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* (VTEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative E. coli (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterohaemorrhagic *E. coli* (EHEC). VTEC 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 pilus, 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 EAE (CVD432+ and/or *aggR*+) and none of them belonged to serotype O104; eight patients had aEPEC (*eae*+ and *bfp*+) and one was ETEC (*ela*+). *Campylobacter jejuni* was detected in two patients that were coincident 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 our study, the most frequent bacterial enteropathogen responsible for this illness was DEC. This result is in concordance with previous studies, in which DEC pathotypes were the main cause of travellers’ diarrhoea throughout the world, except for individuals coming from Southeast Asia where Campylobacter spp. is the main bacterial enteropathogen isolated (Riddle et al., 2006; Shah et al., 2009). EAEC was the most frequent pathotype isolated in our study. This result is expected because EAEC is probably the most common bacterial cause of diarrhoea in the developing world at present (Chattaway et al., 2011). In addition, asymptomatic carriage is very common in certain regions of Africa, suggesting the possibility of asymptomatic carriers who are food handlers passing these strains onto travellers (Oundo et al., 2008).

Molecular identification of EAEC strains is cumbersome because there is no unique stable chromosomal marker defined for diagnosis. However, detection of the CVD432 marker and the best-studied virulence factor aggR are used in the majority of studies, as in ours. A limitation of this approach is that CVD432 shows a good specificity but it is insufficiently sensitive (Kaur et al., 2010; Okeke, 2009; Chattaway et al., 2011). Recently, a limited number of chromosomal genes have been described that can be suitable targets for the detection of EAEC and are typically associated with virulence (Boisen et al., 2012; Taniuchi et al., 2012). These new genes can be adjuncts or alternatives to the traditional CVD432 and aggR molecular markers and could help contribute to understanding the true burden and impact of EAEC on human health.

Patients infected with eEPEC came from India, sub-Saharan Africa and Peru, where this pathotype is one of the main agents responsible for infantile diarrhoea (Wani et al., 2006; Okeke, 2009; Ochoa et al., 2011). Although causative agents of travellers’ and infantile diarrhoea are similar in many cases, the extent to which the aetiology of these two syndromes overlaps is not precisely known (Guerrant et al., 2005). Unfortunately, there are limited studies on patients with travellers’ diarrhoea coming from these regions, where EPEC is not always assessed (Riddle et al., 2006; Shah et al., 2009).

Only one case of ETEC was found in our study. Some authors have suggested an epidemiological change in the aetiology of DEC, with a decrease in the isolation of ETEC in recent years (Shah et al., 2009). Nevertheless, our study presented several limitations, so the prevalence of bacterial enteropathogens as obtained in our study must be interpreted cautiously. Firstly, patients returned from many different regions, so the number of returnees from any one specific area was too small to draw conclusions from. Secondly, the sample was biased because only patients with a more severe case of travellers’ diarrhoea usually seek medical care. Thirdly, we only included lactose-fermenting colonies for PCR testing, but a number of DEC pathotypes including EPEC, EACE and EIEC do not ferment lactose and could therefore remain undetected. Finally, PCR was performed on only a limited number of colonies from a bacterial culture; PCR directly from stool samples could have been a better approach.

In conclusion, our data highlighted the main role of DEC as an aetiopathogen in travellers’ diarrhoea and the need to identify these strains in the appropriate...
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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