Available online at www.sciencedirect.com
ScienceDirect
International Journal of Medical Microbiology 299 (2009) 343–353
IJMM
www.elsevier.de/ijmm

Escherichia coli encoding Shiga toxin 2f as an emerging human pathogen

Rita Prager*, Angelika Fruth, Ute Siewert, Ute Strutz, Helmut Tschäpe

Robert Koch-Institute, Burgstr. 37, D-38855 Wernigerode, Germany

Received 16 July 2008; received in revised form 24 September 2008; accepted 19 October 2008

Abstract

Escherichia coli harbouring the *stx*2f gene have been previously reported in pigeons. Here we demonstrate the presence of this allele in human diarrhoeagenic *E. coli* strains originally classified as atypical enteropathogenic *E. coli* (aEPEC). Thirty-two *stx*2f-positive *E. coli* serotyped as O63:H6, O128:H2, O132:H34, O145:H34, and O178:H7 were found to belong to a large number of clonal groups due to their different MLST-, PFGE- and virulence patterns. The appearance of various *stx*2f-positive clonal lineages among *E. coli* reveals emerging clinical significance. Therefore, it seems to be prudent to include *stx*2f into the diagnostic scope employed for laboratory investigation of enteric infections.

Keywords: Emerging; Shiga toxin 2f; *E. coli*; Pigeon

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) cause a wide spectrum of infectious diseases in humans as well as in animals ranging from mild diarrhoea to life-threatening oedema disease in pigs and haemolytic uremic syndrome (HUS) in humans particularly in children (Paton and Paton, 1998). Generally, the pathogenicity of STEC is associated with a broad range of virulence factors (Prager et al., 2005). Of utmost clinical importance is their production of various toxin variants which differ with respect to their cytotoxicity, the toxin receptor specificity, and the antigenicity of the toxin subunits A or B and which may serve as a reliable diagnostic feature (Paton and Paton, 1998). The Shiga toxin 1 (Stx1) group consists of 3 variants (Stx1, Stx1c, Stx1d). The more heterogeneous Shiga toxin 2 (Stx2) group comprises an expanding number of variants such as Stx2, 2c, $2d_{act}$, 2d, 2e, 2f, 2g (Karch et al., 2005). For instance, the Stx2c or $Stx2d_{act}$ (mucus-activatable toxin) variants are associated with an increased risk of the strains to develop the life-threatening HUS syndrome in children (Paton et al., 1999; Friedrich et al., 2002; Karch et al., 2005; Bielaszewska et al., 2006), whereas other toxin variants, e.g. Stx2d (non-activatable) or Stx2e (restricted to virulence in pigs), are less severe for causing disease in humans (Pierard et al., 1998; Friedrich et al., 2002; Beutin et al., 2004). Highly virulent STEC often carry the pathogenicity island LEE (characterised by the gene *eae*) and possess large virulence plasmids (pO157-like or pO113-like) (Burland et al., 1998; Brunder et al., 2006; Paton and Paton, 2002, 2005) with various virulence genes. In contrast, STEC-lacking LEE or large virulence plasmids appear to be of less clinical or epidemic relevance (Prager et al., 2005).

*Corresponding author. Tel.: +49 0 3943 679 239; fax: +49 0 3943 679 207.
E-mail address: pragerr@rki.de (R. Prager).

1438-4221/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijmm.2008.10.008

Stx2f was described as a new Stx2 variant in 2002 with a clear link to *E. coli* strains from pigeons as their reservoir (Dell'Omo et al., 1998; Schmidt et al., 2000; Kobayashi et al., 2002; Morabito et al., 2001; Großmann et al., 2005). Because the *stx*2f variant was very rarely associated with human infections (Gannon et al., 1990; Friedrich et al., 2002; Jenkins et al., 2003; Isobe et al., 2004; Sonntag et al., 2005; Seto et al., 2007; van Duynhoven et al., 2008), it was thought that *stx*2f might be a pigeon-adapted Shiga toxin variant with a limited impact on diseases in humans.

