Cytolethal distending toxins in Shiga toxin-producing *Escherichia coli*: alleles, serotype distribution and biological effects

Dorothea Orth, Katharina Grif, Manfred P. Dierich and Reinhard Würzner

Correspondence

Dorothea Orth
dorothea.orth@i-med.ac.at

Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University and Austrian Reference Laboratory for Enterohaemorrhagic *Escherichia coli*, Schöpfstr. 41, A-6020 Innsbruck, Austria

To assess the prevalence of cytolethal distending toxin (CDT) among Shiga toxin-producing *Escherichia coli* (STEC), 202 STEC strains were investigated using PCRs targeting various *cdt* alleles (*cdt-I* to *cdt-V*). Seven of the 202 strains contained *cdt-III* and an additional seven contained *cdt-V*. All 14 *cdt*-positive strains produced biologically active CDT, as demonstrated by a progressive distension of cultured Chinese hamster ovary cells. The CDT-positive STEC belonged to eight different serotypes, including sorbitol-fermenting O157:NM (non-motile). The data demonstrate that CDT is present in some STEC serotypes only. However, more studies are required to evaluate whether CDT presence is associated with severe disease.

Received 6 April 2006

Accepted 9 August 2006

INTRODUCTION

The cytolethal distending toxin (CDT) was first described in 1987 as a novel toxin occurring in diarrhoeagenic *Escherichia coli* (Johnson & Lior, 1987). During the years following, closely related toxins were also detected in other intestinal and extraintestinal pathogens, such as *Campylobacter* spp. (Johnson & Lior, 1988a; Pickett *et al.*, 1996), *Shigella* spp. (Okuda *et al.*, 1995), *Salmonella typhi* (Haghjoo & Galan, 2004), *Haemophilus ducreyi* (Cope *et al.*, 1997), *Actinobacillus actinomycetemcomitans* (Mayer *et al.*, 1999) and *Helicobacter* spp. (Chien *et al.*, 2000; Young *et al.*, 2000). The members of the CDT family are usually encoded by a cluster of three genes (*cdtA*, *cdtB*, *cdtC*) which encode the three proteins of the CDT holotoxin (Cortes-Bratti *et al.*, 2001a; Lara-Tejero & Galan, 2001). CDTs cause G1 or G2 cell cycle arrest in mammalian cells (Cortes-Bratti *et al.*, 2001b), leading to the inhibition of proliferation, the characteristic distended cell morphology, and ultimately cell death (Bielaszewska *et al.*, 2005a; Lara-Tejero & Galan, 2001). Because they interfere with the cell cycle, CDTs are classified as cyclomodulins (Nougayrede *et al.*, 2005).

A total of five different *cdt* alleles (*cdt-I*, *cdt-II*, *cdt-III*, *cdt-IV* and *cdt-V*) have been reported in *E. coli* (Janka *et al.*, 2003; Peres *et al.*, 1997; Pickett *et al.*, 1994; Scott & Kaper, 1994; Toth *et al.*, 2003), but there is only limited knowledge available on the epidemiology of the strains harbouring these genes and/or producing CDT. The strains have been

isolated from patients with diarrhoea (Albert *et al.*, 1996; Ansaruzzaman *et al.*, 2000; Johnson & Lior 1988b; Okeke *et al.*, 2000) and non-intestinal diseases (Dobrindt *et al.*, 2003; Johnson & Stell, 2000; Johnson *et al.*, 2002). In several studies, the prevalence of *cdt* among *E. coli* isolated from non-intestinal infections, including urosepsis (8%) (Johnson & Stell, 2000) and meningitis (46%) (Johnson *et al.*, 2002), was higher than that in isolates from patients with diarrhoea (1.6–6.4%) (Albert *et al.*, 1996; Okeke *et al.*, 2000). In addition to human *E. coli* isolates, CDT production and/or *cdt* genes have also been identified, with varying frequencies, in *E. coli* isolated from both diseased and healthy animals, including cattle (Clark *et al.*, 2002; Mainil *et al.*, 2003), pigs (daSilva & daSilva Leite, 2002; Mainil *et al.*, 2003; Toth *et al.*, 2003) and dogs (Mainil *et al.*, 2003; Starcic *et al.*, 2002), as well as various species of wild birds (La Ragione & Woodward, 2002; Morabito *et al.*, 2001; Sonntag *et al.*, 2005a). The fact that different *cdt* alleles were targeted in the various human and animal isolates makes it difficult to draw any conclusions on the epidemiological relationships among CDT⁺ *E. coli* strains from the various sources.

