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Polyclonal Intestinal Colonization with Extended-Spectrum Cephalosporin-Resistant Enterobacteriaceae upon Traveling to India

João Pires1, 2, Esther Kuenzli3, 4, 5, Sara Kasraian1, Regula Tinguely1, Hansjakob Furrer6, Markus Hilty1, 6, Christoph Hatz4, 5 and Andrea Endimiani1*

1 Institute for Infectious Diseases, University of Bern, Bern, Switzerland, 2 Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland, 3 Division for Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland, 4 Swiss Tropical and Public Health Institute, Basel, Switzerland, 5 Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland, 6 Department of Infectious Diseases, Bern University Hospital, University of Bern, Bern, Switzerland

We aimed to assess the intestinal colonization dynamics by multiple extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESC-R-Ent) clones in Swiss travelers to India, a country with high prevalence of these multidrug-resistant pathogens. Fifteen healthy volunteers (HVs) colonized with ESC-R-Ent after traveling to India who provided stools before, after, and at 3- and 6-month follow-up are presented in this study. Stools were enriched in a LB broth containing 3 mg/L cefturoxime and plated in standard selective media (BLSE, ChromID ESBL, Supercarba) to detect carbapenem- and/or ESC-R-Ent. At least 5 Enterobacteriaceae colonies were analyzed for each stool provided. All strains underwent phenotypic tests (MICs in microdilution) and molecular typing to define $\text{bla}$ genes (microarray, PCR/sequencing), clonality (MLST, rep-PCR), and plasmid content. While only three HVs were colonized before the trip, all participants had positive stools after returning, but the colonization rate decreased during the follow-up period (i.e., six HVs were still colonized at both 3 and 6 months). More importantly, polyclonal acquisition (median of 2 clones, range 1–5) was identified at return in all HVs. The majority of the $\text{Escherichia coli}$ isolates belonged to phylogenetic groups A and B1 and to high diverse non-epidemic sequence types (STs); however, 15% of them belonged to clonal complex 10 and mainly possessed $\text{bla}_{\text{CTX-M-15}}$ genes. F family plasmids were constantly found (~80%) in the recovered ESC-R-Ent. Our results indicate a possible polyclonal acquisition of the ESC-R-Ent via food-chain and/or through an environmental exposure. For some HVs, prolonged colonization in the follow-up period was observed due to clonal persistence or presence of the same plasmid replicon types in a new bacterial host. Travel medicine practitioners, clinicians, and clinical microbiologists who are facing the returning travelers and their samples for different reasons should be aware of this important phenomenon, so that better infection control measures, treatment strategies, and diagnostic tests can be adopted.

Keywords: $\text{E. coli}$, travelers, food-chain, environment, pAmpC, ESBL, CTX-M, plasmid
INTRODUCTION

The human gut is increasingly recognized as an important reservoir of extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESC-R-Ent), as increasing colonization rates by these pathogens are observed worldwide (Woerther et al., 2013). In particular, being colonized by ESBL-producing Enterobacteriaceae (ESBL-Ent) constitutes a risk factor to develop future extra-intestinal infections. Despite this fact, silent carriage of these multidrug-resistant (MDR) bacteria in the human gut is still poorly studied (Rogers et al., 2011; Van Der Bij and Pitout, 2012).

The highest colonization prevalence in the healthy population (~70%) has been reported in Southeast Asia (Woerther et al., 2013). Rates increased exponentially over the last decade and can be linked to several main factors: importation of high-risk clones (HiRC) via intercontinental travel, inappropriate use of antibiotics in different settings, high resistance rates in hospitals, poor hygienic measures (including food-handling), and high population density (Woerther et al., 2013). Therefore, it is not surprising that traveling to high prevalence countries constitute a risk factor to become colonized with these organisms (Van Der Bij and Pitout, 2012). Moreover, this importation of bacteria might facilitate the spread of MDR clones in low prevalence regions (Memish et al., 2003; Rogers et al., 2011). The problem has been highlighted in previous studies which reported individuals who acquired infections abroad with organisms encoding for new life-threatening resistance mechanisms (e.g., the first NDM-1 producer in a patient returning to Sweden or the importation of colistin-resistant MCR-1 producers from India), or which described resistance mechanisms in new bacterial hosts and/or genetic structures (Yong et al., 2009; Rogers et al., 2011; Seiffert et al., 2014; Bernasconi et al., 2016).

In recent years, an increased awareness for polymicrobial and/or polybacterial infections has also been achieved due to increased sensitivity of the microbiology diagnostic procedures (Short et al., 2014; Endimiani and Jacobs, 2016). However, we note that very few studies have identified multiple ESC-R-Ent colonizing the gut of healthy individuals (especially returning travelers) (Paltansing et al., 2013; Girlich et al., 2015; Stoeser et al., 2015), and very little attention has been attributed to this phenomenon.

In this work, we aimed to assess the colonization dynamics by multiple ESC-R-Ent clones acquired during a travel to India, a high prevalence country of ESC-R-Ent, to further understand the influence of this phenomenon in the shaping of the population structure of ESC-R-Ent in the human gut.

MATERIALS AND METHODS

Recruitment of Volunteers and Study Design

Fifteen healthy volunteers (HVs) traveling to India were selected from a larger ongoing study to perform this longitudinal pilot analysis. These HVs were chosen because they: (i) provided stool samples before and after traveling (both within 1 week); (ii) were colonized with ESC-R-Ent after returning from their trip, and (iii) provided follow-up samples at 3 and 6 months after the trip.

