Korean J Biol Sci 3: 399-405, 1999

Characterization of the Serotyping and the Plasmid Profile of *E. coli* Isolated from Foods and Clinical Specimens

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Key Words:
E. coli
Serotyping
Plasmid profile
Antimicrobial susceptibility

Characteristics of the food isolates and the clinical specimens isolates of *E. coli* harboring virulence factor and their correlations were analyzed. The predominant serogroup were O8 and O27 in the food isolates and O6 and O18 in the clinical isolates, respectively, showing the different patterns in serogrouping between them. In the test of antibiotic susceptibility, the food isolates were resistant to cephalothin, streptomycin, tetracycline and minocycline, and the clinical isolates were resistant to ampicillin, carbenicillin, streptomycin, cephalothin, trimethoprim/sulfamethoxazole, tetracycline and minocycline, respectively. It shows that *E.coli* isolated from food sources and clinical specimens might be correlated. Plasmid profile in the food and clinical isolates showed wide diversity. Especially, large sized plasmid DNA such as 60 MDa, 90 MDa and 120 MDa were observed. The plasmid DNA (60 MDa) containing a gene encoding hemolysin was found in 43% of the food isolates and 35% of the clinical isolates. To study chromosomal homology, PFGE analysis was performed, showing different restriction patterns by *Xba*I. This result indicates that there were no genetic correlations between the foods and the clinical isolates.

Enterovirulent *E. coli* was classified into 5 groups according to the mechanism of disease - enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAggEC) (Levine, 1987). Each group has a specific serotype. The cellular phenomenon and the response appeared during the process of a disease and the pathological developments were present as the distinct difference (Hitchins et al., 1995; Sears and Kaper, 1996). The groups in *E. coli* can be classified with diversity. Particularly, serotyping is the most fundamental subtyping method which is used in most laboratories. More than 700 serotypes were known in *E. coli* by antigenic properties and the serotypes were classified according to three types of antigens: O (somatic), K (capsule) and H (flagella) antigen. Recently, the K antigen was excluded from serotype classification, but 173 O antigen and 56 H antigen were added. The serotype of pathogenic *E. coli* must be investigated because the prevalence of *E. coli* serotype showed a different pattern according to regional and environmental properties.

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The antibiotic resistantency and the plasmid profile as well as serotyping are frequently used for classification. Although antibiotics resistant patterns were based on the phenotypic properties, the method was highly standardized and were proven to be useful for the subtyping of numerous isolates including nosocomial infection. The factor affecting antibiotic resistance is frequently plasmid DNA (Baldini et al., 1983). Recently, vancomycin-resistant *Staphylococcus aureus*, drug-resistant malaria, multidrug-resistant tuberculosis, multidrug-resistant enterococci- and fluoroquinolon-resistant *Campylobacter* have reemerged and raised a social problem (Anthony, 1998). Their treatment is not easy and occasionally impossible on the infection of human and animals. Therefore, to prevent antibiotic resistant bacteria, the properties of antibiotic resistance in the isolates should be elucidated.

The analysis of the plasmid profile is a simple and useful method for identifying the agent causing a disease. Plasmid DNA is a self-replicating extrachromosomal DNA encoding virulence factor or often carrying antibiotic resistant genes. The plasmid profile has been applied for the subtyping of bacteria such as *E. coli, Salmonella, Shigella, Campylobacter, Vibrio cholerae, Heamophilus influenzae, Neisseria gonorrhoeae, Neisseria meningitidis, Staphylococcus aureus* and *Legionella* species (Holmberg and Wachsmuth, 1989). In particular, the plasmid profile was effectively used to

identify *Shigella* spp. and *E. coli* causing diarrhea isolated from food sources because most enterobacteria contain plasmid DNA. The plasmid profile and restriction enzyme pattern were effectively used to analyze the similarity between the food isolates and isolates of clinical specimens in epidemiological investigations (Dupont and Murray, 1992; Yatsuyanagi et al., 1995). Although the plasmid profile is used as a specific pathogenic marker, there are some limitations in epidemiological investigations because plasmid DNA can move and sometimes naturally disappear in bacterial cells.

