Characterization of an emergent clone of enteroinvasive *Escherichia coli* circulating in Europe

V. Michelacci[1], G. Prosseda[2], A. Maugliani[1], R. Tozzoli[1], S. Sanchez[3], S. Herrera-León[3], T. Dallman[4], C. Jenkins[4], A. Caprioli[1] and S. Morabito[1]

1) *European Union Reference Laboratory for* Escherichia coli, *Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità,* 2) *Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Biology and Biotechnology 'C. Darwin', Sapienza Università di Roma, Rome, Italy,* 3) *Laboratory of Enterobacteriaceae, Service of Bacteriology, National Center of Microbiology, Instituto de Salud Carlos III, 28220, Majadahonda, Madrid, Spain and* 4) *Gastrointestinal Bacteria Reference Unit, Public Health England, London, UK*

Abstract

Enteroinvasive *Escherichia coli* (EIEC) cause intestinal illness indistinguishable from that caused by *Shigella,* mainly in developing countries. Recently an upsurge of cases of EIEC infections has been observed in Europe, with two large outbreaks occurring in Italy and in the United Kingdom. We have characterized phenotypically and genotypically the strains responsible for these epidemics together with an additional isolate from a sporadic case isolated in Spain. The three isolates belonged to the same rare serotype O96:H19 and were of sequence type ST-99, never reported before in EIEC or *Shigella.* The EIEC strains investigated possessed all the virulence genes harboured on the large plasmid conferring the invasive phenotype to EIEC and *Shigella* while showing only some of the known chromosomal virulence genes and none of the described pathoadaptative mutations. At the same time, they displayed motility abilities and biochemical requirements resembling more closely those of the non-pathogenic *E. coli* rather than the EIEC and *Shigella* strains used as reference. Our observations suggested that the O96:H19 strains belong to an emerging EIEC clone, which could be the result of a recent event of acquisition of the invasion plasmid by commensal *E. coli.*

Keywords: Emergence of new pathogenic types, enteroinvasive *Escherichia coli*, genomic characterization, outbreaks of infection, *Shigella*
Original Submission: 8 July 2015; **Revised Submission:** 14 October 2015; **Accepted:** 14 October 2015
Editor: P.T. Tassios
Article published online: 10 November 2015

Corresponding author: V. Michelacci, European Union Reference Laboratory for *Escherichia coli*, Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy
E-mail: valeria.michelacci@iss.it

Introduction

Enteroinvasive *Escherichia coli* (EIEC) are a group of pathogenic bacteria causing intestinal illness upon invasion of the human colonic mucosa [1]. The disease caused by EIEC is a bacillary dysentery with a clinical presentation indistinguishable from that caused by infection with strains of *Shigella* species, involving abdominal cramps, nausea, fever and bloody and mucus diarrhoea [2]. The pathogenesis of EIEC infection involves the colonic epithelial cell penetration preceded by the transcytosis through M cells, the lysis of the endocytic vacuole, intracellular multiplication and extension into adjacent epithelial cells [1].

The main genes conferring the *Shigella* and EIEC invasive phenotype are harboured on a large plasmid and encode the components of a type three secretion system, including *mxi* (Membrane eXcretion of Ipa) and *spa* (Surface Presentation of invasion plasmid Antigens) and a number of translocated effectors, represented by the products of the genes *vir, ipa* (Invasion Plasmid Antigens) and *ipg* (Invasion Plasmid Genes) [3]. Several other virulence genes play accessory roles in the

http://dx.doi.org/10.1016/j.cmi.2015.10.025

pathogenetic process and are differentially distributed in different *Shigella* and EIEC strains, and they encode toxins, proteins interfering with the immune response of the host, factors facilitating the colonization process and iron-uptake systems favouring intracellular growth [3].

The morbidity and mortality of EIEC infections have not been fully assessed but can be inferred from those ascribed to shigellosis. Mortality is especially high among children, and it has been estimated that 99% of the 165 million cases recorded annually worldwide occur in developing countries [4,5]. The high circulation of these pathogens in low-income regions is plausibly linked to the mode of transmission of the infections, which involves the oral–faecal route. In the United States and Europe, where higher hygiene standards are in place, the subjects most often infected are travellers returning from high-incidence countries, children in day care and migrant workers [6]. The information on the incidence of EIEC-associated disease is scant, as the differentiation between these infections and those caused by *Shigella* is difficult and based on the use of multiple tests, such as the PCR targeting the *ipaH* gene, coupled with biochemical and serologic typing [2]. In some cases the infections caused by *Shigella* and EIEC may be transmitted by contaminated food and water, but these appear not to be common sources of infections [7].