All HVs were recruited at the travel clinic of the Swiss Tropical and Public Health Institute (Basel) where they signed an informed consent. Subjects were instructed to self-collect the native stools in sterile plastic containers and send them to the Institute for Infectious Diseases of the University of Bern (Switzerland). Ethical approval was obtained by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ 239/12).

Sample Processing and Isolate Recovery

Stools (20 μg) were enriched overnight in 10 mL of Luria-Bertani (LB) broth containing a 30 μg cefuroxime disc. Enrichments were plated (50 μL) in BLSE, ChromID ESBL (bioMérieux), and a modified Supercarba plate containing imipenem to detect carbapenem- and/or ESC-R-Ent (Viau et al., 2016).

Species identification was obtained by using the MALDI-TOF MS (Bruker). At least 5 Enterobacteriaceae colonies per stool sample were analyzed. Strains were submitted to the double-disk synergism test (DDST) in cation-adjusted Mueller-Hinton plates (Becton Dickinson) supplemented with and without cloxacillin (250 mg/L; Sigma-Aldrich). Isolates yielding DDST results compatible with ESBL or plasmid-mediated AmpC (pAmpC) production were further characterized.

Antimicrobial Susceptibility Testing (AST) and β-lactamase(s) Characterization

ASTs were obtained using the microdilution Sentitre™ GNX2F plate (Trek Diagnostic Systems) and interpreted with EUCAST breakpoints, except for doxycycline and minocycline for which CLSI guidelines were used (CLSI, 2016; Eucast, 2016).

β-lactamase genes (bla) were identified using the CT103XL microarray (Check-Points). PCR/sequencing of the blaESBL and blapAmpC genes was carried out as previously done (Seiffert et al., 2013).

Analysis of Clonality and Plasmid Content

The repetitive extragenic palindromic PCR (rep-PCR) was used to assess clonal relatedness (Seiffert et al., 2013). Multilocus Sequence Typing (MLST) for E. coli (http://mlst.ucc.ie/mlst/dbs/Ecoli) and K. pneumoniae (http://bigdb.web.pasteur.fr/klebsiella/klebsiella.html) were also performed. The phylogenetic group (PhG) for E. coli was also obtained (Escobar-Páramo et al., 2004).

Plasmids’ incompatibility groups were assessed with the multiplex PCR based replicon typing (PBRT) kit (Diatheva).

RESULTS

Demographic and Clinical Characteristics of Travelers

As shown in Table 1, HVs were mostly females (73.3%) and middle-aged adults (average of 44 years). None of them was vegetarian or took anti-acids regularly. The average length of stay in India was 17.6 days (range 9–30 days). Four subjects reported having suffered from travelers’ diarrhea. None of the HVs took
antibiotics during their trip. During the 6-month follow-up period, several HVs traveled to other destinations (including India again).

**Colonization Timeline and Individual ESC-R-Ent Clones**

Only three out of 15 HVs were colonized by ESC-R-Ent before traveling, whereas all of them had positive stools after return (Table 2). During the follow-up period, six subjects were colonized at both 3 and 6 months.

Among the 15 HVs, a total of 143 isolates yielded DDST phenotypes compatible with either ESBL or ß-lactamases. For non-ß-lactams, higher rates of resistance (35–40%) were observed for doxycycline, levofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole (STX), whereas resistance to minocycline and aminoglycosides was ≤20% and none of the isolates was resistant to tigecycline.

Dynamics of molecular characteristics were observed (Table 2). Three clones were identified after returning from the trip, 22 (88%) yielded an individual ST. Nevertheless, 4 isolates (15%) belonged to the clonal complex (CC) 10. The PhG A was predominant (50%), followed by B1 and D (27 and 19%, respectively), being B2 PhG almost absent.

At 3 months, B2 or B1 PhGs were not identified, whereas PhGs A and D were equally represented. At 6 months, PhG D was the most frequent (57%), followed by A (29%) and B1 (14%) strains.

With regard to plasmids, those included in the F family were the most frequently identified (~80%), even though few other replicon types were observed (i.e., X1, 11, B/O, and K). F plasmids were also seen more frequently at 3 and 6 months, whereas other types identified after traveling were no longer recorded (Table 2).

**Shifts in Molecular Characteristics Over Time**

Shifts in the molecular characteristics of the ESC-R-Ent recovered at the different time points were observed (Table 2). Three months after traveling, six HVs (HV #26, 34, 43, 59, 97, and 100) were decolonized regardless of the number of clones identified immediately after return. For three HVs not colonized at 3 months (HV #23, 37, 41), ESC-R-Ent were identified at 6 months; compared to each other, these strains carried a different ß-lactamase type in a different ST and had a different plasmid content. For one subject (HV #29) colonized before traveling, the clone recovered just after the trip was different than the initial one; however, 3 months later the initial clone (D2-ST648-CTX-M-14) reappeared again (Supplemental Figure 1).

