Epidemiol. Infect. (2000), **127**, 221–227. © 2001 Cambridge University Press
DOI: 10.1017/S0950268801006069 Printed in the United Kingdom

An outbreak of diarrhoea due to multiple antimicrobial-resistant Shiga toxin-producing *Escherichia coli* O26:H11 in a nursery

N. HIRUTA[1,3]*, T. MURASE[2] AND N. OKAMURA[3]

[1] *Yokosuka City Institute of Public Health, Yonegahama Dohri 2-7, Yokosuka, Kanagawa 238-0011, Japan*
[2] *Kanagawa Prefectural Public Health Laboratory, Yokohama, Kanagawa 241-0815, Japan*
[3] *Department of Clinical Microbiology and Immunology, School of Allied Health Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8519, Japan*

(*Accepted 14 June 2001*)

SUMMARY

An outbreak due to Shiga toxin-producing *Escherichia coli* O26:H11 (STEC) occurred at a nursery in southeastern Japan in 1997. Thirty-two children had watery or bloody diarrhoea but none of them suffered from haemolytic-uremic syndrome. All of the STEC O26 were isolated during the period from 23 July to 22 August from 24 children, 3 nurses, and 2 food samples. These organisms had *stx*1 and *eae* genes but none of the other genes for which we tested (*stx*2, *bfp*, and EAF plasmid). They also possessed multiple antimicrobial resistances, which were encoded by a transmissible plasmid, and showed mostly identical genomic pulsed-field gel electrophoretic patterns. The results of this investigation suggested that contaminated food was the main contributing factor to this multiple antimicrobial-resistant STEC O26 infection, and person-to-person transmission also contributed to the spread of this outbreak.

INTRODUCTION

Since the finding in 1977 of cytotoxin in *Escherichia coli*, some strains of *E. coli* belonging to the classical serogroups of enteropathogenic *E. coli* (EPEC) have been shown as Shiga toxin-producing *E. coli* (STEC) [1–3]. First the classical EPEC serogroups O26 and O128 strains were shown to carry *stx* genes but none of those possessed the plasmid-coded EPEC adherence factor EAF [4]. Now more than 100 serotypes [5] of *E. coli* which produce Shiga toxin have been associated with human intestinal infections including haemorrhagic colitis and haemolytic uraemic syndrome. The most important serogroup is STEC O157 but serogroups O26, O55, O111 and O128 have also been associated with outbreaks [6, 7] and sporadic infections [7–9]. These strains of STEC, which possess the *eae* gene encoding production of intimin associated with intimate adherence to intestinal epithelium and possess the 60-MDa plasmid, are also called enterohaemorrhagic *E. coli* (EHEC) [7]. Outbreaks of *E. coli* O26 or O111 have occurred in Italy (O111), USA (O111), Australia (O111), and Japan (O111, O26) [10–15]. Although the cases of O26 and O111 STEC infection are increasing in Japan [16], the true frequency of sporadic cases and outbreaks of non-O157 STEC infections remains unknown. Non-O157 STEC usually ferment sorbitol and there are no convenient selective and/or differential media, such as sorbitol MacConkey agar used for the isolation of STEC O157.

In July 1997, we investigated an outbreak due to multiple antimicrobial-resistant STEC O26:H11 strains which were isolated from the stools of children and staff (nurses), and from prepared foods for children. Polymerase chain reaction (PCR) assay [17] enabled detection of STEC O26 in this outbreak. Here

* Author for correspondence.

we report the epidemiology of the outbreak and the microbiological investigation of the *E. coli* O26 isolates, using pulsed-field gel electrophoresis (PFGE) as a genetic tool. We will also show that the multiple antimicrobial resistance was encoded on a transmissible plasmid.