Historically, EIEC have not been associated with large outbreaks in industrialized countries. More commonly, EIEC causes sporadic cases that affect specific risk groups. However, recently an upsurge of cases of EIEC infections has been observed in Europe. In 2012, a large outbreak of bloody diarrhoea occurred among the employees of the fire brigade of the city of Milan, Italy [8]. The episode involved more than 100 cases of infections, and the additional symptoms most commonly reported were fever, abdominal cramps and vomiting [8]. Laboratory investigations showed the presence of a positive PCR amplification of the *ipaH* gene in several stool samples, and an *E. coli* strain positive for the presence of *ipaH* and belonging to serotype O96:H19 was isolated from six cases [8]. Cooked vegetables were suspected as the source for infection following a case–control study [8]. In 2013, an EIEC isolate of the same serotype was isolated from a sporadic case of traveller's diarrhoea in Spain (data not published). Finally, an outbreak of gastrointestinal disease occurred in the East Midlands in the United Kingdom involving 50 people and was suspected to be caused by the consumption of contaminated salad vegetables [9]. Again, an EIEC of serogroup O96 was isolated from some of the patients [9].

We carried out biochemical and phenotypic characterization of the EIEC strains from the Italian and the UK outbreaks and from the sporadic case in Spain, as well as their whole genome sequencing. Our results show that these isolates belong to the same emerging EIEC clone.

Materials and Methods

Bacterial strains

The EIEC isolates involved in the study included the strains EF432, EF433 and EF434 isolated in Italy in 2012 [8], H142690012 isolated in the United Kingdom 2014 [9] and CNM-2113/13 isolated from a case of severe diarrhoea that occurred in Spain in 2014 (data not published). All the five EIEC strains possessed the *ipaH* gene, which is the hallmark for EIEC as well as for *Shigella* spp. strains, as assessed by conventional PCR [10]. The strain EF432 was chosen as representative of the clone that caused the Italian outbreak of infections and was used in all the characterization experiments, while the remaining two Italian isolates (EF433 and EF434) were only included in the pulsed-field gel electrophoresis (PFGE) cluster analysis.

The reference EIEC strains 6.81 and 4608 [11], the *Shigella flexneri* strain M90T [11], the *E. coli* K12 strain MG1655 [12] and the non-pathogenic human *E. coli* isolate ECOR1, part of the ECOR reference collection [13], were included in the study for comparative purposes.

Genomic characterization of EIEC isolates

Whole genome sequencing. Whole genome sequencing of the EIEC strains was carried out to reach the coverage of at least 20 times per isolate. The genomes of the EIEC strains EF432, CNM-2113/13 and 6.81 were sequenced with an Ion Torrent Personal Genome Machine (Life Technologies, Carlsbad, CA, USA). The genome of the EF432 was sequenced in six different runs (three runs of a 200 bp library and three runs of a 400 bp library), while the genomes of CNM-2113/13 and 6.81 strains were sequenced in one and two 400 bp runs, respectively. Sequencing of the EIEC strain H142690012 was carried out by the PHE Genome Sequencing Unit using the Nextera library preparation and the Illumina HiSeq 2500 in fast-run mode according to manufacturers' instructions. The sequencing reads have been uploaded in the European Molecular Biology Laboratory (EMBL)–European Bioinformatics Institute sequence database (EMBL European Nucleotide Archive, accession no. ERP010762).

The reference sequences of EIEC 4608 (accession no. JTCO00000000), *Shigella sonnei* Ss046 (accession no. NC_007384, NC_007385, NC_009345, NC_009346, NC_009347), *S. boydii* CDC3083-94 (accession no. CP001063, CP001058, CP001059, CP001060, CP001061, CP001062), *S. dysenteriae* Sd197 (accession no. NC_007606, NC_007607,