Persistent clones were also observed in HVs #56 and #80 as the same ST, plasmid content, and ß-lactamases were identified at 3 and 6 months, respectively (Supplemental Figure 1). In the case of HV #83, there was a shift of clones and ß-lactamases from the stools collected after traveling to those collected at 3 months; the same clone (D2-ST648) was identified after 6 months. Persistence of the ß-lactamase in the same mobile genetic element (MGE) was suspected in the case of HV #50, since the same replicon types identified in the ST155 strain detected after traveling was identified in the ST394 detected at 3 and 6 months (Table 2, Supplemental Figure 1).

**Carriage of Multiple ESC-R-Ent**

After returning from India, 9 (60%) subjects carried more than one ESC-R-Ent, with a mean of 1.9 clones and a median of 2 clones (overall range of 1–5). Different E. coli STs were frequently identified colonizing the same individual harboring the same ESBL type. Nonetheless, carriage of different ß-lactamases was also observed (HV #34 and #43).

At 3 and 6 months, carriage of multiple clones was identified only in HV #68 and HV #37, respectively. In particular, HV #68 had three different CTX-M-15 E. coli producers at 3 months, whereas HV #37 had at 6 months two different STs harboring dissimilar CTX-M-types.

When different species were found together with E. coli, they could carry the same bla (i.e., CTX-M-15-producing K. pneumoniae for HV #26), or a different gene (i.e., VEB-6-producing P. mirabilis for HV #37).

**DISCUSSION**

In this study, we prospectively followed a group of 15 HVs who returned from India colonized at intestinal level with more than one ESC-R-Ent clone. We emphasize that only a few studies have explored the issue of the polyclonal colonization of travelers with
| HV | Age/Sex | Job          | VG | AC | Previous trips in the last 12 months | Trips in India | 3 months after travel | 6 months after travel |
|----|---------|--------------|----|----|-----------------------------------|---------------|-----------------------|-----------------------|
|    |         |              |    |    | Countries                        | H  | S/C | AB | Days | H  | S/C | AB | New Travel | S/C | AB | New Travel |
| 23 | 67/f    | IT specialist | –  | –  | ITA (17 d); CRO (21 d); GER (2 d); MAL (7 d) | 14 | –  | –  | –    | –  | –  | –  | ITA (20 d) | –  | –  | –          |
| 26 | 50/f    | Speech therapist | –  | –  | ITA (28 d); FRA (7 d) | 14 | LD | –  | –    | –  | –  | –  | ITA (5 d) | –  | –  | –          |
| 29 | 31/m    | Chemist      | nd | nd | SPA (3 d); UK (3 d) | 15 | –  | –  | –    | –  | –  | –  | FRA (6 d) | –  | –  | –          |
| 34 | 36/f    | Art historian | –  | –  | UK (3 d); NED (4 d); CRO (2 d); AUT (2 d); DNK (5 d); GER (nd) | 27 | –  | –  | –    | –  | –  | –  | PER and BOL (21 d) | –  | –  | –          |
| 37 | 43/f    | Economist     | –  | –  | ITA (nd); MOR (nd); JOR (nd) | 14 | –  | –  | –    | –  | –  | –  | –          | –  | –  | –          |
| 41 | 68/f    | Translator    | –  | –  | USA (14 d) | 15 | –  | –  | –    | –  | –  | –  | –          | –  | –  | –          |
| 43 | 37/f    | Art historian | –  | –  | AUT (5 d) | 17 | LD | Na, AbC | –    | –  | –  | – | –          | –  | –  | –          |
| 50 | 33/m    | Chef         | –  | –  | –          | 30 | –  | –  | –    | –  | –  | –  | – | –          | –  | –  | –          |
| 56 | 42/m    | Sales manager | –  | –  | ICE (14 d); BEL (5 d); UK (3 d) | 9  | –  | –  | –    | –  | –  | –  | – | –          | –  | –  | –          |
| 59 | 50/f    | Economist     | –  | –  | ITA (14 d); CRO (21 d) | 14 | –  | –  | –    | –  | –  | –  | – | –          | –  | –  | –          |
| 68 | nd/m    | Personal manager | –  | –  | IND (7 d) | 20 | –  | LD | –    | –  | –  | –  | – | –          | –  | –  | –          |
| 80 | 26/f    | Student       | –  | –  | CRC (21 d); PRT (14 d); BEL (7 d) | 19 | –  | –  | –    | –  | –  | –  | – | –          | –  | –  | –          |
| 83 | 34/f    | Jurist        | –  | –  | GER (14 d); NED (7 d) | 23 | LD | –  | –    | –  | –  | –  | – | –          | –  | –  | –          |
| 97 | 42/f    | Biologist     | –  | –  | GRE (10 d); GER (nd) | 20 | –  | –  | –    | –  | –  | –  | – | –          | –  | –  | –          |
| 100| 57/f    | Librarian     | –  | –  | –          | 14 | –  | –  | –    | –  | –  | –  | – | –          | –  | –  | –          |

ID: identification code for the traveler; m, male; f, female; d, days; VG, vegetarian; AC, regular anti-acid intake; –, negative answer; nd, not defined; H, hospitalization; S/C, symptoms or complaints; AB, antibiotic intake; AUT, Austria; BEL, Belgium; BOL, Bolivia; CRC, Costa Rica; CRO, Croatia; DNK, Denmark; FRA, France; GER, Germany; GRE, Greece; ICE, Iceland; IND, India; ITA, Italy; JOR, Jordan; KHM, Cambodia; LAN, Laos; MAL, Malaysia; MDG, Madagascar; MOR, Morocco; NED, Netherlands; PER, Peru; PRT, Portugal; SPA, Spain; UK, United Kingdom; USA, United States of America; VNM, Vietnam; LD, light diarrhea; SvD, severe diarrhea; Na, nausea; AbC, abdominal cramps; Met, metronidazole.