Although some *E. coli* isolates identified as *cdt*⁺/CDT⁺ also possess *stx* genes (Clark *et al.*, 2002; Johnson & Lior 1988b; Morabito *et al.*, 2001; Sonntag *et al.*, 2005a), such strains originate mostly from animals, i.e. cattle (Clark *et al.*, 2002) and pigeons (Morabito *et al.*, 2001; Sonntag *et al.*, 2005a). The presence of *cdt* genes in Shiga toxin (Stx)-producing *E. coli* (STEC) from humans has rarely been observed (Bielaszewska *et al.*, 2004; Clark *et al.*, 2002; Johnson & Lior, 1988b). However, *cdt-V* has been detected in the majority (87%) of sorbitol-fermenting (SF) STEC

Abbreviations: CDT, cytolethal distending toxin; CHO, Chinese hamster ovary; HUS, haemolytic uraemic syndrome; NM, non-motile; NSF, non-sorbitol fermenting; SF, sorbitol fermenting; STEC, Shiga toxin-producing *Escherichia coli*.

O157:NM (non-motile) strains in Germany (Janka *et al.*, 2003) and in four similar isolates in Austria (Orth *et al.*, 2006). In the present study, we assessed the prevalence of *cdt* in STEC isolates belonging to a broad spectrum of serotypes and originating from various sources.

METHODS

Bacterial strains. Two hundred and two STEC strains from humans (*n*=150), animals (*n*=38) and food (*n*=14) were investigated in this study. One hundred and thirty-two strains were positive for the intimin-encoding *eae* gene, which is located at the locus of enterocyte effacement (LEE); 70 strains were *eae* negative. The isolates were recovered at the Austrian Reference Laboratory for Enterohaemorrhagic *Escherichia coli* during routine diagnostic and epidemiological investigations between 2003 and 2005. The isolation of STEC strains from stools was performed as described previously (Friedrich *et al.*, 2002). The 150 STEC strains originating from human stools were recovered from 12 patients with haemolytic uraemic syndrome (HUS), 101 patients with diarrhoea, 22 asymptomatic individuals and 15 individuals with unknown clinical diagnosis. The animal isolates all originated from cattle, except for one isolate that was found in a goat. The food isolates were recovered from raw meat (*n*=13) and raw milk (*n*=1). The 202 isolates belonged to 61 different serotypes (Table 1). Sixty strains contained *stx*₁ only, 67 strains *stx*₁ and *stx*₂, and 75 strains *stx*₂ only, as detected by PCR using primers KS7 and KS8, and LP43 and LP44 (Table 2).

PCRs. The PCR primers, target sequences and PCR conditions are listed in Table 2. *E. coli* strains 6468/62 (O86:H34; *cdt*-I⁺) (Scott & Kaper, 1994), 9142/88 (O128:H⁻; *cdt*-II⁺) (Pickett *et al.*, 1994), 1404 (O78; *cdt*-III⁺) (Peres *et al.*, 1997), 28c (O78; *cdt*-IV⁺) (Toth *et al.*, 2003), and 493/89 (O157:NM; *cdt*-V⁺) (Janka *et al.*, 2003) were used as positive controls. They were kindly provided by H. Karch (University of Münster), who had received the strains from D. A. Scott (University of Maryland School of Medicine), C. L. Pickett (University of Kentucky Medical Center) and E. Oswald (École nationale vétérinaire de Toulouse), or were isolated in his laboratory (strain 493/89).

CDT bioassay. CDT was assayed using Chinese hamster ovary (CHO) cells and a modification of the procedure described by Scott

& Kaper (1994). Briefly, supernatants of bacterial cultures grown overnight (37 °C, 180 r.p.m.) in cell culture medium (Ham's F12 with 10% fetal calf serum) were filter-sterilized (0.22 µm pore-size filters; Corning), and 1 ml portions of twofold dilutions of the filtrates were added in duplicate to 1 × 10³ CHO cells freshly seeded in 1.5 ml Ham's F12 medium in six-well tissue culture plates (Falcon 3502; Becton Dickinson). The assay mixes were incubated for 4 days at 37 °C in 5% CO₂ and examined daily for typical cell distension (Scott & Kaper, 1994). The CDT titre was defined as the highest filtrate dilution that caused distension, evaluated as described elsewhere, in 50% of CHO cells (Bielaszewska *et al.*, 2004, 2005a). SF STEC O157:NM strain 493/89 (CDT-V) served as a positive control, and CHO cells not exposed to culture filtrates as a negative control.

