In Vitro Susceptibility of Four Serotypes of Enterohaemorrhagic *Escherichia coli* to Antimicrobial Agents

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We evaluated the *in vitro* susceptibility of four serotypes of enterohaemorrhagic *Escherichia coli* (*E. coli* O26, *E. coli* O111, *E. coli* O157, and *E. coli* O165) with diverse DNA patterns to antimicrobial agents. The minimum inhibitory concentrations (MIC) determined in a total of 83 strains using Mueller–Hinton agar under aerobic and anaerobic conditions were 0.015–0.12 µg/ml for ciprofloxacin, 0.06–1 µg/ml for norfloxacin, 2–64 µg/ml for fosfomycin without glucose-6-phosphate (G-6-P), 0.25–32 µg/ml for fosfomycin with G-6-P, 2–≥256 µg/ml for kanamycin, 0.125–2 µg/ml for cefoperazone, and 0.06–1 µg/ml for ceftazidime. The MIC of ciprofloxacin, norfloxacin, cefoperazone, and ceftazidime were low in all strains examined.

Key words enterohaemorrhagic *Escherichia coli*; susceptibility; ciprofloxacin; norfloxacin; cefoperazone; fosfomycin

Escherichia coli (*E. coli*) serotype O157:H7 was first recognized as a human pathogen during a 1982 investigation of two outbreaks of diarrhea, and is estimated to cause more than 20000 cases of infection and as many as 250 deaths each year in the United States.^{1,2} In Japan, the incidence of this infection is also high as was observed in a large outbreak stemming from a school lunch in Sakai City in 1996 in which 12680 people were infected with *E. coli* O157:H7, and 3 of them died.³

There is continuing controversy as to whether antimicrobial therapy should be performed for *E. coli* O157:H7 infection. Some investigators have suggested that administration of antimicrobial agents for this infection promotes release of verotoxin from this serotype or tends to induce secondary infection, thus arguing against antimicrobial therapy.^{4,5} Others have reported no effects of administration of antimicrobial agents for *E. coli* O157:H7 infection on the development of hemolytic uremic syndrome (HUS) or the microorganism discharge period.^{6,7} In contrast, some studies have shown a significant decrease in the incidence of HUS after administration of appropriate antimicrobial agents such as fluoroquinolones for *E. coli* O157:H7 infection, or a significant decrease in the mortality rate after administration of a fluoroquinolone to this infection in a mouse model.^{8–10}

In general, effective oral antimicrobial agents should be administered for bacterial enterocolitis.⁹ Enterohaemorrhagic *E. coli* has many O serotypes (1, 2, 4, 5, 6, 18, 25, 26, 38, 39, 45, 50, 55, 82, 84, 91, 103, 111, 113, 114, 115, 117, 118, 121, 128, 145, 146, 153, 163, 165) in addition to O157. However, there are no data on drug susceptibility of enterohaemorrhagic *E. coli* other than *E. coli* O157.^{11–13} Therefore, we evaluated *E. coli* O26, O111, and O165 which could be acquired in addition to *E. coli* O157.

MATERIALS AND METHODS

Susceptibility Tests The minimum inhibitory concentrations (MIC) were determined for the 83 strains of enterohaemorrhagic *E. coli* after 18 h incubation at 35 °C by dilution on Mueller–Hinton agar (Difco, U.S.A.) under both aerobic and anaerobic conditions. As a control, *E. coli* NIHJ JC-2 was used. The following agents were tested: ciprofloxacin

(Bayer Yakuhin, Ltd.), norfloxacin (Kyorin Pharm.), fosfomycin Na (Meiji Seika Kaisha, Ltd.) with or without 25 µg/ml of glucose-6-phosphate (G-6-P; Oriental Yeast Co., Ltd.) added,¹⁴ kanamycin sulfate (Meiji Seika Kaisha, Ltd.), cefoperazone Na (Pfizer Pharm, Inc.), and ceftazidime (Glaxo Japan Co.). These agents were provided in the form of a bulk powder. Anaerobic incubation was performed using a disposable O₂ absorbing and CO₂ generating agent (AnaeroPack®; Mitsubishi Gas Chemical Co.). The inocula (*ca.* 10⁴ colony forming units/spot) were plated using a multipoint incubator (Sakuma Co.). MIC were defined as the lowest concentration of agent inhibiting visible growth.

