers were 5.2 (95% CI 2.1–31.1) times more likely than non-travelers to have an ESBL-producing E. coli cultured from their stool. The highest rates of ESBL positivity were associated with travel to Africa or the Indian subcontinent. Among the 31 ESBL-producing E. coli isolated, 22 produced CTX-M-15, 8 produced CTX-M-14, 1 produced CTX-M-8, 12 were positive for aac(6′)-Ib-cr, and 8 belonged to clone ST131.

Conclusions. Our study confirms that foreign travel, especially to the Indian subcontinent and Africa, represents a major risk for rectal colonization with CTX-M-producing E. coli and contributed to the worldwide spread of these bacteria.

In Gram-negative pathogens, β-lactamase production remains the most important contributing factor to β-lactam resistance. The extended-spectrum β-lactamases (ESBLs) have the ability to hydrolyze and cause resistance to the cephalosporins and monobactams. The TEM and SHV families were the predominant types of ESBLs during the 1980s and 1990s. However, since the late 1990s CTX-M ESBL enzymes have emerged worldwide among Enterobacteriaceae, in particular Escherichia coli, and have become the most widespread type of ESBL in the world. CTX-M-producing E. coli are important causes of community-onset urinary tract infections, bacteremia, and intra-abdominal infections. Currently, the most widespread and prevalent type of CTX-M enzyme is CTX-M-15.

A very interesting phenomenon about E. coli that produces CTX-M-15 was described in 2008 from researchers in France and Spain. They identified [using a technique called multilocus sequencing typing (MLST)] a sequence type (ST) named ST131 among several CTX-M-15-producing E. coli isolated from countries such as Spain, France, Canada, Portugal, Switzerland, Lebanon, India, Kuwait, and Korea. These two initial studies showed that ST131 had emerged seemingly independently in different parts of the world at the same time. Their findings suggested that the emergence of E. coli that produce CTX-M-15 and those that belonged to ST131 could be either due to the ingestion...
of contaminated food/water sources and/or are being imported into various countries via returning travelers.

The objective of this study was to assess the prevalence and characterize ESBL-producing *E. coli* among stool samples submitted from travelers as compared to non-travelers.

**Materials and Methods**

**Patients, Bacterial Isolates, and Antimicrobial Susceptibilities**

Consecutive diarrheal stool samples submitted to Calgary Laboratory Services (CLS) for routine testing during 2009 were studied. Stools submitted to CLS for routine investigations must state if a patient recently traveled. Travel (defined as being present that country for at least 5 days and the stool submitted within 6 months after their return) was identified by the requisition information and verbally confirmed by phoning the patient. The countries visited are shown in Table 2. Patients did not know their status of colonization. Once a travel case was identified, the next stool from a non-traveler from the community was included. A non-traveler had not been outside of Canada for at least 6 months before submitting a stool specimen. These were then tested for routine stool pathogens and cultured on a selective media for ESBL-producing Gram-negatives using chromID-ESBL selection Agar (bioMerieux Inc., Hazelwood, MO, USA). Only *E. coli* grew on the agar and five different colonies per plate were tested for ESBL production.

ESBL production was confirmed phenotypically by using the CLSI criteria for ESBL screening and disk confirmation tests. Antimicrobial susceptibility was determined with the VITEK 2 instrument (Vitek AMS; bioMerieux Vitek Systems Inc., Hazelwood, MO, USA). The MICs of the following drugs were determined: amoxicillin/clavulanic acid (AMC), piperacillintazobactam (TZP), ertapenem (ERT), amikacin (AMK), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), and trimethoprim-sulfamethoxazole (SXT). Throughout this study, results were interpreted using CLSI criteria for broth dilution.

**β-Lactamase Identification**

Isoelectric focusing, which included cefotaxime hydrolysis and determination of inhibitor profiles on polyacrylamide gels, was performed on freeze–thaw extracts as previously described. Polymerase chain reaction (PCR) amplification and sequencing for *bla*<sub>CTX-M</sub>, *bla*<sub>OXAm</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>SHV</sub> were carried out on the isolates with a GeneAmp 9700 ThermoCycler instrument (Applied Biosystems, Norwalk, CT, USA) using PCR conditions and primers as previously described.

**Plasmid-Mediated Quinolone-Resistant Determinants**

The amplification of *qnrA*, *qnrS*, and *qnrB* genes was performed in all ESBL-positive isolates with multiplex PCR. *aac(6′)-Ib* and *qepA* were amplified in a separate PCR using primers and conditions as previously described. The variant *aac(6′)-Ib-cr* was further identified by digestion with BstF5I11 (New England Biolabs, Ipswich, MA, USA).