RESULTS AND DISCUSSION

Frequency of *cdt* genes among STEC O157

We used a spectrum of PCR procedures to target various *cdt* alleles, previously identified in *E. coli* strains (*cdt*-I, *cdt*-II, *cdt*-III, *cdt*-IV and *cdt*-V), in STEC belonging to 61 different serotypes. In the *E. coli* O157 group, none of the 72 non-sorbitol-fermenting (NSF) *E. coli* O157:H7/NM strains possessed *cdt*. Friedrich and colleagues have recently shown that *cdt*-V-positive O157:H7 strains belong to particular phage types (PTs), although the presence of *cdt* within these PTs is not obligatory (Friedrich *et al.*, 2006). Thus, they conclude that the proportion of *cdt*-V-positive STEC O157:H7 may depend on the PTs tested.

In contrast, five of eight SF STEC O157:NM strains were positive for *cdt*-V (Table 3). The association of *cdt*-V with SF STEC O157:NM confirms the data of Janka *et al.* (2003). However, in contrast to these authors, who found *cdt*-V in six out of 100 NSF *E. coli* O157:H7 (Janka *et al.*, 2003), we found neither *cdt*-V nor any of the other *cdt* alleles in any of the 72 NSF *E. coli* O157:H7/NM strains. In addition to *cdt*, SF *E. coli* O157:NM also possess additional loci which are

Table 1. Distribution of serotypes of the 202 investigated STEC strains

ONT, O non-typeable.

Serotype	Number of strains
O157:H7/NM	72
O26:H11/NM	18
O146:H28/H21/NM	9
SF O157:NM, O125:H8/H7/H4, O103:H21/H7/H2/NM	8
O145:NM	7
O177:NM, O111:H8/NM, O114:H18, O113:H4	4
O175:H40/H8, O127:H40, O116:NM, O91:H21/NM, O76:H21/H19	3
O174:H21/H2, O163:H19/NM, O158:H40/H18, O128:H8/H2,	2
O100:NM, O75:H8, O8:H25, O6:H10, O5:NM	
O185:H28, O182:H16, O143:H11, O139:H25, O119:H4, O118:H11,	1
O112:H18, O84:H2, O82:H11, O55:H7, O22:NM, O20:NM,	
O17:H45, Orough:H21	
ONT:H28/H18/H12/H8/NM	9

Table 2. PCR primers and conditions used to detect *eae*, *stx* and *cdt* genes

Primer	Sequence (5'–3')	Target	PCR conditions*			PCR product (bp)	Reference
			Den.†	Ann.‡	Ext.§		
cdtI-f	TGG TGA GAA TCG GAA CTG	<i>cdt-IA</i>	30 s	51 °C	60 s	418	Bielaszewska <i>et al.</i> (2004)
cdtI-r	CAT TCC ATC AGG TTT GTC						
cdtII-f	AAT CCC TAT CCC TGA ACC	<i>cdt-IIA</i>	30 s	52 °C	60 s	542	Bielaszewska <i>et al.</i> (2004)
cdtII-r	GTT CTA TTG GCT GTG GTG						
cdtIII-f	AAA CAG GAC GGT AAT AAT GAC TAA TA	<i>cdt-III</i>	30 s	54 °C	180 s	2230	Clark <i>et al.</i> (2002)
cdtIII-r	GTG ATC TCC TTC CAT GAA AAT ATA GT						
CDT-IVs	CCTGATGGTTCAGGAGGCTGGTTC	<i>cdt-IVB</i>	60 s	55 °C	60 s	350	Toth <i>et al.</i> (2003)
CDT-IVas	TTG CTC CAG AAT CTA TAC CT						
c338f	AGC ATT AAA TAA AAG CAC GA	<i>cdt-VA</i>	30 s	52 °C	60 s	1329	Janka <i>et al.</i> (2003)
c2135r	TAC TTG CTG TGG TCT GCT AT						
c1309f	AGC ACC CGC AGT ATC TTT GA	<i>cdt-VB</i>	30 s	52 °C	60 s	1363	Janka <i>et al.</i> (2003)
c2166r	AGC CTC TTT TAT CGT CTG GA						
P105	GTC AAC GAA CAT TAG ATT AT	<i>cdt-VC</i>	30 s	49 °C	60 s	748	Janka <i>et al.</i> (2003)
c2767r	ATG GTC ATG CTT TGT TAT AT						
SK1	CCC GAA TTC GGC ACA AGC ATA AGC	<i>eae</i>	30 s	52 °C	60 s	863	Friedrich <i>et al.</i> (2002)
SK2	CCC GGA TCC GTC TCG CCA GTA TTC G						
KS7	CCC GGA TCC ATG AAA AAA ACA TTA TTA ATA GC	<i>stx₁B</i>	30 s	52 °C	40 s	285	Friedrich <i>et al.</i> (2002)
KS8	CCC GAA TTC AGC TAT TCT GAG TCA ACG						
LP43	ATC CTA TTC CCG GGA GTT TAC G	<i>stx₂A</i>	30 s	57 °C	60 s	584	Friedrich <i>et al.</i> (2002)
LP44	GCG TCA TCG TAT ACA CAG GAG C						