METHODS

Case investigation

On 11 July, the index case (female, 1 year old) suffering from diarrhoea consulted a physician and was subsequently admitted to hospital on 19 July when she re-presented with bloody diarrhoea. On 23 July, the laboratory of the Yokosuka City Institute of Public Health received a strain of *E. coli* O26 from the hospital in order to confirm the presence of Shiga toxin. On 24 July Shiga toxin was confirmed in this *E. coli* O26:H11 strain. It was found that the index case had been cared for in a nursery until 18 July. Enteropathogens other than *E. coli* O26:H11 were not isolated from this patient. Preventative measures were taken in the nursery collaboratively with the Yokosuka Municipal Health Center. Investigations of the child care for each age group were conducted. The environment such as the baby rooms, dispensary, kitchen and lavatory was investigated, and the overall level of hygiene of the nursery was evaluated. All families of the cases were interviewed using a standard questionnaire enquiring as to clinical symptoms, severity of illness and diarrhoea. Samples obtained for microbiological investigation included 206 stools (from 94 children, 14 nurses, 3 cooks, 90 household contacts, and 5 food suppliers), 53 prepared foods, 70 food stuffs, and 16 environmental swabs. Attempts to isolate *E. coli* O26 finished on 22 August since no further *E. coli* O26 had been isolated.

Microbiological investigation

Isolation of STEC O26. Stool samples were inoculated directly onto appropriate agar media such as Deoxycholate Hydrogen-sulphide Lactose (DHL) agar (Eiken Chemical, Tokyo, Japan) or MacConkey agar (Difco Laboratories, Detroit, USA) and incubated for 18–24 h at 37 °C. Food samples (25 g) were mixed with 225 ml modified *E. coli* broth (peptone, 20·0 g; bile salts, 1·12 g; lactose, 5·0 g; dipotassium phosphate, 4·0 g; potassium phosphate, 1·5 g; sodium chloride, 5·0 g in a liter of distilled water) (Kyokuto, Tokyo, Japan) supplemented with novobiocin (0·25% w/v) and incubated for 18–24 h at 42 °C. A loopfull of post-enrichment culture was inoculated onto DHL agar or MacConkey agar and incubated for 18–24 h at 37 °C. At least four lactose-positive *E. coli* colonies were identified using conventional methods [18]. The *E. coli* cultures isolated from both human and non-human sources were determined for O and H serotypes using the commercial *E. coli* serotyping kit (Denka Seiken Co., Tokyo, Japan).

All *E. coli* isolates belonging to the serogroup O26 were examined for the possession of Shiga toxin and virulence genes by multiplex PCR with two sets of primers (Table 1). The primer oligonucleotides and their conditions for amplification are shown in Table 1 [17, 19, 20]. After amplification, 10 μl of the reaction mixture was analysed by agarose gel electrophoresis. Strains which were shown by PCR to be positive for *stx* gene(s) were confirmed for their production of Shiga toxin by using the reverse passive latex agglutination (RPLA) kit (Denka Seiken Co., Tokyo, Japan).

Antimicrobial susceptibility test. Minimum inhibitory concentrations (MICs) of 10 antimicrobial agents (ampicillin, tetracycline, kanamycin, streptomycin, gentamicin, nalidixic acid, ofloxacin, fosfomycin, chloramphenicol, trimethoprim-sulfamethoxazole) all of which were determined using Etest (AB BIODISK, Solna, Sweden) [21]. The MIC of sulfamethoxazole was measured by NCCLS standardized agar dilution method.

PFGE. PFGE was performed using the restriction enzymes *Xba*I, *Sfi*I, and *Spe*I supplied by the manufacture (Takara Shuzo Co. Tokyo, Japan). PFGE was carried out as described previously by Izumiya *et al*. [22] with some modification. In brief, the bacterial DNAs were digested with the restriction enzymes and PFGE was performed with 1% agarose gel by using CHEF Mapper apparatus (Bio–Rad Laboratories, Richmond, USA) in 0·5% TBE (Tris–borate–EDTA) buffer at 10 °C at 200 V. A linearly ramped switching time from 0·5 to 40 s was applied for 20 h. After PFGE, the gels were stained with ethidium bromide and were photographed under UV illumination.