Recently, subtyping method by pulsed-field gel electrophoresis (PFGE) has been used extensively to pursue the epidemiological survey and to find the cause of disease occurrence against various pathogenic bacteria such as *Yersinia enterocolitica* (Buchrieser et al., 1994; Najdenski et al., 1994), *Clostridium difficile* (Chachaty et al., 1994; Kristjansson et al., 1994), *V. vulnificus* (Buchrieser et al., 1995), *V. cholerae* O1 (Mahalingam et al., 1994), *S. aureus* (Bannerman et al., 1995), *Listeria monocytogenes* (Proctor et al., 1995) and *E. coli* O157:H7 (Bohm and Karch, 1992; Meng et al., 1996, Izumiya et al., 1997).

In this experiment, serotyping, antibiotic resistant ability, plasmid profile and subtyping by PFGE against food isolates and the clinical specimens isolates harboring virulence factor were performed and the possibilities of human infection through these foods were investigated. The serotypes and genetic correlations between the food isolates and isolates of clinical specimens were determined, and the prevalence of antibiotic resistant bacteria and the factors related to virulence were examined.

Materials and Methods

Bacterial strains

E. coli V517 was obtained from National Institute of Health, Korea, and *E. coli* K-12 W3350/R222 (70 MDa marker) and *E. coli Rst*-1 (120 MDa marker) were kindly supplied by Kifu University, Japan.

One hundred twenty-one *E. coli* isolates and 67 strains were isolated from beef, pork, chicken meats and their processing foods. From 1995 to 1997, fifty four strains were isolated as test strains from clinical specimens.

Serotyping test

Antiserums used to confirm the O and H serotype were provided by the National Institute of Infectious Disease, Japan. Antiserums (O1 ~ O173) were used to confirm the O serogroup. Isolates were inoculated in antibiotic medium No. 3 (Difco) supplemented with 1% (v/v) TTC (2,3,5-triphenyltetrazolium chloride) and incubated at 35℃ for 24 h. Smooth colonies were chosen under the light microscope (×100) and inoculated onto tryptic soy agar slant (Difco) and incubated overnight at 35℃. Cultures were suspended in 2.5 ml of saline solution and sterilized at 121℃ for 15 min. After cooling, the suspension was centrifuged at 3,000 rpm for 15 min, resuspended in 2 ml of saline solution, and then the suspension was kept at room temperature for 30 min. Each drop of the mixed serum and the suspension was added onto the agglutination plate and shaken for 5 min at 500 rpm.

Antiserum (H1 ~ H56) were used to confirm serotype H. After the test strains were inoculated into motility GI medium (Difco) and incubated at 35℃ for 24 h, isolates having motility were reinoculated in the same medium. These isolates were inoculated in 7 ml of brain heart infusion broth (BHI, Difco) and incubated at 35℃ for 24 h and then the same volume of saline solution containing 1% formalin was added. A drop of serum and 0.5 ml of BHI broth culture were mixed in a small tube, incubated at 50℃ water bath for 1 h, and tested for agglutination.

Antibiotic susceptibility test

Antibiotic susceptibility test was carried out by the disc diffusion method (Isenberg, 1992). One-hundred twenty-one isolates were inoculated in 10 ml of Mueller Hinton broth (Difco), respectively and incubated overnight at 35℃. The concentration of the culture was adjusted to MacFarland No. 0.5 and the cultures were inoculated onto Mueller Hinton Agar (MHA, Difco) with a sterile cotton swab. As noted in Table 1, antibiotic discs (BBL) were placed onto MHA and incubated overnight at 35℃. Clear zones were measured and the results were analyzed by National Committe for Clinical Laboratory Standardization (NCCLS, 1984).

Table 1. Antibiotics and their potency

Antibiotics family	Antibiotics	Disc potency
β-Lactams	ampicillin (AM)	10 μg
	carbenicillin (CB)	100 μg
	ampicillin/sulbactam (SAM)	10/10 μg
	cephalothin (CF)	30 μg
Aminoglycosides	gentamycin (GM)	10 μg
	neomycin (N)	30 μg
	streptomycin (S)	10 μg
	tobramycin (NN)	10 μg
	kanamycin (K)	30 μg
	amikacin (AN)	30 μg
Tetracyclines	tetracycline (TE)	30 μg
	minocycline (MI)	30 μg
Peptides	polymycin B (PB)	300 U
Quinolones	ciprofloxacin (CIP)	5 μg
	norfloxacin (NOR)	10 μg
	nalidixic acid (NA)	30 μg
Miscellaneus	chloramphenicol (C)	30 μg
	trimethoprim/ sulfamethoxazole (SXT)	1.25 μg/ 23.75 μg

Analysis of plasmid profile

Plasmid DNA was isolated using QIAGEN plasmid mini kit (LRS Laboratories, Inc.). The purified plasmid DNAs were electrophoresed in 1% agarose gel at 60 mA and the gel was stained with ethidium bromide (0.5 μg/ml).

