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Absence of *Escherichia coli* Phylogenetic Group B2 Strains in Humans and Domesticated Animals from Jeonnam Province, Republic of Korea▽

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Multiplex PCR analyses of DNAs from genotypically unique *Escherichia coli* strains isolated from the feces of 138 humans and 376 domesticated animals from Jeonnam Province, South Korea, performed using primers specific for the *chuA* and *yjaA* genes and an unknown DNA fragment, TSPE4.C2, indicated that none of the strains belonged to *E. coli* phylogenetic group B2. In contrast, phylogenetic group B2 strains were detected in about 17% (8 of 48) of isolates from feces of 24 wild geese and in 3% (3 of 96) of isolates obtained from the Yeongsan River in Jeonnam Province, South Korea. The distribution of *E. coli* strains in phylogenetic groups A, B1, and D varied depending on the host examined, and there was no apparent seasonal variation in the distribution of strains in phylogenetic groups among the Yeongsan River isolates. The distribution of four virulence genes (*eaeA*, *hlyA*, stx_1, and stx_2) in isolates was also examined by using multiplex PCR. Virulence genes were detected in about 5% (38 of 707) of the total group of unique strains examined, with 24, 13, 13, and 9 strains containing *hlyA*, *eaeA*, stx_2, and stx_1, respectively. The virulence genes were most frequently present in phylogenetic group B1 strains isolated from beef cattle. Taken together, results of these studies indicate that *E. coli* strains in phylogenetic group B2 were rarely found in humans and domesticated animals in Jeonnam Province, South Korea, and that the majority of strains containing virulence genes belonged to phylogenetic group B1 and were isolated from beef cattle. Results of this study also suggest that the relationship between the presence and types of virulence genes and phylogenetic groupings may differ among geographically distinct *E. coli* populations.

Escherichia coli is a normal inhabitant of the lower intestinal tract of warm-blooded animals and humans. While the majority of *E. coli* strains are commensals, some are known to be pathogenic, causing intestinal and extraintestinal diseases, such as diarrhea and urinary tract infections (42). Phylogenetic studies done using multilocus enzyme electrophoresis and 72 *E. coli* strains in the *E. coli* reference collection showed that *E. coli* strains can be divided into four phylogenetic groups (A, B1, B2, and D) (20, 41, 48). Recently, a potential fifth group (E) has also been proposed (11). Since multiplex PCR was developed for analysis of phylogenetic groups (6), a number of studies have analyzed a variety of *E. coli* strains for their phylogenetic group association (10, 12, 17, 18, 23, 54). Duriez et al. (10) reported the possible influence of geographic conditions, dietary factors, use of antibiotics, and/or host genetic factors on the distribution of phylogenetic groups among 168 commensal *E. coli* strains isolated from human stools from three geographically distinct populations in France, Croatia, and Mali. Random-amplified polymorphic DNA analysis of the intraspecies distribution of *E. coli* in pregnant women and neonates indicated that there was a correlation between the distribution of phylogenetic groups, random-amplified polymorphic DNA groups, and virulence factors (54). Moreover, based on comparisons of the distribution of *E. coli* phylogenetic groups among humans of different sexes and ages, it has been suggested that *E. coli* genotypes are likely influenced by morphological, physiological, and dietary differences (18). In addition, climate has also been proposed to influence the distribution of strains within *E. coli* phylogenetic groups (12). There are now several reports indicating that there is a potential relationship between *E. coli* phylogenetic groups, age, and disease. For example, *E. coli* isolates belonging to phylogenetic group B2 have been shown to predominate in infants with neonatal bacterial meningitis (27) and among urinary tract and rectal isolates (55). Also, Nowrouzian et al. (39) and Moreno et al. (37) reported that strains belonging to phylogenetic group B2 persisted among the intestinal microflora of infants and were more likely to cause clinical symptoms.