Antimicrobial resistance transfer experiment and plasmid detection. The four strains from the multiple antimicrobial-resistant *E. coli* O26:H11 were selected as the donors. Conjugative matings were performed between multiple antimicrobial-resistant *E. coli* O26:H11 and the nalidixic acid-resistant *E. coli* K-12 CSH-

https://doi.org/10.1017/S0950268801006069 Published online by Cambridge University Press

Table 1. *The primers used in polymerase chain reaction (PCR)*

Primers sequence (5′–3′)		Size of amplified product (bp)	Reference	Amplifying condition
Set 1				
*stx*1: V1	AGTTAATGTGGTGGCGAA	811	Kobayashi *et al.* (17)	30 cycles:
V2	GACTGCGTCAGTGAGGTT			94 °C; 30 s
*stx*2: V3	TTCGGTATCCTATTCCCG	471	Kobayashi *et al.* (17)	55 °C; 30 s
V4	TCTCTGGTCATTGTATTA			72 °C; 30 s
Set 2				
EAF-B	TGGATCGCCAATGTTCTTGG	801	Yatsuyanagi *et al.* (20)	25 cycles:
EAF-SR	ATGGGGACCATGTATTATCA			94 °C; 30 s
eaeK1	GCTTAGTGCTGGTTTAGGAT	489	Ito *et al.* (19)	50 °C; 30 s
eaeK4	TCGCCGTTCAGAGATCGC			72 °C; 60 s
bfps	GAAGTAATGAGCGCAACGTC	234	Ito *et al.* (19)	
bfpas	ACATGCCGCTTTATCCAACC			

2 recipient. The transconjugants were also mated with another rifampicin-resistant *E. coli* K-12 strain. The plasmid was extracted by the method of Kado and Liu [23] and detected by agarose gel electrophoresis.

Statistical analysis

The results of the incidence of diarrhoea in the children in this outbreak were evaluated using the Fischer's exact test.

RESULTS

Epidemiological investigation

Thirty-two out of 94 (34%) children in the nursery reported diarrhoea during the 3 weeks in July. None of the other persons (nurses, cooks, household contacts, and food suppliers) reported diarrhoea. The index case had diarrhoea from 11 July, and the STEC O26 was detected in 16 children all of whom had diarrhoea between 11 and 29 July (Fig. 1). Various press releases reported the detection of STEC O26 in the first patient on 24 July, in the contaminated food on 28 July, and the spread of STEC to 3 nurses and 23 children (29 July–5 August). Nurse staffing ratios for each age group were as follows; 5 nurses for 17 one-year-olds, 2 nurses for 14 two-year-olds, and 2 nurses each for 21 three-year-olds, 21 four-year-olds and 21 five-year-olds. Environmental investigation showed that there was no separation between the kitchen and a hallway. Most of the patients had watery diarrhoea. Two children had bloody stools. None of the patients progressed to haemolytic-uremic syndrome (HUS). The mean age of children with diarrhoea was 2·5 years with a range of 1–6 years. There was a significant difference in the diarrhoea incidence ($P < 0{\cdot}006$), but not in the detection rate of STEC O26, between one-year-olds and the other age groups.

Microbiological investigation

A total of 29 STEC O26 strains were isolated. These consisted of 16 strains from 32 children with diarrhoea, 8 strains from 62 children without diarrhoea, 3 from 14 nurses, and 2 food samples (mixed vegetable with bean sprouts and spinach, and sliced watermelon) from 53 prepared foods. Since not all samples were collected from children during the time they were suffering from diarrhoea, the delay in stool screening may have resulted in a low isolation rate among the children with a history of diarrhoea. None of the samples from cooks, household contacts and food suppliers contained STEC O26. All of the 1-year-old infants from whom STEC were isolated had diarrhoea. Of the 29 STEC O26 strains 28 possessed both *stx*1 and *eae* genes and one only the *stx*1 gene. RPLA tests confirmed PCR tests showing the presence of Shiga toxin. Interestingly, *E. coli* O26 strains, which neither produced Shiga toxin nor agglutinated with any commercial anti-H sera (22 sera including H11), were also isolated from 6 (6·4%) children, 1 (20%) food supplier, and 2 (3·8%) food samples prepared between 14 and 15 July. These *stx*-negative *E. coli* O26 strains did not possess *eae*, *bfp* or EAF plasmid genes, and could be distinguished from classical EPEC of serogroup O26. There were no

https://doi.org/10.1017/S0950268801006069 Published online by Cambridge University Press