Pulsed-field gel electrophoresis

Subtyping by pulsed-field gel electrophoresis (PFGE) was performed as described by the Centers for Disease Control and Prevention (CDC) (1996). Test strains were inoculated in 3 ml of BHI broth and incubated overnight at 35℃ with shaking (150 rpm). Then 1 ml of culture mixture was centrifuged at 10,000 rpm for 2 min. The collected cells were suspended in SE buffer (75 mM sodium chloride, 25 mM EDTA, pH 8.0) and the cell density was adjusted to O.D. 1.2 at 610 nm. The suspension was mixed gently with equal volume of 1% chromosomal grade ultra-pure DNA agarose (FMC) and solidified on ice in the CHEF disposable plug mold (Bio-Rad) for 30 min. Plug was added into 1 ml of lysis buffer {(50 mM Tris, pH 8.0, 50 mM EDTA, pH 8.0), 1% sarkosine, proteinase K (1 mg/ml)} and incubated overnight at 60℃ in water bath. Plug was washed for 30 min with 1 ml of TE buffer containing 30 μl of 0.1 M phenyl methyl sulfonyl fluoride (PMSF) in isopropanol twice. After rinsing with the sterilized water the plug was washed with TE buffer 4 times for 30 min. Plug was cut to a 2.5 mm thick slice. Sliced plug was treated with 4 μl *Xba*I (10 u/μl) and incubated overnight at 35℃ in a shaking water bath. Gel was made with 1% fast lane agarose (FMC). The temperature of the buffer (0.5 X TBE) was 14℃, pump speed was 60, and lambda ladder (BMB) was used as a marker. PFGE condition was as follows; initial A time 5 sec, final A time 35 sec, start ratio 1.0, run time 20 h. Electric current was applied at 200 volt using CHEF DR II. After electrophoresis, gel was stained with ethidium bromide (10 mg/ml) and DNA was visualized by fluorescence.

Results

Serotyping test

As shown in the test results for the serotype of the isolates, 42 kinds of O serogroups were observed such as O1, O25, O55, O63, O78, O81, O114, O153, etc. (Table 2).

The predominant serogroups were O8 and O27 in the food isolates and O6 and O18 in the clinical isolates, indicating differences in the serogroup predominance between the food isolates and the clinical isolates.

Antibiotic susceptibility test

The most pathogenic *E. coli* isolated from food and and clinical specimens showed a multi-resistant pattern (Table 3). Among 121 isolates, 5 strains (4.1%) weren't resistant to all antibiotics, 7 strains (5.8%) were resistant to one antibiotic, and 90% of isolates were resistant to more than two antibiotics. Especially, 18 (14.8%) isolates were resistant to more than 9 antibiotics, suggesting that it might be due to abuse of antibiotics. The food isolates were resistant to cephalothin (58%), streptomycin (70%), tetracycline (73%) and minocycline (60%) but susceptible to chloramphenicol (87%), gentamycin (84%), tobramycin (78%), norfloxacin (93%), ciprofloxacin (91%), trimethoprime/sulfamethoxazole (67%), ampicillin/sulbactam (84%) and amikacin (97%). The clinical isolates were resistant to ampicillin (72%), carbenicillin (74%), cephalothin (81%), gentamycin (46%), streptomycin (76%), tetracycline (65%), minocycline (54%), and trimethoprim-sulfamethoxazole (57%), but susceptible to ampicillin/sulbactam (63%), gentamycin (52%), neomycin (54%), polymycin B (54%), ciprofloxacin (87%), norfloxacin (87%), chloramphenicol (69%), nalidixic acid (69%) and amikacin (98%). Particularly, food and clinical isolates showed common resistant patterns against some antibiotics such as carbenicillin, cephalothin, streptomycin, tetracycline and minocycline.