Boyd and Hartl (2) reported that among the *E. coli* strains in the *E. coli* reference and the diarrheagenic *E. coli* collections, strains in phylogenetic group B2 carry the greatest number of virulence factors, followed by those in group D. Virulence factors carried by group B2 strains are thought to contribute to

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their strong colonizing capacity; a greater number of virulence genes have been detected in resident strains than in transient ones (38). Moreover, a mouse model of extraintestinal virulence showed that phylogenetic group B2 strains killed mice at greater frequency and possessed more virulence determinants than strains in other phylogenetic groups, suggesting a link between phylogeny and virulence genes in *E. coli* extraintestinal infection (45). In contrast, Johnson and Kuskowski (25) suggested that a group B2 ancestral strain might have simply acquired virulence genes by chance and that these genes were vertically inherited by group members during clonal expansion. However, numerous studies published to date suggest that there is a relationship between the genomic background of phylogenetic group B2 and its association with virulence factors (12, 28, 35, 39, 45).

Both enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC, respectively) strains are among the most important food-borne pathogens worldwide, often causing severe gastrointestinal disease and fatal infections (13). While EPEC strains cause diarrhea and generally do not produce enterotoxin, they possess an adherence factor which is controlled by the chromosomal gene *eaeA*, encoding intimin (8). Unlike the EPEC strains, however, the EHEC strains typically contain the *hlyA*, stx_1, and stx_2 virulence genes, encoding hemolysins and Shiga-like type 1 and 2 toxins, respectively, and *eaeA*. The ability to detect EHEC has been greatly facilitated by the use of multiplex PCR (13, 44, 53). Several studies have shown that strains producing Shiga-like toxin 2 are more frequently found in cases of hemolytic-uremic syndrome than are those containing Shiga-like toxin 1 (30, 43, 46, 49).

In the study reported here, we examined the distribution of phylogenetic groups and the prevalence of virulence genes in 659 genotypically unique *E. coli* strains isolated from humans and domestic animals in South Korea. In addition, we also tested 48 and 96 nonunique *E. coli* isolates from wild geese and the Yeongsan River, respectively, for phylogenetic distribution and virulence gene profiles. Here, we report that contrary to what has been previously reported in other parts of the world, no *E. coli* strains belonging to phylogenetic group B2 were found in domesticated animals and in humans from Jeonnam Province, South Korea. We also report that among the strains we examined, virulence genes were mainly found in phylogenetic group B1 strains isolated from beef cattle. Results of these studies may prove to be useful for the development of risk management strategies to maintain public health.

MATERIALS AND METHODS

Isolation of *E. coli* from humans and domesticated animals, Jeonnam Province, South Korea. The sources of *E. coli* isolates, the number of isolates obtained from each source, and the number of individual hosts sampled are listed in Table 1. The human isolates were obtained from randomly selected stool samples collected from healthy humans, and patient isolates were obtained in August 2008 from diarrheic patients at a hospital located in Jeonnam Province, South Korea. The data obtained from studies done with *E. coli* isolates from healthy humans and from patients with diarrhea were analyzed separately. The *E. coli* strains from domesticated animals were obtained in May 2006 by using fecal swabs from chickens, ducks, swine, and beef and dairy cattle collected at farms in Jeonnam Province, South Korea. According to the Korea Food and Drug Administration, antibiotics, such as tetracycline and penicillin, are regularly fed to domesticated animals as feed additives (31). Wild goose isolates were obtained from fecal swabs collected in December 2007 in Jeonnam Province, South Korea, where migrating birds from Siberia rest every winter. Fecal swabs were stored in tubes on ice and streaked within 6 h of collection onto mFC agar (Difco, Detroit, MI) plates (which contain 5 g of proteose peptone, 10 g of tryptose, 3 g of yeast extract, 5 g of sodium chloride, 1.5 g of bile salts, 12.5 g of lactose, 0.1 g of lactose, 0.1 g of methyl blue, 0.1 g of rosolic acid, and 15 g of agar per liter) and incubated at 44.5°C for 18 h. Subsequently, three to five blue colonies appearing on mFC agar plates, per fecal sample, were further streaked for purification onto mFC agar plates and incubated overnight at 44.5°C. All isolates were verified as previously described (9) to be *E. coli* and preserved at −70°C in LB freezing buffer (47).