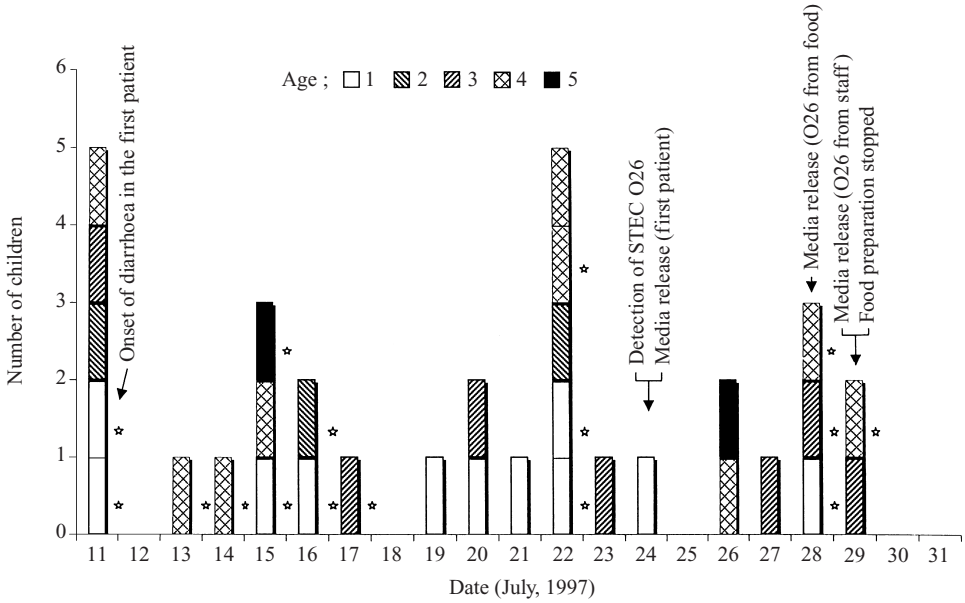

Fig. 1. Onset of diarrhoea of the children (ages 1–5). ☆: Children from whom STEC was isolated.