*All PCRs were performed in 30 cycles with final extension of 5 min at 72 °C.

†Den., denaturing (all reactions at 94 °C).

‡Ann., annealing (all reactions 60 s).

§Ext., extension (all reactions at 72 °C).

||Complete.

absent from NSF *E. coli* O157:H7/NM. These include *efa1*, *sfpA* and a mosaic island composed of the loci of the genome of *E. coli* O157:H7 strain EDL933 and the *Shigella* resistance

locus (Friedrich *et al.*, 2004; Janka *et al.*, 2002, 2005). However, SF STEC O157:NM lack the gene clusters which encode urease and tellurite resistance in *E. coli* O157:H7

Table 3. Distribution of *cdt-I*, *cdt-II*, *cdt-III*, *cdt-IV* and *cdt-V* alleles among STEC strains, and CDT production

Serotype	Strain no.	Origin	Clinical diagnosis	<i>stx₁</i>	<i>stx₂</i>	<i>eae</i>	<i>cdt-I</i>	<i>cdt-II</i>	<i>cdt-III</i>	<i>cdt-IV</i>	<i>cdt-V</i>	CDT titre (CHO cells)
SF O157:NM	10	Human	HUS	–	+	+	–	–	–	–	+	1:2
SF O157:NM	13	Cattle	Healthy	–	+	+	–	–	–	–	+	1:8
SF O157:NM	14	Cattle	Healthy	–	+	+	–	–	–	–	+	1:4
SF O157:NM	15	Human	Asymptomatic	–	+	+	–	–	–	–	+	1:4
SF O157:NM	17	Human	HUS	–	+	+	–	–	–	–	+	1:8
O91:H21	69	Cattle	Healthy	+	+	–	–	–	–	–	+	1:16
O76:H21	92	Cattle	Healthy	–	+	–	–	–	–	–	+	1:16
O158:H40	163	Human	Diarrhoea	+	–	+	–	–	+	–	–	1:2
O128:H8	33	Human	Bloody diarrhoea	+	–	–	–	–	+	–	–	1:16
O127:H40	98	Cattle	Healthy	–	+	+	–	–	+	–	–	1:8
O127:H40	100	Human	Diarrhoea	+	–	+	–	–	+	–	–	1:4
O127:H40	102	Human	Diarrhoea	+	–	+	–	–	+	–	–	1:16
O119:H4	196	Human	Bloody diarrhoea	+	+	–	–	–	+	–	–	1:16
O118:H11	124	Human	Diarrhoea	+	–	+	–	–	+	–	–	1:64

(Bielaszewska *et al.*, 2005b; Friedrich *et al.*, 2005), which implies that different spectra of virulence factors are present.

Frequency of *cdt* genes among STEC non-O157

Of the 122 STEC isolates belonging to 58 different non-O157 serotypes, nine (7.4%) tested positive for *cdt* genes. Serotypes of the *cdt*-positive strains are shown in Table 3. In contrast to SF STEC O157:NM, which contained only *cdt-V*, seven of nine non-O157 STEC contained *cdt-III*, and two of these harboured *cdt-V* (Table 3). We demonstrated the presence of *cdt-III* in three STEC strains belonging to serotype O127:H40. Interestingly, *E. coli* isolates of serotype O127:NM have been the most prevalent CDT⁺ strains found among enteropathogenic *E. coli* (EPEC) in several studies (Albert *et al.*, 1996; Ansaruzzaman *et al.*, 2000). The presence of only *cdt-III* and *cdt-V* in this study is in agreement with earlier studies from Germany (Bielaszewska *et al.*, 2004) and North America (Pickett *et al.*, 2004), in which only these two alleles have been identified. However, *cdt-I* was the only *cdt* allele found in STEC strains isolated in Iran (Bouzari *et al.*, 2005). Altogether, these data suggest that differences may exist in *cdt* alleles occurring in STEC strains from different geographical regions. Neither in our study nor in earlier studies from other countries (Bielaszewska *et al.*, 2004, 2005c; Pickett *et al.*, 2004; Prager *et al.*, 2005) were *cdt* genes identified in STEC of the major non-O157 serogroups associated with human disease, such as O26, O103, O111 and O145, which, however, are mostly *eae* positive (Bielaszewska *et al.*, 2005c; Friedrich *et al.*, 2002).