a Relatives traveling together.
TABLE 2 | Molecular features of the recovered individual clones detected during the study period among the healthy volunteers (HVs) traveling to India.

| HV | Before traveling | After traveling | 3 months after traveling | 6 months after traveling |
|----|------------------|-----------------|--------------------------|-------------------------|
|    | PhG-ST<sup>a</sup> | β-lactamase (Plasmid replicon types)<sup>b</sup> | PhG-ST<sup>a</sup> | β-lactamase (Plasmid replicon types)<sup>b</sup> | PhG-ST<sup>a</sup> | β-lactamase (Plasmid replicon typing)<sup>b</sup> |
| 23 | – – – | B1-ST200 (CC40) | Dha-1-like (Fll) | – – | B1-ST2521 | CTX-M-1 (N) |
| 26<sup>c</sup> | – – – | D2-ST3052 | CTX-M-15 (Fll) | – – | – – | – – |
| 29 | D2-ST648<sup>(CC648)</sup> | CTX-M-14 (Fll, FIA, FIB) | D2-ST349 | CTX-M-15 (Xll) | D2-ST648 (CC648) | CTX-M-14 (Fll, FIA, FIB) | – – | – – |
| 34 | – – – | B1-ST29 | Dha-1-like (Fll) | SHV-12 (Xll) | – – | – – | A1-ST48 (CC10) | CTX-M-24-like (Xll) |
| 37 | – – – | A1-ST-New | CTX-M-15+TEM-3-like (Fll, FIB, P, Hll) | – – | – – | – – | A1-ST744 | CTX-M-15 (Fll) |
| 41 | – – – | A1-ST2325 | CTX-M-15 (Fll, FIB) | CTX-M-15 (Fll) | – – | – – | D2-S641 | CTX-M-1 (P, Hll) |
| 43 | – – – | B1-ST602 (CC446) | CTX-M-15 (Fll) | – – | – – | – – | – – | – – |
| 50 | B23-ST131<sup>(CC131)</sup> | CTX-M-15 (Fll, FIA, FIB) | B1-ST155 (CC155) | CTX-M-15 (Fll, B/O, K) | D1-ST394 (CC394) | CTX-M-15 (Fll, B/O, K) | D1-ST394 (CC394) | CTX-M-15 (Fll, B/O, K) |
| 56 | – – – | B2-ST-New | A1-ST617 (CC10) | Dha-1-like (Fll, FIA, B/O) | – – | – – | – – | – – |
| 59<sup>c</sup> | – – – | A1-ST652 | CTX-M-15 (Fll) | D1-ST-New | CTX-M-15 (Fll) | D1-ST-New | CTX-M-15 (Fll) | – – |
| 68 | – – – | A1-ST410 (CC23) | CTX-M-15 (Fll, FIA, FIB) | – – | – – | – – | – – |
| 80 | – – – | A1-ST10 (CC10) | CTX-M-15 (Fll, FIA, FIB) | A1-ST48 (CC10) | CTX-M-15 (Fll, Hll2) | CTX-M-15 (Fll, Hll2) | CTX-M-15 (Fll, Y) | – – |
| 83 | – – – | A1-ST10 (CC10) | CTX-M-15 (Fll, B/O, K) | A1-ST48 (CC10) | CTX-M-15 (Fll, Hll2) | CTX-M-15 (Fll, Hll2) | CTX-M-15 (Fll, Y) | – – | – – |
| 97 | – – – | A1-ST641 | CTX-M-15 (Fll, B/O, K, Hll1) | – – | – – | – – | D2-ST648 (CC648) | CMY-42 (Fll, FIA, FIB, I1) |
| 100 | – – – | B1-ST155 (CC155) | CTX-M-15 (Fll, FIB) | B1-ST-New | CTX-M-15 (Fll, FIB, B/O, K, Hll1) | – – | – – | – – |

Note. *–*, negative for ESC-R-Ent.

<sup>a</sup>PhG, phylogenetic group; ST, sequence type. Clonal complex (CC) is shown within brackets. Phylogenetic group is shown only for E. coli clones. Species other than E. coli are specified. The new STs could not be assigned because whole-genome sequencing for these strains was not performed as requested by the curator of the E. coli MLST scheme (see Supplemental Table 1).

<sup>b</sup>Only β-lactamases conferring resistance to extended-spectrum cephalosporins are shown.

