Diarrhoeagenic *Escherichia coli* pathotypes in travellers attending a tropical medicine unit in a Spanish hospital

Travellers’ diarrhoea is the most common travel-related illness. More than 60% of cases are caused by a variety of bacterial enteropathogens, of which diarrhoeagenic *Escherichia coli* (DEC) is a significant contributor (Riddle *et al.*, 2006; Shah *et al.*, 2009). The major distinguishing factors between pathogenic and non-pathogenic *E. coli* strains is the presence of virulence genes, which encode various known mechanisms of pathogenicity. Based on these virulence factors and the patient clinical picture, at least five pathotypes of DEC have been described: verocytotoxin (VT)-producing *E. coli* (VT-EPEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterogaggregative *E. coli* (EAEC). VT-EPEC strains produce VT1 and/or VT2. ETEC is defined by the presence of heat-labile and heat-stable *E. coli* enterotoxins, encoded by *eltA* and *estA* genes, respectively. EPEC is associated with the virulence factor intimin, encoded by the *eae* gene. The *eae* gene may also be present in VTEC strains. EPEC is divided into two types according to the presence of bundle-forming pilis, a fimbrial adhesin that is a virulence determinant of typical EPEC but is absent from atypical EPEC (aEPEC). EIEC strains are related closely to *Shigella* spp. in terms of phylogeny and pathogenesis, and are characterized by the presence of the *ipaH* gene (Kaper *et al.*, 2004). EAEC strains are characterized by a DNA fragment sequence known as CVD432 that usually also contains the virulence gene *aggR*. Serotyping and biochemical analysis have been widely applied in the diagnosis of gastrointestinal pathogens, but cannot be used for identifying DEC pathotypes. For this reason, DEC infections are often undiagnosed. Identification of the characteristic virulence genes, by hybridization or PCR, is an obvious choice for DEC diagnosis (Persson *et al.*, 2007).

ETEC and EAEC are the main DEC pathotypes responsible for travellers’ diarrhoea throughout the world; however, pathotypes show geographical differences in their distribution, which may require different strategies of antimicrobial therapy or vaccination. Most studies on travellers’ diarrhoea caused by DEC have been performed in patients coming from the traditional tourist destinations; however, data about this illness involving subjects from developing areas such as sub-Saharan African regions are scarce (Riddle *et al.*, 2006; Shah *et al.*, 2009; Okeke, 2009). Our aim was to investigate the prevalence of DEC and other enterobacterial pathogens in patients with travellers’ diarrhoea in a tropical medicine unit with a significant proportion of subjects coming from sub-Saharan Africa.

From June 2009 to October 2010, stool samples from patients suffering from acute diarrhoea that were seen in the Tropical Medicine Department, Hospital Carlos III, were investigated. Specimens were collected within 72 h of the onset of symptoms. Culturing of bacterial enteropathogens was carried out by using six standard media: MacConkey, Salmonella–Shigella, Yersinia and Campylobacter agar, and selenite broth (bioMérieux). After overnight incubation at 37 °C, lactose-fermenting colonies with the typical appearance of *E. coli* were selected for further analysis. Isolates were identified by biochemical assays using MicroScan Gram-negative combo panel NUC 45 (Siemens).

The DNA template for PCR was obtained by picking up to ten colonies from a pure overnight bacterial culture. A commercial multiplex PCR (DEC PCR kit; Statens Serum Institut) was used to detect genes associated with DEC, VTs 1 (*vtx1*) and 2 (*vtx2*), intimin (*eae*), heat-stable enterotoxin (*estA*) and heat-labile enterotoxin (*eltA*), and the invasive plasmid antigen (*ipaH*), following the manufacturer’s instructions. In addition, other virulence markers related to DEC including CVD432, *aggR* and *bfp* were detected by PCR using primers and conditions previously described (Blanco *et al.*, 2006; Mora *et al.*, 2011). Molecular serotyping was performed using the O104 antigen-associated gene (wzx-O104) (Mora *et al.*, 2011). Epidemiological data including recent travels and country of destination were also recorded.

A total of 84 patients were included in this study. The mean age of the study population was 36.3 ± 12.8 years and the gender distribution was 51.2% for women. Seventy (83.3%) patients were native Spanish travellers and the rest were immigrants living in Spain who had travelled to their country of origin. Almost half of the patients (*n*=36, 42.9%) came from sub-Saharan African destinations: 15 from the central region (nine from Equatorial Guinea), 14 from the west zone (eight from Mali, Senegal and Nigeria) and seven from the east region (four from Ethiopia). The rest of the patients came from: Asia (*n*=17, 20.2%), mostly from India; Central America (*n*=15, 17.9%), mainly from Haiti; South America (*n*=13, 15.5%), mostly from Peru and Ecuador; and the Maghreb (*n*=3, 3.6%).

Enteropathogenic bacteria were isolated in 19 (22.6%) patients, five of whom presented with coinfection. The epidemiological features of the patients and genetic characterization of the isolates are shown in Table 1. The majority of patients were Spanish travellers returning from sub-Saharan Africa and India. DEC was identified in 15 patients: nine patients had EAEC (CVD432<sup>+</sup> and/or *aggR*<sup>+</sup>) and none of them belonged to serotype O104; eight patients had aEPEC (*eae*<sup>+</sup> and *bfp*<sup>+</sup>) and one was ETEC (*eltA*<sup>+</sup>).* Campylobacter jejuni* was detected in two patients that were coinfected with DEC. *Salmonella* spp. and *Shigella sonnei* (*ipaH*+) were identified in two patients each and were the only bacteria isolated in these patients.

Travellers’ diarrhoea continues to be a worldwide disease that affects millions of...
In conclusion, our data highlighted the importance of investigating EAEC strains in diarrhoea cases in travellers. The use of polymicrobial diarrhoea cultures in travellers’ diarrhoea in Africa and the problematic issue in the diagnosis of DEC and EAEC need more attention in future studies. In addition, further studies on the use of molecular markers (e.g., aggR) should be investigated to confirm the reported results. This will help the clinicians in the field, as the results of our study are in line with the general diagnostic approach in the field of diarrhoea in travellers, where polymicrobial cultures are the most common in all regions. We also suggest that further studies be conducted to investigate the role of EAEC strains in the development of gastroenteritis, as well as the presence of EAEC strains in diarrhoea cases in travellers, in the developing world. This will help in the development of new diagnostic techniques and the development of new treatment strategies for EAEC strains in travellers’ diarrhoea.
clinical setting. Identification of virulence genes by multiplex PCR is a fast and simple method and is a useful tool for routine diagnostics.

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