Table 2. Serotypes of *E. coli* isolated from food and clinical specimens

O serogroup	H antigen	No. of isolates	O serogroup	H antigen	No. of isolates
1	NM	2(F)	63	NM	1(S), 3(F)
1	6	1(S)	74	1	1(S)
1	7	1(S)	78	6	1(S)
2	NM	1(S)	78	4	1(F), 1(S)
5	NM	3(F)	81	NM	3(S)
5	21	1(S)	86a	AGG	1(F)
6	NM	2(S), 2(F)	103	NM	1(F)
6	UT	2(S)	114	40	1(F)
6	45	1(S)	114	4	1(F)
6	12	3(S), 2(F)	114	NM	1(F)
7	4	1(S)	115	40	1(F)
8	NM	5(F), 1(S)	119	NM	1(F)
8	20	1(F)	125	UT	1(S)
16	NM	1(S)	125	NM	1(F)
18	NM	3(S)	146	NM	1(F)
18	11	1(S)	146	UT	1(F)
18	12	2(S)	147	NM	1(F)
18	5	1(S)	148	18	1(F)
18	33	1(S)	153	NM	1(F)
20	NM	1(S)	153	AGG	1(F)
20	UT	1(S)	153	9	1(F)
25	NM	1(F), 2(S)	153	42	1(F)
25	4	1(S)	157	7	1(F)
26	4	1(F)	158	6	1(S)
27	2	2(F)	159	UT	1(F)
27	NM	4(F)	161	11	1(S)
27	AGG	2(S)	162	NM	1(F), 1(S)
27	7	1(F)	162	50	1(F)
29	12	1(S)	162	43	1(F)
32	21	1(F)	164	UT	1(F)
36	21	1(F)	168	NM	1(F)
38	26	1(F)	168	10	1(F)
44	NM	1(S), 1(F)	169	7	1(F)
44	45	1(S)	172	NM	1(S)
55	NM	2(S)	UT	NM	1(S), 6(F)
55	26	1(F)	UT	5	1(S)
55	8	1(F)	UT	26	2(F)
62	7	1(F)	UN		5(S)
			Total		121

* F; strains isolated from foods, S; strains isolated from clinical specimens, UT; untypable, UI; unidentified, NM; nonmotile, Agg; agglutinate to all H antiserum

Table 3. Multi-resistant patterns of *E. coli* isolated from food and clinical specimens

Multi-resistance pattern	No. of isolates(%)
CF-GM	2(1.6)
CF- S	4(3.3)
S-TE	2(1.6)
TE-MI	3(2.5)
AM-CB-CF	4(3.3)
CB-CF- N	1(0.8)
CF- S-TE	2(1.6)
CF-TE-MI	2(1.6)
GM-NN- S	1(0.8)
N-TE- K	2(1.6)
N-NN- S	1(0.8)
S-TE-MI	4(3.3)
CF- S-TE-MI	4(3.3)
CF- N-TE- K-MI	2(1.6)
N-NA- S-TE-MI	2(1.6)
AM-CB-NA- S-TE-MI	1(0.8)
AM-CB-CF-GM-TE-MI	1(0.8)
AM-CB-CF-NA- S-TE	4(3.3)
AM-CB-CF- S-TE-MI	3(2.5)
AM-CB-CF-NA-SXT-TE-MI	3(2.5)
AM-CB- N- S-SXT-TE- K	2(1.6)
AM-CF-GM-NOR-CIP-S-SXT	2(1.6)
CB-CF- N-NN-PB-TE-MI	1(0.8)
CB-CF- N- S-TE- K-MI	1(0.8)
CF-GM-NN- S-TE-MI-SAM	1(0.8)
AM- C-CB-NA-S-SXT-TE-MI	1(0.8)
AM-CB-GM-NA-NN- S-TE-MI	2(1.6)
AM-CB-CF- N-PB-S-SXT-TE	2(1.6)
AM-CB-CF- N-NA- S-TE-MI	1(0.8)
AM-CB-CF-S-SXT-TE-SAM-MI	2(1.6)
AM-CB-CF-GM-NN-TE- K-MI	1(0.8)
AM-CB-CF-GM-NN- S-SXT- K	5(4.1)
CB-CF-GM-NN- S-TE-SAM-MI	1(0.8)
CB- N-NA-CIP-S-TE- K-MI	2(1.6)
AM- C-CB-CF-GM- N- S-TE- K	2(1.6)
AM-CB-CF-NA-NOR-CIP- S-SXT-TE	2(1.6)
AM-CB-CF- N-NA- S-SXT-TE- K-MI	5(4.1)
AM-CB-CF-GM- N-NA-NN- S-TE- K-MI	1(0.8)
AM-CB-CF-GM- N-NA-S-SXT-TE- K-MI	2(1.6)
AM- C-CB-CF-NA-S-SXT-TE-MI-NOR-CIP	6(4.9)