Distribution of *cdt*-positive STEC among human and environmental isolates

cdt genes, including *cdt-III* and *cdt-V*, were found in nine (6.0%) of 150 human isolates, in five (13.2%) of 38 animal

isolates, and in none of 14 food isolates. The origins of the STEC isolates harbouring *cdt-III* and *cdt-V* are shown in Table 3. Eight of the nine human *cdt*-positive isolates originated from diseased persons, including two patients with HUS and two patients with bloody diarrhoea (Table 3). The presence of *cdt-V* in STEC strains isolated from patients with severe disease, including HUS, supports reports by other investigators (Bielaszewska *et al.*, 2004; Janka *et al.*, 2003; Prager *et al.*, 2005). Our finding of *cdt* in STEC isolated from cattle is in agreement with the report of Clark *et al.* (2002). Moreover, the absence of *cdt* from the goat STEC isolate investigated in our study is in accordance with previous studies by other authors (Morabito *et al.*, 2001; Sonntag *et al.*, 2005a) which suggest species-specific differences in the prevalence of *cdt* among STEC of animal origin. Specifically, whereas *cdt* genes are regularly found in STEC strains isolated from pigeons (Morabito *et al.*, 2001; Sonntag *et al.*, 2005a), STEC isolated from pigs with oedema disease or diarrhoea lack *cdt* (Sonntag *et al.*, 2005b). Since *cdt* in STEC occurs in a variety of serotypes from a number of different origins, it is possible that these genes spread horizontally. Janka and colleagues have shown that the *cdt-V* gene in SF STEC O157:NM is flanked by sequences of bacteriophage P2 (Janka *et al.*, 2003), suggesting that the gene might have been acquired by phage transduction (Janka *et al.*, 2003). In contrast, the *cdt-III* gene cluster in STEC has been shown to be plasmid-borne (Bielaszewska *et al.*, 2004). Thus, conjugation could represent a mechanism by which *cdt-III* is spread among STEC strains.

Association of *cdt* genes with the *eae* gene

Ten (7.6%) of 132 *eae*-positive and four (5.7%) of 70 *eae*-negative STEC possessed *cdt-III* or *cdt-V*, demonstrating that there was no significant difference in the prevalence of

Fig. 1. Photomicrographs of CHO cells after 4 days' incubation with culture filtrates from CDT-V⁺ control strain 493/89 (a), STEC strain 69 (O91:H21) producing CDT-V (b), and STEC strain 33 (O128:H8) producing CDT-III (c). (d) CHO cells incubated for 4 days in Ham's F12 medium only, in the absence of any CDTs. Bars, 75 µm.

cdt between *eae*-positive and *eae*-negative STEC from our collection. Moreover, *eae* was found in the same frequency among *cdt*-III-positive and *cdt*-V-positive STEC strains (Table 3). These data demonstrate that there is no association between a particular *cdt* allele and the presence of *eae* in STEC. Our finding of *cdt* in non-O157 STEC carrying *eae* extends the findings of previous studies in which *cdt* in non-O157 STEC was restricted to *eae*-negative STEC strains (Bielaszewska *et al.*, 2004; Prager *et al.*, 2005).

CDT-III and CDT-V expression in STEC

Of the 14 STEC strains harbouring *cdt*-III or *cdt*-V genes, all produced active CDT according to the CHO cell assay. This was evidenced by a progressive distension in CHO cells for up to 4 days after exposure to culture filtrates of the STEC strains. The CDT titres ranged from 1:2 to 1:64 (Table 3). There was no difference in CDT titre between strains harbouring *cdt*-III and those harbouring *cdt*-V (Table 3). The typical distending effects of STEC isolates producing CDT-III or CDT-V are demonstrated in Fig. 1.

In conclusion, we demonstrate that two different *cdt* alleles encode biologically active CDT in STEC of particular serotypes, some of which are associated with severe human diseases, including HUS and bloody diarrhoea. Further investigation is necessary to clarify the mechanisms that govern the *cdt* genetic coding and spread among STEC strains, and to determine the role of CDT in the pathogenesis of STEC-mediated diseases.

