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Characterization of Shiga toxin-producing *Escherichia coli* isolated from aquatic environments

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Abstract

This study reports the phenotypic and genotypic characterization of 144 Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from urban sewage and animal wastewaters using a Shiga toxin 2 gene variant (stx_2)-specific DNA colony hybridization method. All the strains were classified as *E. coli* and belonged to 34 different serotypes, some of which had not been previously reported to carry the stx_2 genes (O8:H31, O89:H19, O166:H21 and O181:H20). Five stx_2 subtypes (stx_2, stx_{2c}, stx_{2d}, stx_{2e} and stx_{2g}) were detected. The stx_2, stx_{2c}, stx_{2d} and stx_{2e} subtypes were present in urban sewage and stx_{2e} was the only stx_2 subtype found in pig wastewater samples. The stx_{2c} and stx_{2g} were more associated with cattle wastewater. One strain was positive for the intimin gene (*eae*) and five strains of serotypes were positive for the adhesin encoded by the *saa* gene. A total of 41 different seropathotypes were found. On the basis of occurrence of virulence genes, most non-O157 STEC strains are assumed to be low-virulence serotypes.

Keywords: *Escherichia coli*; STEC; stx_2; VT2 verotoxin; VTEC, Water

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) strains are an important cause of severe disease in humans, resulting in haemorrhagic colitis (HC) and haemolytic uraemic-syndrome (HUS) [1,2]. Domestic ruminants seem to be the principal reservoir of infectious STEC [3,4]. Transmission occurs through consumption of undercooked meat, unpasteurized dairy products and vegetables or water contaminated by faeces from carriers [2].

One of the most important pathogenicity factors produced by STEC strains is Shiga toxin (Stx). It contains two major groups called Stx_1, similar to Stx of *Shigella dysenteriae* type 1, and Stx_2 [5]. Whilst Stx_1 is highly conserved, Stx_2 encompasses 11 distinct variants [6]. Adherence to the intestinal epithelium and colonization of the gut by STEC is another important component of the pathogenic process. This property is encoded on a pathogenicity island termed the locus for enterocyte effacement (LEE) [7]. Besides chromosomal markers, most STEC strains isolated from humans (both LEE positive and LEE negative) also carry large (>90 Kb) plasmids encoding proteins such as the enterohaemorrhagic *E. coli* enterohaemolysin (EHEC-HlyA) [8].

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STEC strains belong to diverse serotypes, O26:H11, O103:H2, O111:H8 and O157:H7 being the most common, and present a wide range of phenotypic characteristics [9]. For instance, the ability to ferment sorbitol or β-D-glucuronidase activity, negative in O157 but positive in several non-O157 strains [9]. Usually, the information reported and the methods developed for isolation of STEC are based on the study of strains isolated from human or animal faeces using selective methods. It is assumed that the clinical strains are representative of what could be found in the natural environment of the same geographical area. This hypothesis should nevertheless be verified with environmental studies to confirm that no differential selection has occurred and that the strains isolated in the clinic correspond to those present in the environment. However, the isolation of STEC in the environment is difficult, due to the low proportion of pathogens compared with the generally high microbial concentration; consequently, confirmation of the presumptive causative agent involved in an outbreak is not always achieved [10].

A suitable method for detection and isolation of potential STEC strains from the environment has been developed [11]. This method permits the isolation of STEC from coliform bacteria grown on Chromocult® coliform agar (Merck, Darmstadt, Germany) using a specific probe against *stx*$_2$*A*. Using this method, environmental strains could be detected and isolated without any selection associated with their serotype and phenotypic or molecular characteristics. In this study, we have characterized STEC strains isolated from urban sewage and animal faecal wastes from three different animal abattoirs (cattle, swine and a mixed animal slaughterhouse). Complete characterization was performed, including phenotypic, serological and molecular characterization, providing new information about STEC strains occurring in the environment.

2. Materials and methods

2.1. Strains and media

The *E. coli* O157:H7 strain ATCC 43889, which produces Stx$_2$, and *E. coli* O157:H7 strain ATCC 43888, which does not produce either Stx$_1$ or Stx$_2$ and does not possess the genes for these toxins, were used as positive and negative controls, respectively, in the hybridization protocol. Bacteria were grown in Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) at 37 °C for 24 h. Chromocult® coliform agar (Merck, Darmstadt, Germany) was used for recovery of *E. coli* (EC) and total coliforms (TC) at 37 °C. The positive and negative control strains used in the PCR studies are shown in Table 1.

2.2. Isolation of stx$_2$-carrying E. coli from environmental samples

Raw sewage samples of urban origin, mostly contaminated by human faecal wastes, and wastewater samples from three different abattoirs (cattle, pig, and a mixed cattle, lamb, goat and poultry slaughterhouse) were used for the isolation of *stx*$_2$-strains by colony hybridization. Samples were collected aseptically and transferred into sterile containers according to standard procedures [12]. The samples were then placed in coolers, transported to the laboratory, and kept at 4 °C. Analysis was performed within 6 h of sampling. The characteristics of the samples and the sampling sites have been previously described [13]. The *stx*$_2$ gene-carrying bacteria, *E. coli* organisms, faecal coliforms (FC) and total coliforms (TC) presented similar values through all the period of the study, showing only minor variations. TC, FC, and *E. coli* were present in quantities up to 10^6, 10^5, and 10^5 CFU ml^{-1}, respectively. Tenfold dilutions of these samples were performed with Ringer 1/4 solution (Oxoid). Aliquots of 250 μl were spread on 140-mm diameter Chromocult® agar plates. Incubation was performed at 37 °C for 24 h. Those plates from dilutions presenting heavy but non-confluent colony growth were selected for colony hybridization [11].

2.3. Preparation of digoxigenin-labeled stx$_2$A-specific gene probes

A 378 bp DNA fragment of the *stx*$_2$-*A* gene generated by amplification with primers UP 378 and LP 378 (Table 2) was labeled by incorporating digoxigenin-11-deoxyuridine-triphosphate (Roche Diagnostics, Barcelona,

Table 1
Characteristics of the control strains used

Strain	Serotype	Origin	Virulence genes	Reference
E. coli C600 (933W)	–	Laboratory strain	*stx*$_2$	[39]
E. coli DH5α	–	Gibco-BRL collection	–	[40]
E. coli B2F1	O91:H21	HUS	*stx*$_{2c}$ (*stx*2vha, *stx*2vhb)	[41]
E. coli OX3:H21	O174:H21	Sudden infant death	*stx*$_{2d}$	[41]
E. coli FAC9	ONT	Porcine edema disease	*stx*$_{2e}$	[41]
E. coli ED431	ONT	Pigeons	*stx*$_{2f}$	[36]
E. coli ATCC 43895	O157:H7	ATCC	*stx*$_1$,*stx*$_2$, *eae*, *ehxA*	[42]
E. coli E14b887	O113:H21	Laboratory isolate	*stx*$_2$, *saa*, *ehxA*	[43]