* AM; ampicillin, CB; carbenicillin SAM; ampicillin/sulbactam, CF; cephalothin, GM; gentamycin, N; neomycin, S; streptomycin, NN; tobramycin K; kanamycin, TE; tetracycline, MI; minocycline, PB; polymycin B, CIP; ciprofloxacin, NOR; norfloxacin, NA; nalidixic acid, C; chloramphenicol, SXT; trimethoprim/sulfamethoxazole.

Analysis of plasmid profile

Most isolates except 14 isolates among 121 isolates contained various sizes of plasmid DNA (Fig. 1). Plasmid profiles for all the isolates were described in Table 4. Twenty-eight strains (41.8%) out of 67 strains isolated from the food sources contained ~60 MDa plasmid DNA, and 7 strains (10.4%) and 14 strains (20.9%) had as large as 90 MDa and 120 MDa, plasmid DNA respectively. Nineteen strains (35.2%) among 54 strains isolated from clinical specimens contained 60 MDa plasmid DNA and 13 strains (24.1%) and 8 strains (14.8%) had large-sized plasmid DNA of 90 MDa and 120 MDa, respectively.

The polymorphism of the plasmid profile may be correlated to antibiotic resistant characteristics. Especially, 60 MDa DNA seem to hold antibiotic resistance and confer on the function of intestinal attachment and invasion in EHEC, ETEC, and EPEC groups.

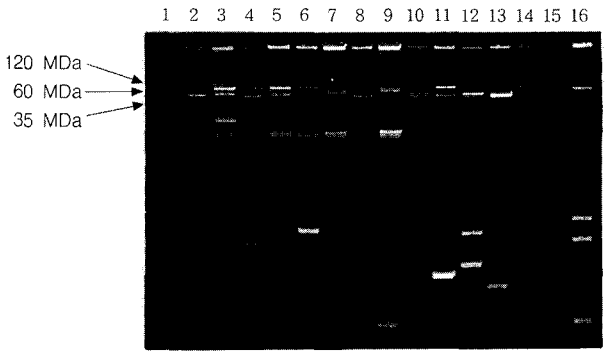

Fig. 1. Plasmid profile of *E. coli* isolated from clinical specimens. Lane 1; *E. coli* V517(size marker), lane 2; *E. coli* ATCC 43894, lane 3; S53, lane 4; S54, lane 5; S42, lane 6; S17, lane 7; S44, lane 8; S38, lane 9; S49, lane 10; S55, lane 11; S16, lane 12; S18, lane 13; S15, lane 14; S56, lane 15; S52, lane 16; S43.

Pulsed-field gel electrophoresis (PFGE)

To investigate the chromosomal homology between food and clinical isolates, subtyping by PFGE was carried out. Ten to twenty restriction fragments were observed by digesting the plasmid DNAs with *Xba*I as shown in Fig. 2.

According to the results of PFGE, pathogenic *E. coli* isolated from foods and clinical specimens showed diverse patterns and also, the isolates showing the same serotype showed different patterns. Therefore, genetic correlation was not found in sporadic isolates.