<sup>c</sup>Volunteers traveling together.
TABLE 3 | Distribution of β-lactamase types, antibiotic resistance rates and E. coli phylogenetic groups for the 15 volunteers.

| Overall period | Before Traveling | After Traveling | 3 months after traveling | 6 months after traveling |
|----------------|------------------|-----------------|--------------------------|-------------------------|
| (n = 46, n° isolates, %) | (n = 3 n° isolates, %) | (n = 28, n° isolates, %) | (n = 8, n° isolates, %) | (n = 7, n° isolates, %) |
| **β-LACTAMASE TYPES** | | | | |
| CTX-M-Group 1 | 36 (73.9) | 2 (66.6) | 23 (82.1) | 6 (75) | 5 (71.4) |
| CTX-M-15 | 34 (73.9) | 2 (66.6) | 23 (82.1) | 6 (75) | 3 (42.9) |
| CTX-M-1 | 2 (4.3) | – | – | – | – |
| CTX-M-Group 4 | 3 (6.5) | 1 (33.3) | – | – | 2 (28.6) |
| CTX-M-14 | 2 (4.3) | 1 (33.3) | – | – | 1 (14.3) |
| CTX-M-24-like | 1 (2.2) | – | – | – | 1 (14.3) |
| SHV-12 | 1 (2.2) | – | 1 (3.6) | – | – |
| VEB-6 | 1 (2.2) | – | 1 (3.6) | – | – |
| DHA-1-like | 3 (4.3) | – | 3 (10.7) | – | – |
| CMY-42 | 2 (4.3) | – | – | 1 (12.5) | 1 (14.3) |
| **ANTIBIOTIC RESISTANCE** | | | | |
| Amikacin | 1 (2.2) | 0 | 1 (3.6) | 0 | 0 |
| Tobramycin | 11 (23.9) | 2 (66.7) | 5 (17.9) | 3 (37.5) | 1 (14.3) |
| Gentamicin | 6 (13) | 2 (66.7) | 3 (10.7) | 1 (12.5) | 0 |
| Ciprofloxacin | 19 (41.3) | 3 (100) | 10 (35.7) | 3 (37.5) | 3 (42.9) |
| Levofloxacin | 23 (50.0) | 3 (100) | 11 (39.3) | 5 (62.5) | 4 (57.1) |
| Trimethoprim-Sulfamethoxazole | 16 (34.8) | 3 (100) | 10 (35.7) | 1 (12.5) | 2 (28.6) |
| Doxycycline | 21 (45.7) | 1 (33.3) | 11 (39.3) | 5 (62.5) | 4 (57.1) |
| Minocycline | 10 (21.7) | 1 (33.3) | 6 (21.4) | 2 (25) | 1 (14.3) |
| Tigecycline | 0 | 0 | 0 | 0 | 0 |
| Colistin | 1 (2.2) | 0 | 1 (3.6) | 0 | 0 |
| Ticarcillin/clavulanate | 10 (21.7) | 2 (66.7) | 4 (14.3) | 2 (25) | 2 (28.6) |
| Piperacillin/tazobactam | 0 | 0 | 0 | 0 | 0 |
| Cefotaxime | 44 (95.7) | 3 (100) | 26 (92.9) | 8 (100) | 7 (100) |
| Ceftazidime | 42 (91.3) | 3 (100) | 27 (96.4) | 8 (100) | 4 (57.1) |
| Cefepime | 17 (37) | 2 (66.7) | 10 (35.7) | 2 (25) | 3 (42.9) |
| Aztreonam | 42 (91.3) | 3 (100) | 24 (85.7) | 8 (100) | 7 (100) |
| Imipenem | 0 | 0 | 0 | 0 | 0 |
| Ertapenem | 0 | 0 | 0 | 0 | 0 |
| Meropenem | 0 | 0 | 0 | 0 | 0 |
| Doripenem | 0 | 0 | 0 | 0 | 0 |
| **E. coli PHYLOGENETIC GROUPS** | | | | |
| A | 20 (45.5) | 1 (33.3) | 13 (50) | 4 (50) | 2 (28.6) |
| B1 | 8 (18.2) | 0 | 7 (26.9) | 0 | 1 (14.3) |
| D | 14 (31.8) | 1 (33.3) | 5 (19.2) | 4 (50) | 4 (57.1) |
| B2 | 2 (4.5) | 1 (33.3) | 1 (3.8) | 0 | 0 |

aOne isolates co-carries a TEM-3-like.
bBased on EUCAST breakpoints except for doxycycline and minocycline for which CLSI was used.

As expected, the most prevalent β-lactamase types identified in this study were the CTX-M-15 (Kennedy and Collignon, 2010; Tängdén et al., 2010; Peirano et al., 2011; Östholm-Balkhed et al., 2013; Paltansing et al., 2013; Kuenzli et al., 2014; Sole et al., 2014; Lübbert et al., 2015; Ruppé et al., 2015; Valverde et al., 2015; Barreto Miranda et al., 2016), whereas other β-lactamases (i.e., SHV-12-like, CMY-2-like, and DHA-type) were rarely detected (Östholm-Balkhed et al., 2013; Kuenzli et al., 2014; Sole et al., 2014). The frequent observation of F plasmids was also consistent with the great dispersion of these MGEs.

Antibiotic Susceptibility and β-lactamase Diversity
The resistance profiles of the recovered ESC-R-Ent isolates (i.e., higher rates of resistance to fluoroquinolones, STX, and aminoglycosides) clearly emphasized the limited option available for antibiotic therapy in case of infection due to these pathogens. These life-threatening Gram-negative pathogens (Paltansing et al., 2013; Girlich et al., 2015; Stoesser et al., 2015).
Population Structure Shifts among ESC-R-Ent in Returning Travelers

Prior to travel, the HiRC B23-ST131 and D2-ST648 were identified. These PhGs/STs have been found worldwide colonizing and/or causing infections in humans, both companion and food-producing animals, and wildlife (Woodford et al., 2011; Ewers et al., 2012, 2014; Pitout, 2012; Nakane et al., 2016). Interestingly, in the HVs analyzed these initial clones were replaced after the trip to India (Table 2).