ACKNOWLEDGEMENTS

We thank Professor H. Karch (University of Münster) for providing us with *cdt*-positive control strains and for stimulating discussions. We thank C. Ortner, A. Rief and G. Hechenblaikner for excellent technical assistance. This study was supported by the Network of Excellence EuroPathoGenomics (LSHB-CT-2005-512061).

REFERENCES

- Albert, M. J., Faruque, S. M., Faruque, A. S., Bettelheim, K. A., Neogi, P. K., Bhuiyan, N. A. & Kaper, J. B. (1996). Controlled study of cytotolethal distending toxin-producing *Escherichia coli* infections in Bangladeshi children. *J Clin Microbiol* **34**, 717–719.
- Ansaruzzaman, M., Albert, M. J., Nahar, S., Byun, R., Katouli, M., Kuhn, I. & Mollby, R. (2000). Clonal groups of enteropathogenic *Escherichia coli* isolated in case-control studies of diarrhoea in Bangladesh. *J Med Microbiol* **49**, 177–185.
- Bielaszewska, M., Fell, M., Greune, L., Prager, R., Fruth, A., Tschäpe, H., Schmidt, M. A. & Karch, H. (2004). Characterization of cytotolethal distending toxin genes and expression in Shiga toxin-producing *Escherichia coli* strains of non-O157 serogroups. *Infect Immun* **72**, 1812–1816.
- Bielaszewska, M., Sinha, B., Kuczius, T. & Karch, H. (2005a). Cytotolethal distending toxin from Shiga toxin-producing *Escherichia coli* O157 causes irreversible G2/M arrest, inhibition of proliferation, and death of human endothelial cells. *Infect Immun* **73**, 552–562.
- Bielaszewska, M., Tarr, P. I., Karch, H., Zhang, W. & Mathys, W. (2005b). Phenotypic and molecular analysis of tellurite resistance among enterohemorrhagic *Escherichia coli* O157:H7 and sorbitol-fermenting O157:NM clinical isolates. *J Clin Microbiol* **43**, 452–454.
- Bielaszewska, M., Zhang, W., Tarr, P. I., Sonntag, A. K. & Karch, H. (2005c). Molecular profiling and phenotype analysis of *Escherichia coli* O26:H11 and O26:NM: secular and geographic consistency of enterohemorrhagic and enteropathogenic isolates. *J Clin Microbiol* **43**, 4225–4228.
- Bouzari, S., Oloomi, M. & Oswald, E. (2005). Detection of the cytotolethal distending toxin locus *cdtB* among diarrhoeagenic *Escherichia coli* isolates from humans in Iran. *Res Microbiol* **156**, 137–144.
- Chien, C. C., Taylor, N. S., Ge, Z., Schauer, D. B., Young, V. B. & Fox, J. G. (2000). Identification of *cdtB* homologues and cytotolethal distending toxin activity in enterohepatic *Helicobacter* spp. *J Med Microbiol* **49**, 525–534.
- Clark, C. G., Johnson, S. T., Easy, R. H., Campbell, J. L. & Rodgers, F. G. (2002). PCR for detection of *cdt*-III and the relative frequencies of cytotolethal distending toxin variant-producing *Escherichia coli* isolates from humans and cattle. *J Clin Microbiol* **40**, 2671–2674.
- Cope, L. D., Lumbley, S., Latimer, J. L. & 8 other authors (1997). A diffusible cytotoxin of *Haemophilus ducreyi*. *Proc Natl Acad Sci U S A* **94**, 4056–4061.
- Cortes-Bratti, X., Frisan, T. & Thelestam, M. (2001a). The cytotolethal distending toxins induce DNA damage and cell cycle arrest. *Toxicon* **39**, 1729–1736.
- Cortes-Bratti, X., Karlsson, C., Lagergard, T., Thelestam, M. & Frisan, T. (2001b). The *Haemophilus ducreyi* cytotolethal distending toxin induces cell cycle arrest and apoptosis via the DNA damage checkpoint pathways. *J Biol Chem* **276**, 5296–5302.
- daSilva, A. S. & daSilva Leite, D. (2002). Investigation of putative CDT gene in *Escherichia coli* isolates from pigs with diarrhea. *Vet Microbiol* **89**, 195–199.
- Dobrindt, U., Agerer, F., Michaelis, K. & 7 other authors (2003). Analysis of genome plasticity in pathogenic and commensal *Escherichia coli* isolates by use of DNA arrays. *J Bacteriol* **185**, 1831–1840.
- Friedrich, A. W., Bielaszewska, M., Zhang, W.-L., Pulz, M., Kuczius, T., Ammon, A. & Karch, H. (2002). *Escherichia coli* harbouring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J Infect Dis* **185**, 74–84.
- Friedrich, A. W., Nierhoff, K. V., Bielaszewska, M., Mellmann, A. & Karch, H. (2004). Phylogeny, clinical associations, and diagnostic utility of the pilin subunit gene (*sfpA*) of sorbitol-fermenting, enterohemorrhagic *Escherichia coli* O157:H[−]. *J Clin Microbiol* **42**, 4697–4701.
- Friedrich, A. W., Köck, R., Bielaszewska, M., Zhang, W., Karch, H. & Mathys, W. (2005). Distribution of the urease gene cluster among and urease activities of enterohemorrhagic *Escherichia coli* O157 isolates from humans. *J Clin Microbiol* **43**, 546–550.
- Friedrich, A. W., Lu, S., Bielaszewska, M., Prager, R., Bruns, P., Xu, J. G., Tschape, H. & Karch, H. (2006). Cytotolethal distending toxin in *Escherichia coli* O157:H7: spectrum of conservation, structure, and endothelial toxicity. *J Clin Microbiol* **44**, 1844–1846.
- Haghjoo, E. & Galan, J. E. (2004). *Salmonella typhi* encodes a functional cytotolethal distending toxin that is delivered into host cells by a bacterial-internalization pathway. *Proc Natl Acad Sci U S A* **101**, 4614–4619.
- Janka, A., Bielaszewska, M., Dobrindt, U. & Karch, H. (2002). Identification and distribution of the enterohemorrhagic *Escherichia*