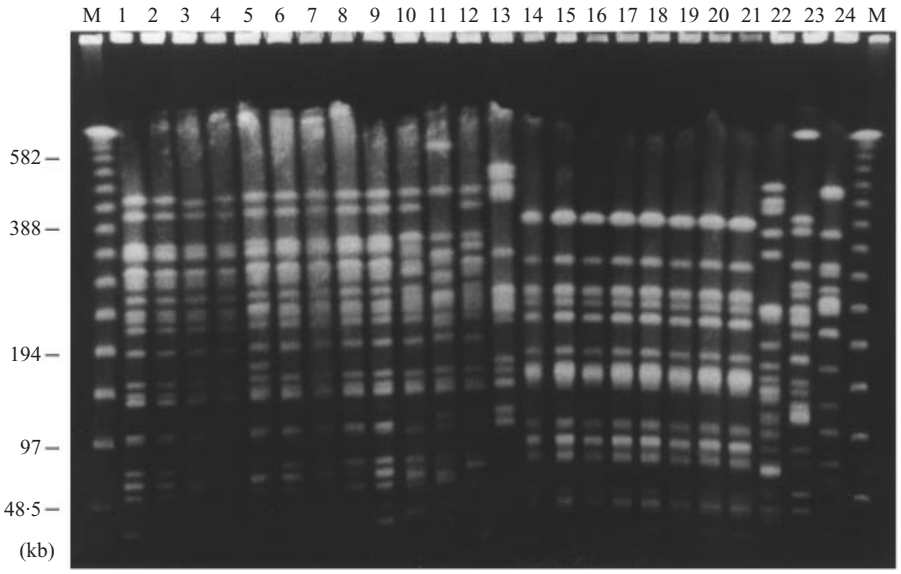

Fig. 2. Pulsed-field gel electrophoresis patterns of *E. coli* O26 isolates from a day nursery outbreak. Figure shows patterns for chromosomal digestion of *E. coli* O26 with *Xba*I. Lanes 1–9, strains of O26:H11 STEC from children (lanes 1–4), nurses (lanes 5–7), mixed vegetable with bean sprouts and spinach (lane 8), and sliced watermelon (lane 9) of outbreak cases; lanes 10–12, control strains of O26:H11 STEC from sporadic cases; lanes 13–21, strains of Shiga toxin-non producing *E. coli* O26 from food supplier (lane 13), children (lanes 14–19), served rice (lane 20), and noodle (lane 21) of outbreak cases in the nursery; lanes 22–24, control strains of Shiga toxin-non producing *E. coli* O26 from sporadic cases; lane M, lambda DNA ladder as a molecular size marker.

children who possessed both STEC O26:H11 and *stx*-negative O26.

PFGE analysis was performed on the genomic DNAs of both STEC O26 and *stx*-negative *E. coli* O26 strains from the children, nurses, a food supplier and prepared foods in this outbreak (Fig. 2). The PFGE patterns of the STEC O26 digested with the restriction enzyme *Xba*I were almost identical (lanes 1–9). Although the *eae*-negative STEC O26 strain had the identical electrophoretic pattern with that of the other *eae*-positive STEC O26 strains, it showed a slightly different electrophoretic pattern from that of the other *eae*-positive STEC O26 when genomic DNAs were digested with *Sfi*I, and *Spe*I (data not shown). These results suggested that the *eae* gene of this strain was either deleted or a variant. The PFGE

https://doi.org/10.1017/S0950268801006069 Published online by Cambridge University Press

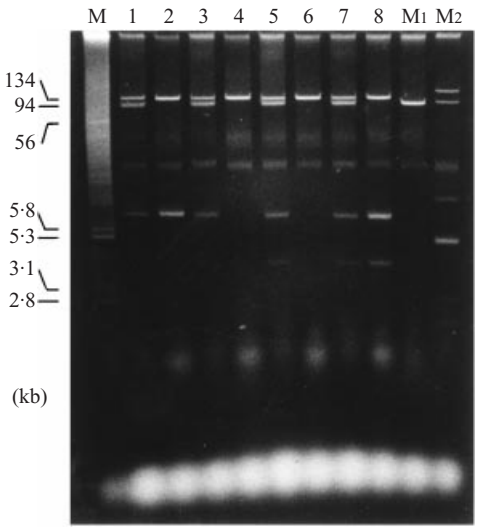

Fig. 3. Agarose gel electrophoresis patterns of plasmids of multiple antimicrobial-resistant *E. coli* O26:H11 strains and *E. coli* K-12 transconjugants. Lanes 1, 3, 5, and 7, donor strains of *E. coli* O26:H11; lanes 2, 4, 6, and 8, recipient strains of transconjugants *E. coli* K-12; lanes M, M1, and M2, molecular size marker *E. coli* V517, *E. coli* TM199 (NR1) and *Shigella sonnei* 98038 respectively.

patterns of the Shiga toxin-nonproducing *E. coli* O26 strains (lanes 13–21) were all identical except for the strain isolated from the food supplier (lane 13). Control *E. coli* O26 strains from sporadic cases (lanes 10–12, 22–24) differed from the outbreak strains in banding patterns.