Post-travel, the high heterogeneity of clones identified indicated numerous possible exposure sources with ESC-R-Ent. Given that isolates belonged mostly to A and B1 PhGs and to CC10, CC155, or CC23, we speculate that the acquisition of these clones could be linked to environmental exposure and/or the food-chain (Paltansing et al., 2013; Kuenzli et al., 2014; Valverde et al., 2015). Moreover, the fact that we found ESBL-Ent coding for the same β-lactamase enzymes, which are frequently reported in food-items and river waters, strengthens this hypothesis (Bajaj et al., 2015; Upadhyay et al., 2015; Zurluhu et al., 2015).

However, we should note that despite being unlikely, human-to-human transmission cannot be excluded, since people living in tropical or sub-tropical climates have PhG A E. coli strains as the predominant lineages in the gut (Escobar-Páramo et al., 2004; Tenailleon et al., 2010). One Indian study identified CTX-M-15-producing E. coli of PhG A as the most frequent ESBL-E. coli lineage colonizing the gut of healthy individuals (Escobar-Páramo et al., 2004; Dureja et al., 2014). Furthermore, the identification of A1-ST3203 in two HVs traveling together (#26 and #59), but having different pre-travel colonization status (Table 2), may suggest that the acquisition arises from a common exposure source or human-to-human transmission. Overall, the increasing recovery of PhG A and B1 E. coli is worrisome, due to their expansion in the clinical setting associated with the production of ESBLs (Woodford et al., 2011; Rodrigues et al., 2015).

In the stools collected at 3 and 6 months, several epidemiological shifts were observed. The persistence of the same clone was observed until 3 months for HV #80 and until 6 months for HV #56. Nevertheless, the identification of the same replicon types in a different ST, as observed for HV #50, suggests that in vivo conjugation may occur (van Schaik, 2015). However, we cannot exclude that the different clones identified during the follow-up can also be associated to new independent acquisitions, as several of these HVs have traveled to high prevalence countries during this period (HV #23, 37, and 68; Table 1).

Among the different STs that could be consistently identified over time, ST648 and members of CC10 were the most frequent (Table 2). In addition, the majority of plasmids recovered belonged to the F family. This combination of clones and plasmids, which can easily adapt to the human host, could explain their persistence in the gut (Paltansing et al., 2013; Titelman et al., 2014). Intrinsic characteristics of these genetic backgrounds or factors conferring increased fitness (e.g., usage of alternative metabolic pathways), might also contribute to the persistent intestinal colonization (Alteri and Mobley, 2012).

Colonization Dynamics: Hypothetical Pathways

According to our observations spanning a 6-month period, several pathways can be drawn when an initially non-colonized HV travels to a high prevalence setting: (i) clonal persistence after returning from the trip (e.g., HV #56); (ii) persistence of the MGE (e.g., HV #50); (iii) detection of a previously unidentified clone or independent new acquisition (e.g., HV #83), and (iv) clonal clearance (e.g., HV #26) from the intestinal tract after several months (Table 2).

In the case of previously colonized HVs, an additional pathway can be identified, as the initial clone can be still present after traveling, but below the detection limit and then re-emerges after some time (e.g., HV #29) (Paltansing et al., 2013). This phenomenon has been observed in patients colonized after an infection (Titelman et al., 2014).

Polyclonality: Clinical and Ecological Implications

In our study, a median of 2 clones were identified in the stools of travelers after the trip, indicating that a polyclonal acquisition occurs when visiting a high ESC-R-Ent prevalence area. Interestingly, HV #68 returned to India in the follow-up period and then he was found colonized with three unique MDR clones, emphasizing the risk of polyclonal acquisition in this region. This overall phenomenon has been already perceived by other authors (Östholm-Balkhed et al., 2013; Paltansing et al., 2013; Lübbert et al., 2015; Ruppé et al., 2015; Valverde et al., 2015). However, only one study reported a mean of 1.8 clones among the travelers (being the polyclonal acquisition identified in 46% of them), but no bacterial population structure analysis was performed (Ruppé et al., 2015).

In our opinion, there are several important implications associated with the polyclonal acquisition of ESC-R-Ent. On the clinical level, physicians should be aware that extra-intestinal infections in patients who recently traveled in high ESC-R-Ent prevalence region might be due to MDR clones that are persisting in the gut. Therefore, the antibiotic treatment has to be tailored according to the ASTs results provided by the clinical laboratory and not empirically given (Kennedy and Collignon, 2010). At the diagnostic laboratory level, colony picking from the selective agar plates can lead to the identification of just one MDR isolate (e.g., identifying an ESBL-Ent ciprofloxacin susceptible, but not the ESBL-Ent resistant to the antibiotic), hindering the implementation of proper antibiotic treatment regimens (Kennedy and Collignon, 2010; Endimiani and Jacobs, 2016). At the colonization level, different pathways are possible. The genetic background of the bacterium could have a significant influence on its ability to persist in the gut. Moreover, in vivo conjugation can also propagate resistance mechanisms and/or novel adaptive characteristics into new bacterial hosts with
stronger ability to persistently colonize the human gut (e.g., the HfKCs ST131 and ST648), further potentiating the overall spread of these MDR pathogens (Baquero et al., 2011; van Schaik, 2015). Moreover, it has also been demonstrated that bacteria with certain genetic backgrounds (ST131, ST648, and those of PhG A) do not suffer fitness upon acquisition of MDR plasmids. Therefore, particular combinations of very fitting STs with MDR plasmids might favor more successful colonization overtime (Johnson et al., 2015; Schaufler et al., 2016).