**Pulsed-Field Gel Electrophoresis**

Genetic relatedness of the ESBL-producing isolates was examined by PFGE following the extraction of genomic DNA and digestion with XbaI using the standardized *E. coli* (O157:H7) protocol established by the Centers for Disease Control and Prevention, Atlanta, GA. Cluster designation was based on isolates showing approximately 80% or greater relatedness, which corresponds to the “possibly related (4–6 bands difference)” criteria of Tenover and colleagues.

**Identification of Clone ST131**

ST131 were detected in the ESBL-producing isolates using a PCR for the *pabB* allele, recently described by Clermont and colleagues. MLST was performed on those isolates that tested positive for ST131 using seven conserved housekeeping genes (*adk, fumC, gyrB, iza, mdh, purA, and recA*). A detailed protocol of the MLST procedure, including allelic type and ST assignment methods, is available at MLST databases at the ERI, University College Cork web site (http://mlst.ucc.ie/mlst/dbs/Ecoli).

**Phylogenetic Groups**

The ESBL-positive isolates were assigned to one of the four main *E. coli* phylogenetic groups (A, B1, B2, and D) by the use of a multiplex PCR-based method.

**Statistical Analysis**

The analysis was performed using Stata 9.2 (StataCorp, College Station, TX, USA). Samples submitted from travelers and non-travelers were treated as independent samples and grouped for analysis. Proportions were compared using Fisher’s exact test and continuous data using the Student’s *t*-test. For all comparisons a *p*-value <0.05 was deemed to represent statistical significance.

**Results**

**Patients, Bacterial Isolates, and Antimicrobial Susceptibilities**

A total of 226 samples were included; 207 (92%) of samples were submitted from community-based collection sites and 19 (8%) were submitted from emergency departments. The mean age (±SD) was 37.5 ± 22.5 years and 126 (57%) were females. Among the 113 foreign travelers, 21 traveled to Asia, 18 traveled within North America (17 Mexico and 1 USA), 18 to Caribbean/Central America, 17 to Africa, 12 to India, 10 to Europe, 8 to South America, 6 to the Middle East, and 3 to Australia/New Zealand.
Colonization of Travelers

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of different characteristics of travelers and non-travelers</th>
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<tbody>
<tr>
<td>Factor</td>
<td>Travelers (n = 113)</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>34.7 ± 19.4</td>
</tr>
<tr>
<td>Male gender</td>
<td>54 (48%)</td>
</tr>
<tr>
<td>ESBL positive*</td>
<td>26 (23%)</td>
</tr>
<tr>
<td>Submitting location</td>
<td>Community 109 (92%)</td>
</tr>
<tr>
<td>Inpatient ward</td>
<td>3 (3%)</td>
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ESBL = extended-spectrum β-lactamase.

*6/26 and 2/5 of the ESBL-producing Escherichia coli from travelers and non-travelers, respectively, belonged to ST131.

Among the 226 stool samples, 191 (85%) were negative, 31 (14%) were positive for ESBL-producing *E. coli*. A number of factors were compared among travelers and non-travelers and are shown in Table 1. Notably, travelers were 5.2 (95% CI 2.1–31.1) times more likely than non-travelers to have an ESBL-producing *E. coli* cultured from their stool (Table 1). Among the 31 isolates, 27 (87%) were non-susceptible (ie, intermediate or resistant) to SXT, 23 (74%) to AMC, 12 (39%) to TZP, 26 (84%) to CIP, 21 (68%) to TOB, 18 (58%) to GEN, and 2 (6%) to AMK. No resistance was detected to ERT or MER. We used the latest CLSI breakpoints for ERT (0.25 μg/mL) and MER (1 μg/mL).

Among the 113 foreign travelers, the location of travel was associated with the likelihood of positivity of stool for ESBL-producing *E. coli* as shown in Table 2. The highest rates of ESBL positivity were associated with travel to Africa or the Indian subcontinent (p = 0.001).

### β-Lactamase Gene Identification and PMQR Determinants

All the *E. coli* isolates were positive for blaCTX-M genes: 22 produced CTX-M-15, 8 produced CTX-M-14, and 1 produced CTX-M-8. Some of the CTX-M-producing isolates also produced TEM-1 (ie, those with CTX-M-14 and -15) and OXA-1 (only those with CTX-M-15) β-lactamases. No other types of ESBLs were present. Travelers to South America (n = 1) were colonized with CTX-M-8; Asia (n = 6) and Caribbean/Central America (n = 1) with CTX-M-14 and -15, while patient from India (n = 7), Middle East (n = 1), and Africa (n = 9) were colonized with CTX-M-15-producing *E. coli*. CTX-M-15 (n = 4) and CTX-M-14 (n = 1) were present in the non-travelers. Twelve (39%) of the ESBL-producing *E. coli* isolates (all producing CTX-M-15) were positive for aac(6')-Ib-cr. None of the other PMQR genes were detected.