Antimicrobial susceptibility tests showed that 27 STEC O26 strains had multiple antimicrobial resistances to ampicillin (MIC, $> 256\ \mu g/ml$), tetracycline ($> 256\ \mu g/ml$), kanamycin ($> 256\ \mu g/ml$), streptomycin (512 to $> 1024\ \mu g/ml$) and sulfamethoxazole ($> 256\ \mu g/ml$), and the other two strains also had the same resistance patterns except for sensitivity to kanamycin. They were sensitive to fosfomycin which, in Japan, is widely used for the treatment of children with diarrhoea.

Plasmid profiles of STEC O26 outbreak strains were mostly identical, and there were four plasmid bands of molecular weight of 134, 94, 8·3, and 4·2 kb respectively (Fig. 3). Antimicrobial resistance transfer experiments were done between outbreak strains as donors and nalidixic acid-resistant *E. coli* K-12 CSH-2 as a recipient. The 24 transconjugants which grew on antibiotic medium No. 3 agar plates containing ampicillin ($54\ \mu g/ml$) and nalidixic acid ($50\ \mu g/ml$) were all resistant to other unselected antimicrobial agents (tetracycline, kanamycin, streptomycin, and sulfamethoxazole). Agarose gel electrophoresis of the plasmids of the transconjugants suggested that all of the antimicrobial-resistant genes were encoded on one plasmid with apparent molecular weight of 134 kb (Fig. 3).

Control measures

Children with diarrhoea were immediately admitted to the Yokosuka City Hospital as soon as the *E. coli* O26 isolate was confirmed as STEC. The carriers of STEC O26 were advised not to attend the nursery until such time as STEC O26 was not isolated from their stool. The duration of shedding of the STEC O26 varied with each child from less than 6 to 13 days. The nursery was ordered to stop preparing food for children on 28 July. The kitchen of the nursery was cleaned and disinfected on 28 July, and construction to separate the kitchen from the hall was finished on 12 August. No cases of STEC O26 diarrhoea were identified after 22 August.

DISCUSSION

Our investigation showed that an outbreak due to STEC O26:H11 occurred among the children and nurses in the nursery. We were also able to isolate the STEC O26:H11 from food samples served to the children. The antimicrobial susceptibility patterns, PFGE patterns of genomic DNA, and agarose gel electrophoretic patterns of plasmid DNAs of the STEC O26 outbreak strains indicated that these isolates were identical or closely related. The food samples positive for STEC O26 were served on 11 July, when the first patient had already had diarrhoea followed by bloody diarrhoea on 19 July. We could not determine whether the source of this outbreak was a child or served foods in the nursery. At that time in Yokosuka City no other nurseries or day-care facilities experienced similar STEC O26 outbreaks and we could not isolate the STEC O26 strains from vegetables sold elsewhere in the city. Further, we could not isolate STEC O26 from cases' household contacts. Although the source remains undetermined, we thought that the contaminated foods, served in the nursery, were the main factor spreading STEC O26, although person to person transmission also contributed to spread in this outbreak. In fact in most reported cases involving nurseries or children's day-care facilities [24, 25], person-to-person transmission is the primary means of spreading the infection [25].

https://doi.org/10.1017/S0950268801006069 Published online by Cambridge University Press

Several reports [26–28] have suggested that STEC O26 and O111 serogroups, which cattle carry, are associated with diarrhoea in humans. However, there was no direct evidence which suggested any link with animals. In Japan we experienced the large outbreaks of STEC O157 in 1996 [22]. Since then, the clinical laboratories in hospitals have been well prepared for the identification of STEC strains. In addition nurseries and schools have been advised by the government to retain samples of previously served foods in the freezer. Thus in our case we could precisely identify the STEC O26 and quickly examine the children in the nursery.

We isolated two different *E. coli* O26 from both humans and food samples. One was STEC O26:H11, and the other was a Shiga toxin-nonproducing *E. coli* O26 which did not possess any virulence genes (*stx*, *eae*, *bfp*, and EAF plasmid, LT, ST, and *invE*) (data not shown). These two types of *E. coli* O26 could be easily distinguished on the basis of their virulence factors by multiplex PCR, and this could be useful for the detection of pathogens such as non-O157 STEC.