- coli* factor for adherence (*efal*) gene in sorbitol-fermenting *Escherichia coli* O157:H⁻. *Int J Med Microbiol* **292**, 207–214.
- Janka, A., Bielaszewska, M., Dobrindt, U., Greune, L., Schmidt, M. A. & Karch, H. (2003). Cytolethal distending toxin gene cluster in enterohemorrhagic *Escherichia coli* O157:H⁻ and O157:H7: characterization and evolutionary considerations. *Infect Immun* **71**, 3634–3638.
- Janka, A., Becker, G., Sonntag, A. K., Bielaszewska, M., Dobrindt, U. & Karch, H. (2005). Presence and characterization of a mosaic genomic island which distinguishes sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:H⁻ from *E. coli* O157:H7. *Appl Environ Microbiol* **71**, 4875–4878.
- Johnson, W. M. & Lior, H. (1987). Response of Chinese hamster ovary cells to a cytolethal distending toxin (CDT) of *Escherichia coli* and possible misinterpretation as heat-labile (LT) enterotoxin. *FEMS Microbiol Lett* **43**, 19–23.
- Johnson, W. M. & Lior, H. (1988a). A new heat-labile cytolethal distending toxin (CLDT) produced by *Campylobacter* spp. *Microb Pathog* **4**, 115–126.
- Johnson, W. M. & Lior, H. (1988b). A new heat-labile cytolethal distending toxin (CLDT) produced by *Escherichia coli* isolates from clinical material. *Microb Pathog* **4**, 103–113.
- Johnson, J. R. & Stell, A. L. (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis* **181**, 261–272.
- Johnson, J. R., Oswald, E., O'Bryan, T. T., Kuskowski, M. A. & Spandjaard, L. (2002). Phylogenetic distribution of virulence-associated genes among *Escherichia coli* isolates associated with neonatal meningitis in the Netherlands. *J Infect Dis* **185**, 774–784.
- La Ragione, R. M. & Woodward, M. J. (2002). Virulence factors of *Escherichia coli* serotypes associated with avian colisepticaemia. *Res Vet Sci* **73**, 27–35.
- Lara-Tejero, M. & Galan, J. E. (2001). CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. *Infect Immun* **69**, 4358–4365.
- Mainil, J. G., Jacquemin, E. & Oswald, E. (2003). Prevalence and identity of *cdt*-related sequences in necrotoxicogenic *Escherichia coli*. *Vet Microbiol* **94**, 159–165.
- Mayer, M. P., Bueno, L. C., Hansen, E. J. & DiRienzo, J. M. (1999). Identification of a cytolethal distending toxin gene locus and features of a virulence-associated region in *Actinobacillus actinomycetemcomitans*. *Infect Immun* **67**, 1227–1237.
- Morabito, S., Dell'Omo, G., Agrimi, U., Schmidt, H., Karch, H., Cheasty, T. & Caprioli, A. (2001). Detection and characterization of Shiga toxin-producing *Escherichia coli* in feral pigeons. *Vet Microbiol* **82**, 275–283.
- Nougayrede, J. P., Taieb, F., De Rycke, J. & Oswald, E. (2005). Cyclomodulins: bacterial effectors that modulate the eukaryotic cell cycle. *Trends Microbiol* **13**, 103–110.
- Okeke, I. N., Lamikanra, A., Steinruck, H. & Kaper, J. B. (2000). Characterization of *Escherichia coli* strains from cases of childhood diarrhea in provincial southwestern Nigeria. *J Clin Microbiol* **38**, 7–12.