Productivity of Verotoxin The production of verotoxin (Shiga-like toxin) was determined by VTEC-RPLA SEIKEN (Denka Seiken Co., Ltd., Tokyo, Japan) based on the reversed passive latex agglutination method.

Pulsed Field Gel Electrophoresis The preparation of high molecular weight chromosomal DNA was performed according to Murray *et al.*¹⁵ Digestion was carried out by placing a small slice of an agarose plug in 200 µl of reaction buffer with 30 units of *Xba* I. Pulsed field gel electrophoresis was performed with the Bio-Rad Gene Path system in a 1% agarose gel in 0.5× TBE buffer at 14 °C with a linear ramp time of 4 to 50 s over a period of 20 h. Thereafter, the gels were stained with ethidium bromide and photographed.

RESULTS

Table 1 shows the 83 examined strains of enterohaemorrhagic *E. coli* (*E. coli* O26, *E. coli* O111, *E. coli* O157, and *E. coli* O165) and their productivity of verotoxin. Of the 83 strains, 82 were isolated in hospitals in Yamaguchi Prefecture (population, about 1600000) in Japan between June, 1997 and December, 1999. One strain was isolated in an outbreak in a home for the aged (27 patients with infection), 13 strains from 13 families (43 patients with familial infection), and 68 strains from 68 patients with sporadic infection. The other strain was isolated in a large outbreak (12680 patients with infection) in Sakai, Osaka Prefecture, Japan in 1996.^{3,16} All 83 strains were confirmed to differ in DNA pattern by pulsed field gel electrophoresis.

Table 2 shows the range of MIC and MIC₉₀ of 6 antimicro-

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Table 1. Characteristics of Eighty-Three Enterohaemorrhagic *Escherichia coli*

Serotype	No. of strains producing		
	Verotoxin I	Verotoxin II	Verotoxin I & II
O26:H11	11		1
O26:NM ^{a)}	1		
O111:NM	3		
O157:H7		26	35
O157:NM		2	3
O165:NM		1	

a) NM: non motility.

bial agents for the 83 strains of enterohaemorrhagic *E. coli* determined using Mueller–Hinton agar under aerobic and anaerobic conditions. The MIC of ciprofloxacin, norfloxacin, cefoperazone, and ceftazidime were $\leq 2 \mu\text{g/ml}$ in all 83 strains under both conditions. The MIC of fosfomycin were lower with than without added G-6-P, and lower under anaerobic conditions than under aerobic conditions, being $\leq 64 \mu\text{g/ml}$ in all 83 strains under both conditions. Kanamycin showed lower MIC under aerobic conditions than under anaerobic conditions and an especially high MIC ($\geq 256 \mu\text{g/ml}$) in 1 of the 83 strains.

DISCUSSION

There have been many reports of the drug susceptibility of

Table 2. Susceptibility of Eighty-Three Enterohaemorrhagic *E. coli* Strains of Four Serotypes to Antimicrobial Agents

