Characterization of *Escherichia coli* O157 : H7 isolates causing haemolytic uraemic syndrome in France

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Forty-seven non-epidemic *Escherichia coli* O157 : H7 isolates causing haemolytic uraemic syndrome in France were characterized. The isolates clustered into 36 clones using PFGE typing. All the isolates harboured *eae* and one or more copies of *stx2* and belonged to phylogenetic group D. Nine per cent were resistant to amoxicillin.

### Introduction

*Escherichia coli* O157 : H7 is a food-borne pathogen that has emerged as a major cause of haemorrhagic colitis (Griffin & Tauxe, 1991; Karmali, 1989; Riley et al., 1983). *E. coli* O157 : H7 colonizes the digestive tract of cattle and is transmitted to humans by food and water, directly from person to person and, occasionally, through occupational exposure (Armstrong et al., 1996). *E. coli* O157 : H7 can cause haemolytic uraemic syndrome (HUS) mainly by secretion of Shiga toxins encoded by the genes *stx1* and/or *stx2* and variants (Karmali et al., 1988; Pierard et al., 1998; Tesh & O’Brien, 1991). Several epidemics have been described worldwide (Allison et al., 2000; Barrett et al., 1994; Fukushima et al., 1999; Keene et al., 1997; Liesegang et al., 2000; Willshaw et al., 1997). Epidemic and sporadic strains have been characterized in North America (Barrett et al., 1994; Bohm & Karch, 1992; Davis et al., 2003; Noller et al., 2003; Preston et al., 2000; Whittam et al., 1988, 1993), the United Kingdom (Allison et al., 2000; Willshaw et al., 1997), Germany (Bohm & Karch, 1992; Liesegang et al., 2000) and Japan (Izumiya et al., 1997; Watanabe et al., 1999). Fewer data are available on French isolates (Giammanco et al., 2002). The aim of this study was to characterize non-epidemic French *E. coli* O157 : H7 isolates causing HUS in children and adults, and to test their antimicrobial sensitivity.

### Methods

We characterized 47 epidemiologically unrelated non-epidemic *E. coli* O157 : H7 isolates from stools of children (n = 35) and adults (n = 12) with sporadic HUS collected from various regions of France from 1992 to 2002. We also analysed the complete-genome-sequenced North American reference strain EDL933 (ATCC 43895) (Perna et al., 2001) and the O55 : H7 reference strain CIP 105242 representative of the supposed ancestor clone of *E. coli* O157 : H7 strains (Whittam et al., 1993). PCR detection of the *stx1*, *stx2* and *eae* genes was performed with primers fM1 (5’-CAT TAT GGA ACG GCA GAG GT-3’) and rY4 (5’-ATC TTC TGC GTA CTG CGT TCA-3’) for *eae* detection, VT1-u (5’-GGC CTT AAG AAG AGT CCG TGG GAT TAC G-3’) and VT1-l (5’-GGC CAA TCG AAA GCG ATG CAG CTA TTA ATA A-3’) for *stx1* detection, and VT2-u (5’-CAA CGG TTT CCA TGA CAA CG-3’) and VT2-l (5’-AAC TGC TCT GGA TGC ATC TCT CCA ACA CG-3’) for *stx2* detection as described elsewhere (Bastian et al., 1998; Beaudry et al., 1996; Mariani-Kurkdjian et al., 1997). No subtyping of the *stx2* genes was performed. EDL933 was used as a positive control for PCR of those three genes. Genetic relatedness of the isolates was studied by PFGE using the restriction enzyme XbaI and the CHEF-DRIII apparatus (Bio-Rad) at 6 V cm⁻¹ for 27 h, with pulse times varying linearly between 2 and 49 s. Each electrophoresis was performed including a lambda ladder molecular mass marker (Bio-Rad) and EDL933 for the normalization of gel images. Restriction patterns were analysed with the Finger-printing II software (Bio-Rad) (Dice similarity index and UPGMA method). PFGE patterns were considered clonally related if they had a similarity coefficient higher than 80% (usually corresponding to a difference of no more than four bands in our study) (Tenover et al., 1995). PFGE digests were transferred to nylon membranes (Amersham Pharmacia Biotech) and hybridized with digoxigenin-labelled probes for the *stx2* and *eae* genes, generated from the primers used for the PCR detection as recommended by the manufacturer (Roche Biotech) and trimmed using the E-test (AB BIODISK).

Antimicrobial susceptibility testing was based on the disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS), with amoxicillin (25 μg), amoxicillin + clavulanic acid (20 μg/10 μg), ceftriaxone (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg) and trimethoprim + sulfamethoxazole (1.25 μg/23.75 μg) (Bio-Rad) (NCCLS, 1997). Susceptibility to azithromycin was assessed by MIC determination in the E-test (AB BIODISK).
Fig. 1. Dendrogram calculated from Xba I PFGE patterns, showing the genetic relatedness of the 47 epidemiologically unrelated French sporadic *Escherichia coli* O157 : H7 isolates, the North American reference strain EDL933 (ATCC 43895) (Perna et al., 2001) and the O55 : H7 reference strain (CIP 105242). Presence (+) or absence (−) of stx1 and stx2 genes is indicated. When an isolate harbours more than one copy of the stx2 gene, according to Southern hybridization, the number of copies is indicated in parentheses.
Results

The 47 isolates yielded 43 different PFGE patterns representing 36 different clones at a similarity threshold of 80% (Fig. 1). None of the isolates displayed a PFGE pattern similar to that of the North American clone reference strain EDL933 (ATCC 43895) or of the O55:H7 reference strain. Representative patterns are shown in Fig. 2(a). We observed no association between temporal and/or geographical origin and PFGE patterns.