CONCLUSIONS

A high polyclonal acquisition rate of ESC-R-Ent was observed when HVs traveled to India. These multiple clones can follow different pathways, ranging from clonal clearance to clonal and/or MGE persistence in the gut. The genetic backgrounds of the acquired clones suggest that this acquisition is linked to not yet well-defined non-human sources, with the food-chain and the environment being likely potential sources.

The effective implications of these overall findings are still not completely clear. However, it appears plausible that travel medicine practitioners, clinicians, and clinical microbiologists who are facing the returning travelers and their specimens for different clinical reasons should be aware of this important phenomenon, so that better infection control measures, treatment strategies, and diagnostic tests can be adopted. Studying the factors underlying ESC-R-Ent persistence in the gut is also extremely important to better devise decolonization and colonization prevention strategies in the near future.

AUTHOR CONTRIBUTIONS

Conception and design (EK, CH, AE); acquisition of data (JP, EK, SK, RT); analysis and interpretation of data (JP, EK, HF, MH, CH, AE); drafting the work (JP, AE); revising it critically for important intellectual content (all authors); final approval of the version to be published (all authors); agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (all authors).

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SUPPLEMENTARY MATERIAL

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REFERENCES

Alteri, C. J., and Mobley, H. L. T. (2012). Escherichia coli physiology and metabolism dictates adaptation to diverse host microenvironments. Curr. Opin. Microbiol. 15, 3–9. doi: 10.1016/j.mib.2011.12.004

Bajaj, P., Singh, N. S., Kanaaup, P. K., and Virdi, J. S. (2015). Distribution and molecular characterization of genes encoding CTX-M and AmpC β-lactamases in Escherichia coli isolated from an Indian urban aquatic environment. Sci. Total Environ. 505, 350–356. doi: 10.1016/j.scitotenv.2014.09.084

Baquero, F., Coque, T. M., and De La Cruz, F. (2011). Ecology and evolution as targets for novel eco-eco drugs and strategies to fight antibiotic resistance. Antimicrob. Agents Chemother. 55, 3649–3660. doi: 10.1128/AAC.00013-11

Barreto Miranda, I., Ignatius, R., Pfüller, R., Friedrich-Jänicke, B., Steiner, F., Pires, J., Tinguely, R., Carattoli, A., Hatz, C., Mahajan, S., and Raychaudhuri, S. (2014). Phylogenetic distribution and prevalence of genes encoding class I integrons and CTX-M-15 extended-spectrum β-lactamases in Escherichia coli isolates from healthy humans in Chandigarh, India. PLoS ONE 9:e112551. doi: 10.1371/journal.pone.0112551

Endimiani, A., and Jacobs, M. R. (2016). The changing role of the clinical microbiology laboratory in defining resistance in gram-negatives. Infect. Dis. Clin. North Am. 30, 323–345. doi: 10.1016/j.idc.2016.02.002

Escober-Páramo, P., Grenet, K., Le Menac’h, A., Rode, L., Salgado, E., Amorin, C., et al. (2004). Large-scale population structure of human commensal Escherichia coli isolates. Appl. Environ. Microbiol. 70, 5698–5700. doi: 10.1128/AEM.70.9.5698-5700.2004

Eucaut (2016). European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters Version 6.0. Available online at: www.eucaut.org

Ewers, C., Bethe, A., Semmler, T., Gouenther, S., and Wieler, L. H. (2012). Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin. Microbiol. Infect. 18, 646–655. doi: 10.1111/j.1469-0691.2012.03850.x

Ewers, C., Bethe, A., Stamm, I., Grobbel, M., Kopp, P. A., Guerra, B., et al. (2014). CTX-M-15-D-ST648 Escherichia coli from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? J. Antimicrob. Chemother. 69, 1224–1230. doi: 10.1093/jac/dkt516

Girlich, D., Nordmann, P., Lécuyer, H., Berche, P., Mammarat-Khuong, A., Gross, I., et al. (2015). Multiple colonization with highly resistant bacteria: carbapenemase-producing Enterobacteriaceae, carbapenemase-producing Pseudomonas aeruginosa, carbapenemase-producing Acinetobacter baumannii, and glycopeptide-resistant Enterococcus faecium. Diagn. Microbiol. Infect. Dis. 81, 217–218. doi: 10.1016/j.diagmicrobio.2014.12.002

Johnson, T. J., Singer, R. S., Isaacson, R. E., Danzeisen, J. L., Lang, K., Kobluk, K., et al. (2015). In vivo transmission of an IncA/C Plasmid in escherichia coli depends on tetracycline concentration, and acquisition of the plasmid results in a variable cost of fitness. Appl. Environ. Microbiol. 81, 3561–3570. doi: 10.1128/AEM.04193-14