However, today an increasing number of Stx2f-producing STEC (*stx*2f STEC) isolates is observed among clinical isolates from children suffering from diarrhoea and some of them showing severe clinical symptoms. Thirty-two *stx*2f STEC belonging to 5 serotypes originating from different geographical regions of Germany between 2004 and 2007 out of the strain collection of the National Reference Centre for Salmonella and Other Bacterial Enterics (NRC) were analysed. In this paper, we describe the serotypes, virulence factors, MLST-, and PFGE results of these *stx*2f-producing isolates. Initially a diffuse outbreak due to one *stx*2f-positive strain was taken into consideration due to rather similar genetic properties of 3 isolates but the increasing number of *stx*2f-positive strains turned out to be genetically rather diverse.

Materials and methods

Bacterial strains

Thirty-two *stx*2f-positive, *eae*-positive strains were investigated throughout this study. For comparison, 141 atypical enteropathogenic *E. coli* (aEPEC) defined as *eae*-positive, *stx*-negative, enterohaemorrhagic *E. coli* haemolysin (*ehly*)-negative, EPEC adherence factor (EAF)-negative by means of respective PCR assays using primers SK1/SK2, LP30/31, LP43/44, HlyA1/HlyA4 (Prager et al., 2005), EAF1/25 (Franke et al., 1994), and belonging to various serotypes were also included in this study. Among these, 141 aEPEC 28 were of the same serotypes as the *stx*2f STEC. All strains were isolated from cases of human infection, such as severe enteritis, mild diarrhoea, or from asymptomatic carriers during 2004–2007. Two *stx*2f-positive strains were used as references: T4/97 (O128:H2) originating from pigeon and the human isolate H.I.8. (O128:H2) kindly provided by H. Karch.

Microbiological investigation

Serotyping of the strains was performed as described by Prager et al. (2003). Non-motile strains were analysed for their flagellar genotypes by PCR according to Prager et al. (2003). The strains were investigated for the production of Stx with a commercially available enzyme immuno assay (Ridascreen-EIA; R-Biopharm AG, Darmstadt, Germany).

The cytotoxicity assay was performed with Vero cell monolayers in 96-well plates as described by Karmali et al. (1985). The cells were exposed for 48 h to filtered supernatants of bacterial strains grown overnight in an enrichment medium that contained mitomycin C (EHEC Direct Medium, Heipha, Germany).

PCR detection of virulence-related genes

PCRs were carried out according to Prager et al. (2002). All PCR primers, PCR conditions, and target sequences used in this study are described by Sonntag et al. (2005) and Prager et al. (2005). Moreover, all strains were tested for the presence of *stx*2 gene variants by PCR using the primer pair *stx*IIva-u/*stx*IIva-d and by *stx*2-RFLP analysis using the restriction enzyme BglII as described by Lin et al. (1993). The amplicons of the *stx*2f-specific PCR (128-1/128-2) were verified by RFLP using EcoRV (Schmidt et al., 2000), DdeI and HaeII (this publication). The *eae* typing was carried out according to Ramachandran et al. (2003).

A PCR-based detection of virulence plasmid markers in order to identify different *E. coli* pathotypes was performed according to Prager and Tschäpe (2007).

Southern blot

DNA isolation, restriction digestion, agarose gel electrophoresis, blotting, and hybridization were carried out as described in Prager et al. (2002). For the *stx*2f blots, the genomic DNA was digested with the restriction enzyme SalI and a *stx*2f PCR probe from T4/94 (O128:H2) was labelled with Digoxigenin-11-dUTP using a Random Primed Labelling Kit (Roche Diagnostics). For the *cdt*I blots, the restriction enzyme PstI was used, and a *cdt*I PCR probe from the strain T4/94 (O128:H2) was labelled as described above. Plasmid blots were performed as described earlier (Prager et al., 2005) with a labelled *ast*A probe.

Characterization of plasmids

The determination of the plasmid profiles and the incompatibility groups were carried out according to Prager et al. (2005) and Carattoli et al. (2005).

PFGE

PFGE was performed following the PulseNet protocol of CDC, Atlanta (Hunter et al., 2005). The PFGE