All 47 isolates harboured the stx2 and the eae genes and 6 (12.7%) also harboured the stx1 gene. Fourteen strains (30%) carried two copies of stx2 and one carried three copies (Fig. 2b). All the isolates harboured one copy of the eae gene (Fig. 2c). All the isolates harboured the chuA gene and belonged to E. coli phylogenetic group D (Clermont et al., 2000).

Nine per cent of the isolates were resistant to amoxicillin and 4% to amoxicillin + clavulanic acid and trimethoprim + sulfamethoxazole. Resistant isolates belonged to different pulsotypes. None of the isolates was resistant to ceftriaxone or quinolones (nalidixic acid and ciprofloxacin). Azithromycin MICs ranged from 3 to 12 μg ml⁻¹. The concentrations at which 50% of isolates were inhibited [MIC₅₀] and at which 90% of isolates were inhibited [MIC₉₀] were 6 and 8 μg ml⁻¹, respectively.

Discussion

Using a multilocus enzyme electrophoresis method (MLEE), Whittam et al. (1993) found that O157:H7 strains arose recently by clonal expansion from a common progenitor strain closely related to O55:H7 strains. It was recently reported that multilocus sequence typing (MLST), which is a development of MLEE based on nucleotide sequencing, showed a lack of diversity among E. coli O157:H7 isolates with distinct PFGE patterns (Noller et al., 2003). PFGE is thus considered the gold standard for molecular typing of O157:H7 isolates (Preston et al., 2000). Previous PFGE studies of non-epidemic E. coli O157:H7 isolates from North America and Germany showed that epidemiologically unrelated strains displayed limited genetic diversity (Barrett et al., 1994; Bohm & Karch, 1992; Davis et al., 2003; Preston et al., 2000). Using the same method, Bender et al. (1997) found marked heterogeneity among O157:H7 isolates from Minnesota, but this was attributed to these authors’ stringent strain definition. We found that French E. coli O157:H7 isolates showed marked genetic diversity, as our 47 isolates obtained throughout France belonged to 36 clones. Differences between O157:H7 strains are due to discrete insertions or deletions containing a XbaI site that is polymorphic between strains rather than single-nucleotide polymorphism in the XbaI sites themselves (Kudva et al., 2002). Our results are in keeping with those of a recent study based on Italian and French isolates, which also displayed a wide range of PFGE patterns among 13 human isolates, suggesting that

Fig. 2. Representative XbaI PFGE patterns. (a) PFGE patterns. (b) Southern hybridization with an stx2 DNA probe. (c) Southern hybridization with an eae DNA probe. Inverted white triangles indicate fragments hybridizing with the stx2 probe. White triangles indicate fragments hybridizing with the eae probe. Lanes: M, molecular mass marker (lambda ladder; Bio-Rad); 1–8, unrelated E. coli O157:H7 isolates; 4 and 7, isolates harbouring two copies of stx2; 8, isolate harbouring three copies of stx2.
several clones circulate in this part of Europe (Giammanco et al., 2002). All our 47 isolates belonged to phylogenetic group D. Pupo et al. (1997) studied a limited number of O157 : H7 strains and found that they also belonged to group D. Various intestinal pathogenic E. coli derive from phylogenetic groups D, A or B1 (Pupo et al., 1997). In contrast, extraintestinal pathogenic E. coli derive predominantly from group B2 and, to a lesser extent, group D, and from specific clones within these groups (Bingen et al., 1998; Pupo et al., 1997; Rolland et al., 1998). Most of our isolates carried only the stx2 gene, in keeping with the results of Giammanco et al. (2002), who studied a small number of French isolates. Hybridization showed that some isolates had two or more copies of this gene. Bohm & Karch (1992) have previously identified an American isolate harbouring two copies of stx2. Based on the supposed evolution of the O157 : H7 lineage, most of those isolates could belong to the stage between the loss of sorbitol fermentation and acquisition of the stx1 phage (Feng et al., 1998). Most of the isolates tested here were susceptible to all the antibiotics recommended for the treatment of traveller’s diarrhoea (Guerrant et al., 2001). The sensitivity pattern of our isolates fits with previous reports showing that pathogenic E. coli strains are more susceptible than colonization isolates (Johnson et al., 2002). It is also in keeping with the greater susceptibility of French isolates compared to North Italian isolates observed by Giammanco et al. (2002). However, antibiotics are a risk factor for HUS, and their use is therefore contraindicated in patients with STEC infection (Wong et al., 2000). Indeed, antibiotic therapy can increase free Shiga toxin levels in vivo, thus facilitating disease progression (Wong et al., 2000). Current American guidelines on antimicrobial chemotherapy of haemorrhagic diarrhoea are cautious (Guerrant et al., 2001). Recent reports show that azithromycin, a macrolide, yields high eradication rates in children with shigellosis (Basualdo & Arbo, 2003), probably because it reaches high concentrations in stools. Median faecal concentrations of azithromycin exceed 48-fold the MIC90 value of our O157 : H7 isolates (Khan et al., 1997). Macrolides bind selectively to the 50S subunit of the bacterial ribosome and inhibit protein synthesis by blocking transpeptidation. This mechanism of action has been linked to low-level Shiga toxin release and lower lethality in a murine model of E. coli O157 : H7 infection (Hiramatsu et al., 2003). Further clinical studies are needed to determine whether azithromycin reduces the risk of HUS in patients with E. coli O157 : H7 haemorrhagic colitis.

References


Characterization of Escherichia coli O157 : H7