The outbreak strains of STEC O26 possessed resistances to five antimicrobial agents (ampicillin, tetracycline, kanamycin, streptomycin and sulfamethoxazole), which were encoded by a transmissible plasmid. There are reports of isolations of antimicrobial-resistant STEC from both humans and animals [29, 30], and recently in Japan, some reports [31, 32] indicate that in STEC O26 strains, resistance to fosfomycin is increasing. However, to the best of our knowledge, reports of outbreaks due to multiple antimicrobial resistant STEC O26 are very few.

Our analysis of that nursery outbreak suggests that continuous monitoring of such antimicrobial resistant non-O157 STEC is needed.

ACKNOWLEDGEMENTS

Part of this work was presented at the 72nd Meeting of the Japanese Association for Infectious Diseases in 1998. We would like to thank T. Okamoto, J. Yamaguchi, M. Himori, S. Yamai, and K. Ito for their helpful discussions and their technical laboratory assistance.

REFERENCES

1. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1997; **18**: 775–9.
2. Scotland SM, Day NP, Rowe B. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. FEMS Microbiol Lett 1980; **7**: 15–7.
3. Karch H, Heesemann J, Laufs R. Phage-associated cytotoxin production by and enteroadhesiveness of enteropathogenic *Escherichia coli* isolated from infants with diarrhea. J Infect Dis 1987; **155**: 707–15.
4. Smith HR, Scotland SM, Stokes N, Rowe B. Examination of strains belonging to enteropathogenic *Escherichia coli* serogroups for genes encoding EPEC adherence factor and Vero cytotoxins. J Med Microbiol 1990; **31**: 235–40.
5. Bopp CA, Brenner FW, Wells JG, Strockbine NA. *Escherichia*, *Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology, 7th ed. Washington DC: ASM Press, 1999: 459–74.
6. Bielaszewska M, Sramkova L, Janda J, Blahova K, Ambrozova H. Verotoxigenic (enterohaemorrhagic) *Escherichia coli* in infants and toddlers in Czechoslovakia. Infection 1990; **18**: 352–6.
7. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998; **11**: 142–201.
8. Scotland SM, Willshaw GA, Smith HR, *et al*. Virulence properties of *Escherichia coli* strains belonging to serogroups O26, O55, O111 and O128 isolated in the United Kingdom in 1991 from patients with diarrhoea. Epidemiol Infect 1993; **111**: 429–38.
9. Bielaszewska M, Janda J, Blahova K, *et al*. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome in the Czech Republic. Clin Nephrol 1996; **46**: 42–4.
10. Caprioli A, Luzzi I, Rosmini F, *et al*. Community-wide outbreak of hemolytic-uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli*. J Infect Dis 1994; **169**: 208–11.
11. Morabito S, Karch H, Mariani-Kurkdjian P, *et al*. Enteroaggregative, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic–uremic syndrome. J Clin Microbiol 1998; **36**: 840–2.
12. Banatvala N, Debeukelaer MM, Griffin PM, *et al*. Shiga-like toxin-producing *Escherichia coli* O111 and associated hemolytic-uremic syndrome: a family outbreak. Pediatr Infect Dis J 1996; **15**: 1008–11.
13. Henning PH, Tham EB, Martin AA, Beare TH, Jureidini KF. Haemolytic-uraemic syndrome outbreak caused by *Escherichia coli* O111:H−: clinical outcomes. Med J Aust 1998; **168**: 552–5.
14. Tanaka H, Ohseto M, Yamashita Y, *et al*. Bacteriological investigation on an outbreak of acute enteritis associated with verotoxin-producing *Escherichia coli* O111:H−. J Jpn Assoc Infect Dis 1989; **63**: 1187–94.
15. Sakata H, Maruyama S. Result of antibiotic therapy for children with *Escherichia coli* O26:H11 infection. J Jpn Assoc Infect Dis 1998; **72**: 1–4.
16. Watanabe H, Terajima J, Izumiya H, Wada A, Tamura K. Molecular analysis of enterohemorrhagic *Escheri-*