Agent	Serotype (no. of strains)	MIC ($\mu\text{g/ml}$) under an aerobic condition		MIC ($\mu\text{g/ml}$) under an anaerobic condition	
		Range	MIC ₉₀	Range	MIC ₉₀
Ciprofloxacin	O26 (13)	0.015–0.06	0.06	0.06–0.12	0.12
	O111 (3)	0.03	0.03	0.06–0.12	0.12
	O157 (66)	0.015–0.06	0.06	0.06–0.12	0.12
	O165 (1)	0.06	—	0.12	—
	Total (83)	0.015–0.06	0.06	0.06–0.12	0.12
Norfloxacin	O26 (13)	0.06–0.12	0.12	0.25–1	0.5
	O111 (3)	0.12	0.12	0.5	0.5
	O157 (66)	0.06–0.5	0.25	0.5–1	1
	O165 (1)	0.06	—	1	—
	Total (83)	0.06–0.5	0.25	0.25–1	1
Fosfomycin	O26 (13)	16–32	32	4–16	16
	O111 (3)	32–64	64	4–8	8
	O157 (66)	8–32	32	2–16	8
	O165 (1)	32	—	8	—
	Total (83)	8–64	32	2–16	16
Fosfomycin + G-6-P (25 $\mu\text{g/ml}$)	O26 (13)	0.25–32	32	0.25–8	8
	O111 (3)	0.5	0.5	0.25–0.5	0.5
	O157 (66)	0.25–32	2	0.25–4	1
	O165 (1)	4	—	1	—
	Total (83)	0.25–32	2	0.25–8	1
Kanamycin	O26 (13)	4– ≥ 256	8	16– ≥ 256	32
	O111 (3)	4	4	16–32	32
	O157 (66)	2–4	4	16–64	64
	O165 (1)	2	—	16	—
	Total (83)	2– ≥ 256	8	16– ≥ 256	64
Cefoperazone	O26 (13)	0.125–1	1	0.125–2	2
	O111 (3)	0.125–0.25	0.25	0.125–0.25	0.25
	O157 (66)	0.125–1	0.25	0.125–2	0.5
	O165 (1)	0.5	—	0.5	—
	Total (83)	0.125–1	0.5	0.125–2	1
Ceftazidime	O26 (13)	0.125–0.25	0.25	0.125–0.25	0.25
	O111 (3)	0.06–0.15	0.25	0.06–0.25	0.25
	O157 (66)	0.06–0.25	0.25	0.125–1	0.5
	O165 (1)	0.5	—	1	—
	Total (83)	0.06–0.5	0.25	0.06–1	0.25

E. coli O157 among enterohaemorrhagic *E. coli*. Swerdlow *et al.* reported resistance of 1 strain of *E. coli* O157:H7 to streptomycin and tetracycline¹⁷⁾ while Meng *et al.* reported resistance of six of 118 strains (5.1%) of *E. coli* O157 to one or both of these agents.¹⁸⁾ Stock and Wiedemann evaluated 139 *E. coli* strains including 18 strains of enterohaemorrhagic *E. coli* (serotype not described) and observed resistance of 9 strains (6.5%) to kanamycin and resistance of 37 strains (26.6%) to tetracycline, but high sensitivity of all strains to fluoroquinolone and cefoperazone.¹¹⁾ Tsuboi *et al.* reported that growth of all of 43 *E. coli* O157 strains was inhibited at a concentration of $\leq 0.13 \mu\text{g/ml}$ by several agents including ciprofloxacin and norfloxacin.¹²⁾ We previously reported that the minimum bactericidal concentrations (MBC) in 6 strains of *E. coli* O157 are high for fosfomycin, ampicillin, and tetracycline but low for ciprofloxacin, polymyxin B, cefoperazone, and kanamycin.¹³⁾ However, in these studies, drug susceptibility of other enterohaemorrhagic *E. coli* than *E. coli* O157 was not examined. Therefore, we determined MIC of 4 antimicrobial agents widely used for intestinal infection and 2 bile excreting type cephem antibiotics among the 83 strains of four serotypes of enterohaemorrhagic *E. coli* under aerobic and anaerobic conditions.

Kanamycin showed a high MIC in 1 strain. The MIC of ciprofloxacin, norfloxacin, and fosfomycin with or without added G-6-P for all serotypes of enterohaemorrhagic *E. coli* examined, however, were less than the intestinal concentrations reached following their oral administration at routine doses. The MIC of cefoperazone and ceftazidime were also less than the intestinal concentrations reached following their parenteral administration at routine doses.¹⁹⁾ Of these five agents, ciprofloxacin, norfloxacin, cefoperazone, and ceftazidime had marked *in vitro* antimicrobial effects on four serotypes of enterohaemorrhagic *E. coli*.

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