- Okuda, J., Kurazono, H. & Takeda, Y. (1995). Distribution of the cytolethal distending toxin A gene (*cdtA*) among species of *Shigella* and *Vibrio*, and cloning and sequencing of the *cdt* gene from *Shigella dysenteriae*. *Microb Pathog* **18**, 167–172.
- Orth, D., Grif, K., Dierich, M. P. & Würzner, R. (2006). Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157: indications for an animal reservoir. *Epidemiol Infect* **134**, 719–723.
- Peres, S. Y., Marches, O., Daigle, F., Nougayrede, J. P., Herault, F., Tasca, C., DeRycke, J. & Oswald, E. (1997). A new cytolethal distending toxin (CDT) from *Escherichia coli* producing CNF2 blocks HeLa cell division in G2/M phase. *Mol Microbiol* **24**, 1095–1107.
- Pickett, C. L., Cottle, D. L., Pesci, E. C. & Bikah, G. (1994). Cloning, sequencing, and expression of the *Escherichia coli* cytolethal distending toxin genes. *Infect Immun* **62**, 1046–1051.
- Pickett, C. L., Pesci, E. C., Cottle, D. L., Russell, G., Erdem, A. N. & Zeytin, H. (1996). Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* sp. *cdtB* gene. *Infect Immun* **64**, 2070–2078.
- Pickett, C. L., Lee, R. B., Eyigor, A., Elitzur, B., Fox, E. M. & Stockbine, N. A. (2004). Patterns of variations in *Escherichia coli* strains that produce cytolethal distending toxin. *Infect Immun* **72**, 684–690.
- Prager, R., Annemüller, S. & Tschäpe, H. (2005). Diversity of virulence patterns among Shiga toxin-producing *Escherichia coli* from human clinical cases – need for more detailed diagnostics. *Int J Med Microbiol* **295**, 29–38.
- Scott, D. A. & Kaper, J. B. (1994). Cloning and sequencing of the genes encoding *Escherichia coli* cytolethal distending toxin. *Infect Immun* **62**, 244–251.
- Sonntag, A. K., Zenner, E., Karch, H. & Bielaszewska, M. (2005a). Pigeons as a possible reservoir of Shiga toxin 2f-producing *Escherichia coli* pathogenic to humans. *Berl Munch Tierarztl Wochenschr* **118**, 464–470.
- Sonntag, A. K., Bielaszewska, M., Mellmann, A., Dierksen, N., Schierack, P., Wieler, L. H., Schmidt, M. A. & Karch, H. (2005b). Shiga toxin 2e-producing *Escherichia coli* isolates from humans and pigs differ in their virulence profiles and interactions with intestinal epithelial cells. *Appl Environ Microbiol* **71**, 8855–8863.
- Starcic, M., Johnson, J. R., Stell, A. L., van der Goot, J., Hendriks, H. G., van Vorstenbosch, C., van Dijk, L. & Gaastra, W. (2002). Haemolytic *Escherichia coli* isolated from dogs with diarrhea have characteristics of both uropathogenic and necrotoxicogenic strains. *Vet Microbiol* **85**, 361–377.
- Toth, I., Herault, F., Beutin, L. & Oswald, E. (2003). Production of cytolethal distending toxins by pathogenic *Escherichia coli* isolated from human and animal sources: establishment of the existence of a new *cdt* variant (type IV). *J Clin Microbiol* **41**, 4285–4291.
- Young, V. B., Chien, C. C., Knox, K. A., Taylor, N. S., Schauer, D. B. & Fox, J. G. (2000). Cytolethal distending toxin in avian and human isolates of *Helicobacter pullorum*. *J Infect Dis* **182**, 620–623.