Frontiers in Microbiology | www.frontiersin.org 8 July 2016 | Volume 7 | Article 1069
Kennedy, K., and Collignon, P. (2010). Colonisation with *Escherichia coli* resistant to “critically important” antibiotics: a high risk for international travellers. *Eur. J. Clin. Microbiol. Infect. Dis.* 29, 1501–1506. doi: 10.1007/s10096-010-1031-y

Kuenzli, E., Jaeger, V., Frei, R., Neumayr, A., Decrom, S., Hallé, S., et al. (2014). 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.* 14:528. doi: 10.1186/1471-2334-14-528

Lübbert, C., Straube, L., Stein, C., Makarewicz, O., Schubert, S., Mössner, J., et al. (2015). Colonization with extended-spectrum beta-lactamase-producing and carbapenemase-producing *Enterobacteriaceae* in international travelers returning to Germany. *Int. J. Med. Microbiol.* 305, 148–156. doi: 10.1016/j.ijmm.2014.12.001

Mathers, A. J., Peirano, G., and Pitout, J. D. D. (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clin. Microbiol. Rev.* 28, 565–591. doi: 10.1128/CMR.00116-14

Memish, Z. A., Venkatesh, S., and Shibli, A. M. (2003). Impact of travel on *Short, F. L.*, Murdoch, S. L., and Ryan, R. P. (2014). Polybacterial human disease: the ills of social networking. *Trends Microbiol.* 22, 508–516. doi: 10.1016/j.tim.2014.05.007

Sole, M., Pitart, C., Oliveira, I., Fábrega, A., Muñoz, L., Campo, J., et al. (2014). Extended-spectrum beta-lactamase-producing *Escherichia coli* faecal carriage in Spanish travellers returning from tropical and subtropical countries. *Clin. Microbiol. Infect.* 20, O636–O639. doi: 10.1111/1469-0691.12592

Stoesser, N., Xayayeuang, S., Vongsouath, M., Phommasone, K., Elliott, I., Del Ojo Elias, C., et al. (2015). Colonization with *Enterobacteriaceae* producing ESBLs in children attending pre-school childcare facilities in the Lao People’s Democratic Republic. *J. Antimicrob. Chemother.* 70, 1893–1897. doi: 10.1093/jac/dkv021

Tängrén, T., Cars, O., Melhus, Å., and Löwdin, E. (2010). Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob. Agents Chemother.* 54, 3564–3568. doi: 10.1128/AAC.00220-10

Tenaillon, O., Skurnik, D., Picard, B., and Denamur, E. (2010). The population genetics of commensal *Escherichia coli*. *Nat. Rev. Microbiol.* 8, 207–217. doi: 10.1038/nrmicro2298

Tietelman, E., Hasan, C. M., Iversen, A., Nauclér, P., Kais, M., Kalin, M., et al. (2014). Faecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* is common 12 months after infection and is related to strain factors. *Clin. Microbiol. Infect.* 20, OS08–O515. doi: 10.1111/1469-0691.12559

Upadhyay, S., Hussain, A., Mishra, S., Maurya, A. P., Bhattacharjee, A., and Joshi, S. R. (2015). Genetic environment of plasmid mediated CTX-M-15 extended spectrum beta-lactamases from clinical and food borne bacteria in North-Eastern India. *PLoS ONE* 10:e0138056. doi: 10.1371/journal.pone.0138056

Valverde, A., Torrents, M. C., Norman, F., San Martín, E., Moreno, L., Pérez-Molina, J. A., et al. (2015). CTX-M-15 non-ST131 *Escherichia coli* isolates are mainly responsible of faecal carriage with ESBL-producing *Enterobacteriaceae* in travellers, immigrants and those visiting relatives and friends. *Clin. Microbiol. Infect.* 21, 252.e1–4. doi: 10.1016/j.cmi.2014.09.021

Van Der Bij, A. K., and Pitout, J. D. D. (2012). The role of international travel in the worldwide spread of multiresistant *Enterobacteriaceae*. *J. Antimicrob. Chemother.* 67, 2090–2100. doi: 10.1093/jac/dks214

van Schaik, W. (2015). The human gut resistome. *Philos. Trans. R. Soc. B Biol. Sci.* 370:20140087. doi: 10.1098/rstb.2014.0087

Vrau, R., Frank, K. M., Jacobs, M. R., Wilson, B., Kaye, K., Donskey, C. J., et al. (2016). Intestinal carriage of carbapenemase-producing organisms: current status of surveillance methods. *Clin. Microbiol. Rev.* 29, 1–27. doi: 10.1128/CMR.00118-14

Woerther, P.-L., Burdet, C., Chachaty, E., and Andremont, A. (2013). Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. *Clin. Microbiol. Rev.* 26, 744–758. doi: 10.1128/CMR.00023-13

Woodford, N., Turton, J. F., and Livermore, D. M. (2011). Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol. Rev.* 35, 736–755. doi: 10.1111/j.1574-6968.2011.01268.x

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., et al. (2009). Characterization of a new metallo-beta-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.* 53, 5046–5054. doi: 10.1128/AAC.00774-09

Zurfluh, K., Nüesch-Inderbinen, M., Morach, M., Zähler Bernard, A., Hächler, H., and Stephan, R. (2015). Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* isolated from vegetables imported from the dominican republic, India, Thailand, and Vietnam. *Appl. Environ. Microbiol.* 81, 3115–3120. doi: 10.1128/AEM.02528-15

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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