Discussion

Factors which cause the outbreak of diarrheal disease by pathogenic *E. coli* include adhesive element, invasive element, movement, chemotaxis, surface shape of antiphagocytic action, defense against serum sterilization, defense against immune response, and genetic reversible factor. In general, three paradigms of these pathogenic *E. coli* are enterotoxin-producing ETEC and EAEC,

Table 4. Plasmid profile for *E. coli* isolates

Molecular weight (MDa)	Serotype
60	O6:H12(LT+),O63:NM(LT+),O27:H2(LT+), O1:NM(EAF+),O78:H4(LT+),O8:NM(LT+), O157:H7(VT II+),O28:H12(LT+)
90	O6:NM(LT+),O153:H42(LT+),O55:NM(EAF+)
120	O18:H33(LT+),O74:H1(LT+)
120, 50	O114:H4(LT+)
120, 90	O119:NM(EAF+)
120, 90, 60, 7.5, 6, 3.9	O114:NM(LT+)
120, 60, 6.5, 5.3, 2.8	O8:NM(ST+)
120, 50, 6, 2.9	O168:NM(LT+)
90, 60, 8.6, 2.4, 1.7, 1.1	O55:H26(EAF+)
60, 15, 7, 4.9	O36:H21(LT+)
60, 6.8, 1.4, 1.2	O1:NM(EAF+)
55, 6.8, 4.5	O146:NM(EAF+)
55, 6.3, 3, 2.8, 2.6	O153:H9(LT+)
55, 5.5, 4.5, 2.5, 2.3	O1:H7(VT I+)
50, 12, 5.5, 4.8, 3.7	O168:H10((LT+)
50, 4.3, 4, 2.4, 1.4, 1.2	O5:NM(LT+)

LT+; LT-producing *E. coli,* ST+; ST-producing *E. coli,* EAF; EAF harboring *E. coli,* VT I; VT I-producing *E. coli,* VT II; VT II-producing *E. coli*

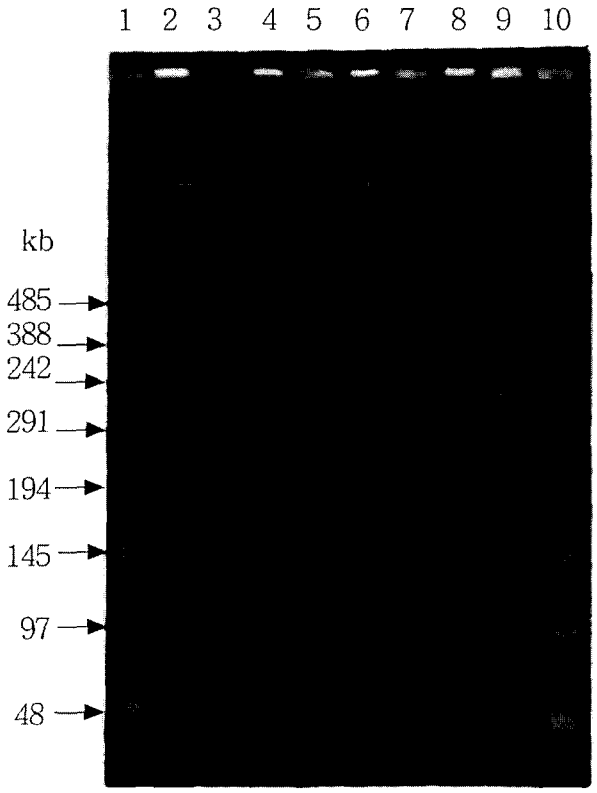

Fig. 2. DNA restriction patterns of enterovirulent *E. coli* isolated from meat as determined by CHEF after cleavage with *Xba*I. Lane 1, 10: λ DNA marker, lane 2; F36(UT), lane 4; F43(O1:NM), lane 9; F22(O63:NM), lane 3; F45(O114:H40) lane 6; F51(O153:H42), lane 8; F52(O114:H4), lane 5; F23 (O8:H20), lane 7; F16(O27:H2).

enteroinvasive EIEC, and EHEC and EPEC adhesive to the cell membrane.

The typical methods to isolate and identify these bacteria are biochemical tests and serotyping. Phenotypic analysis based on the virulence factor, nucleic acid probe and PCR are common molecular biology approaches. The most general method to classify the pathogenic *E. coli* group is serotyping. As shown in this report, the serotype distribution of isolates depended upon their localities.