### Pulsed-Field Gel Electrophoresis

PFGE identified a closely related group of *E. coli* isolates that was designated as clone A (n = 8). The isolates that belonged to clone A had >80% similar PFGE profile. The remaining ESBL-producing isolates were not clonally related, i.e., exhibited <80% similar PFGE profiles and did not show patterns similar to those from clone A.

### Identification of Clone ST131

The PCR for the *pabB* allele of ST131 status identified PFGE clone A (n = 8) as belonging to ST131. ST131 status was confirmed by MLST. ST131 was present in six travelers that returned form Africa (n = 2), India (n = 2), and South-East Asia (n = 2). The PCR for the *pabB* allele was also performed on the remaining ESBL-producing *E. coli* and none tested positive for ST131.

### Phylogenetic Groups

Ten isolates (including the 8 that tested positive for ST131) belonged to phylogenetic group B2, 11 belonged to A, 2 belonged to B1, and the remaining 8 isolates belonged to phylogenetic groups D.

### Discussion

In recent years, international travel had grown by approximately 6% per year. A total of 880 million international tourist arrivals were recorded in 2009 (United Nations World Tourism organization. http://www.world-tourism.org. Accessed on December 10, 2010). This growth has been strongly driven by travelers to newly popular destinations in Asia, Africa, and the Middle East. Approximately 80 million persons from industrialized nations travel to the developing countries each year, and an estimated 200 million persons now reside outside their country of birth.16
It had been suggested that international travel, trade, tourism, and population migration form an important mode for the spread of antimicrobial-resistant bacteria. Antimicrobial-resistant bacteria are more pronounced in developing countries, where several factors select for the development of resistance and encourage for the dissemination of these bacteria. The selection and spread of resistant bacteria in these countries can often be traced to complex socioeconomic behaviors. These include urban migration, overcrowding, and improper sewage disposal.

A previous study from Calgary demonstrated that travel to the Indian subcontinent (ie, India, Pakistan, Africa, and Middle East) was associated with a high risk of urinary tract infection (including urosepsis) with an ESBL-producing *E. coli* in returning travelers. A follow-up study showed that this high risk of infection was mostly due to the acquisition of clone ST131 that produce CTX-M-15.

The results of this study confirm the findings of two recent studies from Sweden; the first study investigated the occurrence of ESBL-producing bacteria in patients with travelers’ diarrhea and found that the overall prevalence of fecal carriage of ESBL-producing bacteria was 24% (58/242). ESBL-producing *E. coli* was especially common among patients returning from India (11/14), Egypt (19/38; 50%), and Thailand (8/38; 22%).

The other study from Sweden included healthy volunteers that traveled outside Northern Europe and collected rectal swabs before and after traveling. Twenty-four of 100 participants with negative pretravel samples were colonized with ESBL-producing *E. coli* after the trip and travel to India was associated with the highest risk for the acquisition of ESBLs (88%; n = 7).

This study together with the Swedish studies confirms that foreign travel, especially to the Indian subcontinent and Africa, represent a major risk for rectal colonization with CTX-M-producing *E. coli* and most likely contribute to the Worldwide spread of these bacteria. Overall, we found that 24/52 (46%) of travelers with diarrhea returning from India, Africa, or Asia were colonized with ESBL-producing organisms. This study was specifically designed to only address potential travel as a possible risk factor. A potential source of selection bias might have come from the controls as patients with diarrhea due to chronic intestinal diseases were not excluded and probably have a lower probability of previous travel because of their disease. It was interesting to note that the prevalence of clone ST131 was similar among travelers and non-travelers. This suggests that ST131 has established itself among ESBL-producing *E. coli* in the Calgary region. Data from Calgary have shown that over 50% of ESBL-producing *E. coli* responsible for bacteremia during 2009 belonged to ST131 (J. Pitout, December 2010, manuscript in review).

The latest data regarding the prevalence of ESBLs in isolates collected during 2007 show some alarmingly high rates of ESBL-producing *E. coli* and *Klebsiella* spp in certain areas of Asia and the Indian subcontinent; rates as high as 55% were reported from China while a staggering 79% of *E. coli* collected in India were positive for ESBLs. An interesting aspect of the data from India was that the ESBL prevalence was equally high among *E. coli* collected from the hospital and community settings. As reports from India indicate that more than 70% of *E. coli* collected from the community is ESBL producers, it is conceivable that foreign travel to high-risk areas such as the Indian subcontinent plays an important role in the spread of this type of resistance across different continents.

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Declaration of Interests

The authors state they have no conflicts of interest to declare.

References