chia coli isolates in Japan and its application to epidemiological investigation. Pediatr Int 1999; **41**: 202–8.
17. Kobayashi K, Seto K, Makino M. Detection of two Shiga-like toxins from *Escherichia coli* O157:H7 isolates by the polymerase chain reaction method. Jpn J Microbiol 1990; **45**: 649–52.
18. Ewing WH. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. New York: Elsevier Science Publishing Co Inc, 1986.
19. Sueyoshi M, Fukui H, Tanaka S, Nakazawa M, Ito K. A new adherent form of an attaching and effacing *Escherichia coli* (*eaeA*+, *bfp*−) to the intestinal epithelial cells of chicks. J Vet Med Sci 1996; **58**: 1145–7.
20. Yatsuyanagi J, Kinouchi Y, Saito S, Sato H, Morita M. Enteropathogenic *Escherichia coli* strains harboring enteroaggregative *Escherichia coli* (EAggEC) heat-stable enterotoxin-1 gene isolated from a food-borne like outbreak. J Jpn Assoc Infect Dis 1996; **70**: 73–9.
21. Baquero F, Canton R, Martinez-Beltran J, Bolmstrom A. The E-test as an epidemiologic tool. Diagn Microbiol Infect Dis 1992; **15**: 483–7.
22. Izumiya H, Terajima J, Wada A, *et al*. Molecular typing of enterohemorrhagic *Escherichia coli* O157:H7 isolates in Japan by using pulsed-field gel electrophoresis. J Clin Microbiol 1997; **35**: 1675–80.
23. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981; **145**: 1365–73.
24. Lerman Y, Cohen D, Gluck A, Ohad E, Sechter I. A cluster of cases of *Escherichia coli* O157 infection in a day-care center in a communal settlement (Kibbutz) in Israel. J Clin Microbiol 1992; **30**: 520–1.
25. Al-Jader L, Salmon RL, Walker AM, *et al*. Outbreak of *Escherichia coli* O157 in a nursery: lessons for prevention. Arch Dis Child 1999; **81**: 60–3.
26. Borie CF, Monreal Z, Martinez J, Arellano C, Prado V. Detection and characterization of enterohaemorrhagic *Escherichia coli* in slaughtered cattle. Zentralbl Veterinarmed [B] 1997; **44**: 273–9.
27. Rios M, Prado V, Trucksis M, *et al*. Clonal diversity of Chilean isolates of enterohemorrhagic *Escherichia coli* from patients with hemolytic–uremic syndrome, asymptomatic subjects, animal reservoirs, and food products. J Clin Microbiol 1999; **37**: 778–81.
28. Holland RE, Wilson RA, Holland MS, *et al*. Characterization of *eae*+ *Escherichia coli* isolated from healthy and diarrheic claves. Vet Microbiol 1999; **66**: 251–63.
29. Prado V, Basualdo W, Arellano C, Martinez J, Levine MM. In vitro susceptibility of enterohemorrhagic *Escherichia coli* to 11 antimicrobials. Relationship between antibiotic resistance and toxigenic genotype. Rev Med Chil 1995; **123**: 1085–90.
30. Burnens AP, Frey A, Lior H, Nicolet J. Prevalence and clinical significance of vero-cytotoxin-producing *Eschrichia coli* (VTEC) isolated from cattle in herds with and without calf diarrhoea. Zentralbl Veterinarmed [B] 1995; **42**: 311–8.
31. Yamamoto T, Wakisaka N. Status of emerging drug resistance in Shiga toxin-producing *Escherichia coli* in Japan during 1996: a minireview. Jpn J Clin Med 1998; **56**: 2718–29.
32. Horii T, Kimura T, Sato K, Shibayama K, Ohta M. Emergence of fosfomycin-resistant isolates of Shiga-like toxin-producing *Escherichia coli* O26. Antimicrob Agents Chemother 1999; **43**: 789–93.