Tamura et al. (1966) reported that the virulence factor and serotype of 3,605 strains of *E. coli* isolated from diarrheal patients in Southeast Asia, India, Middle east, and Japan were investigated and so their results were observed from 1057 ETEC, 132 EIEC, 64 EHEC, and 745 EPEC. Serogroups O6, O8, O15, O25, O27, O63, O73, O78, O114, O119, O126, O127, O128, O146, O148, O153, O159, O161, O167, O168, and O169 belonged to ETEC. Among them, O6 was a predominant serogroup. Serogroup O28, O124, O136 and O114 were included in EIEC and O157 was a predominant serogroup among EHEC. Serogroup O18, O26, O44, O55, O86, O103, O111, O114, O119, O125, O126, O127, O142, O153, O159, O160, O166, and O167 belongs to EAF-positive EPEC. In this experiment, the serogroup of *E. coli* isolated from domestic regions were classified as the pathogenic group and serogroup O6, O8, O18, O20, O25, O27, O29, O32, O36, O63, O78, O114, O115, O148, O153, O159, O162, O168 and O169 belongs to ETEC. Especially, O6, O8, O18 and O27 were predominant serogroups. Serogroup O26, O1 and O157 belonged to EHEC, but O125, O119, O146, O18, O1, O162, O55 and O38 belonged to EPEC, and O164 and O44 were in EIEC. These results indicated a similarity with a serogroup of enterovirulent *E. coli* isolated from Asia. The serogroup of pathogenic *E. coli* isolated from clinical specimens belonged to O1, O6, O18, O20, O25, O27, O44, O55, O78, O125 and O146 types. Among them, serogroup O6 and O18 were predominant. The predominant serogroups in the food isolates were O6 and O27. This result corresponds with Tamura's report (1966) that the predominant serogroup of the clinical specimen isolates was the O6 serotype. However, different serotyping patterns were reported in *E. coli* isolated from Bangladesh (Strockbin et al., 1992), Italy (Giammanco et al., 1996), and Bolivia (Utsunomiya et al., 1995).

Recently, the major cause of food poisoning from ground beef was by EHEC which contaminated beef through the cattle's feces during slaughter. Although, the serotyping of VT-producing EHEC was reported to be 50 types, serogroup O26, O103, O113, O157 were the predominant serotypes of EHEC isolated from cattle raised in Spain (Blanco et al., 1993), and serogroup O26, O111, O145, O157 were predominantly isolated from diarrheal patients in Japan (Tada et al., 1992). These serotypes were frequently isolated from diarrheal patients in Japan, and thus, domestic animals were the most important medium for EHEC infection of human. Serogroup O157:H7, O157: H-, O145:H-, O111:H8, O26: H11 were isolated in Germany (Beutin et al., 1994), serogroups O5, O26, O115, O128, O145, O153 in England were isolated (Chart et al., 1996), and serogroup O1, O26, O157 were confirmed in this study. The distribution pattern of EHEC was not identical regionally, but the major serotypes were O157, O26 and O111. According to Jeong's report (1998), the isolated ratio of *E. coli* O157:H7 was 1.42% in U.S.A and 1.4% in Japan. The results of the distributional survey were 2 strains (0.51%) in 390 cattle's feces and 2 strains (0.48%) among 420 pig's feces in Korea. The isolation rate of *E. coli* O157:H7 from domestic animals in Korea was very low compared to foreign nations, but these results implied the possibility of human infection because *E. coli* O157:H7 in a domestic animal's feces can be transmitted to meats, soil, water, and thus, contamination can occur by other means.

Luscher et al. (1994) identified that *Shigella* and EIEC isolated from a person who traveled to tropical areas were sensitive to antibiotics in the quinolones family such as ciprofloxacin. In this study, foods and clinical isolates were sensitive to quinolone antibiotics such as ciprofloxacin and norfloxacin, and proved to be safe antibiotics for emerging disease resistance.

Antibiotics were used as the useful objects such as in the prevention and treatment of cattle's disease and the feed additives for the improvement of the efficiency of feed stuff. These cattle utilizing the antibiotics must be slaughtered after a given time, and in this case, residual antibiotics are not found in meats. However, if bacteria infected in cattle acquired any antibiotic resistance properties, it will deter effective treatments of a disease. The identification of antibiotic resistant bacteria in cattle and human need to be investigated, and the use of antibiotics shoud be restricted to prevent the spread of resistant bacteria. For example, *Shigella*, EPEC, ETEC isolated from diarrheal patients in Bolivia were resistant to aminobenzil-penicillin (ABPC) and trimethoprim, and it was concluded because these resistant properties obtained from repeated usage of ABPC and SXT by diarrhea patients (Utsunomiya et. al., 1995). *Klebsiella pneumoniae* and *E. coli* having a resistant property against oxyimino β-lacton were caused by repeated infection for ten years since it was confirmed that *Klebsiella* and *E. coli* were resistant to ceftazidine in 1971 (Schiappa, 1996). In the present study, carbenicillin, cephalothin, streptomycin, tetracycline and minocycline commonly showed resistance to the tested strains. Particularly, 90% of the isolates showed multi-resistant patterns of more than two antibiotics, and it originated from the abuse of antibiotics for inappropriate purposes.

It was reported that the resistant properties of antibiotics and plasmid DNA profile are related, and 60 MDa curing strain eliminated resistant properties against ampicillin, chloramphenicol, tetracycline (Baldini et al., 1983). Also, gene of the antibiotics resistant bacteria isolated from patients of urinary infection, pneumonia and meningitis in hospitals in Chicago and New York contained large sized plasmid. The function of 60 MDa plasmid DNA are diverse. Namely, 60 MDa plasmid is related to the responsibility for adherence of EPEC to the cell, causing specific tissue pathogenesis in the pig and is an essential virulence factor for causing a disease (Tzipori et al., 1989). Plasmid DNA is reported to be related to VT function (Yatsuyanagi et al., 1995). Xu et al. (1996) reported EAggEC, VT II, LT were involved in 60 MDa plasmid DNA. In this study, 60 MDa plasmid DNA was detected not only in EHEC and EPEC but also in ETEC isolates as stated above. Large plasmid DNA, 90 MDa and 120 MDa, were isolated in 7 strains (10.4%) and 14 strains (20.9%) among the food isolates, and in 13 strains (20.1%) and 8 strains (14.8%) among the clinical isolates, respectively. Plasmid DNA pattern has limitations as an epidemiological investigation tool because specificity is weak to identify isolates having a different molecular character. The reason is that plasmid DNA is transferred unstably and identified unspecifically. Thus, subtyping by PFGE have been used frequently for epidemiological analysis.

As PFGE can distinguish isolates having a different phenotype from isolates of the same serotype, it has been used for genotypic analysis of the various kinds of pathogenic bacteria. This procedure is very convenient to confirm genotypes since probes are not needed as in Southern hybridization. This method can be compared with the method of ribotyping (Chachaty et al., 1994; Kristjansson et al., 1994) for many bacteriological investigations. DNA restriction pattern by PFGE is characterized by consistent results between laboratories. When food-borne diseases caused by EHEC outbreak occurred in Japan, the subtyping of *E. coli* O157: H7 were enforced by PFGE. DNA was digested with *Xba*I restriction enzyme and electrophoresed, and then the isolates related with the outbreak of a group in 1996 were classified into 6 groups. However, profile of PFGE on total 1700 strains of O157: H7 isolated from sporadic occurrence and the outbreak of a group showed 200 patterns and the diversity of the genotype of *E. coli* O157: H7 (Izumiya et al., 1997). In order to clarify the epidemiological direction of the subtyping of *E. coli* strains by PFGE, PFGE pattern differed between foods and the clinical isolates, and did not have any genetic similarities, but diversities in genotypes. It was confirmed that PFGE patterns of pathogenic *E. coli* in this experiment differed from each other but had the same PFGE pattern were different mutually even if the serotype of the strains and occurrence in a sporadic outbreak were the same (Bohn and Karch, 1992). In CDC (USA) PFGE patterns against bacteria causing food poisoning were analyzed to examine a cause when food poisonings occurred, and these results were arranged systematically and used to for epidemiological problems. In this study, the subtyping by PFGE of the pathogenic *E. coli* isolated from one country was carried out in this experiment. These PFGE patterns expect to be used as the basic materials for an epidemiological investigation when an outbreak caused by pathogenic *E. coli* occur. From the basis of these results, pulseNet system against *L. monocytogenes*, *Salmonella*, *Yersinia enterocolitica*, *Staphyococcus aureus* isolated from one region except pathogenic *E. coli* must be achieved and information needs to be exchanged not only nationally, but also internationally for mutual search and isolation for the causative agent of outbreaks.

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[Received September 13, 1999; accepted October 9, 1999]