TABLE 1. Sources and numbers of *E. coli* isolates used in this study

Source	No. of individual hosts sampled	No. of isolates	No. of unique strains
Human	122	442	141
Patient	16	83	21
Chicken	57	154	60
Duck	93	220	139
Beef cattle	71	266	106
Dairy cattle	38	194	53
Swine	117	226	139
Wild geese	24	48	ND[b]
Freshwater[a]		96	ND

[a] Samples were taken four times per year.
[b] ND, not determined.

Isolation of *E. coli* from Yeongsan River, Jeonnam Province, South Korea. One site on the Yeongsan River, in Jeonnam Province, South Korea, was selected for these studies. The site is a part of a tributary upstream from the Yeongsan River and surrounded by an urbanized area. Environmental *E. coli* strains were obtained using the membrane filtration technique according to U.S. Environmental Protection Agency method 1603 (52). Briefly, 500 ml of surface water was sampled every 3 months from November 2007 to August 2008, and 100-ml, 10-ml, and 1-ml aliquots of surface water were individually filtered onto the surface of 0.45-μm-pore-size membranes (Advantec, Tokyo, Japan). Filtrates were incubated on modified mTEC (membrane-thermotolerant *E. coli*) agar (Difco, Detroit, MI) plates at 35°C for the first 2 h and then at 44.5°C for 16 h. Red or magenta colonies were considered to be *E. coli*, and 24 randomly selected *E. coli* isolates were further streaked and incubated under the same conditions and used for subsequent species verification as described above.

HFERP DNA fingerprinting. Horizontal fluorophore-enhanced repetitive extragenic palindromic-PCR (HFERP) DNA fingerprinting of the *E. coli* strains was done as described previously (29). Briefly, a loopful of bacteria from each strain was suspended in 0.05 N NaOH for 15 min at 95°C, and 1 μl was used as a template for PCR. The HFERP DNA fingerprinting was done using BOXA1R primers labeled with 6-carboxyfluorescein (Genotech Co. Ltd., South Korea) as previously described (29). All gel lanes contained Genescan-2500 6-carboxy-X-rhodamine (Applied Biosystems, Foster City, CA) as an internal size standard. Gel images were captured using a Typhoon 9400 variable mode imager (Molecular Dynamics/Amersham Biosciences, Sunnyvale, CA) using the fluorescence acquisition mode, with the following settings: green excitation laser, 30-nm bandpass filter centered at 610 nm and 526-nm short-pass emission filter in the autolink mode, normal sensitivity, 200-μm/pixel scan resolution, +3-mm focal plane, and 800 V power. Scanned images of HFERP DNA fingerprints were processed using Image Quant (Molecular Dynamics/Amersham Biosciences, Sunnyvale, CA) and converted to 256 grayscale tagged-image file format images. Gel images were normalized and analyzed using Bionumerics, version 5.0, software (Applied Maths, Sint-Martens-Latem, Belgium). Isolates which showed ≥92% similarity from the same host were considered to be clones and were removed from further analyses (22, 29). The percentages of known phylogenetic group strains assigned to their correct phylogenetic groups were calculated by using Jackknife analysis with maximum similarities.

Phylogenetic grouping and virulence gene identification. Only unique strains defined by the HFERP DNA fingerprint analysis were subjected to analyses of phylogenetic groups and virulence gene identification. Phylogenetic grouping was done as previously described by Clermont et al. (6). The presence of the *ibeA* gene (invasion of brain epithelium) among Clermont phylogenetic group D strains harboring the $chuA^+$, *yjaA* mutant, and $TSPE4.C2^+$ genes was examined as previously described (14).

The presence of virulence genes in *E. coli* strains was determined by using

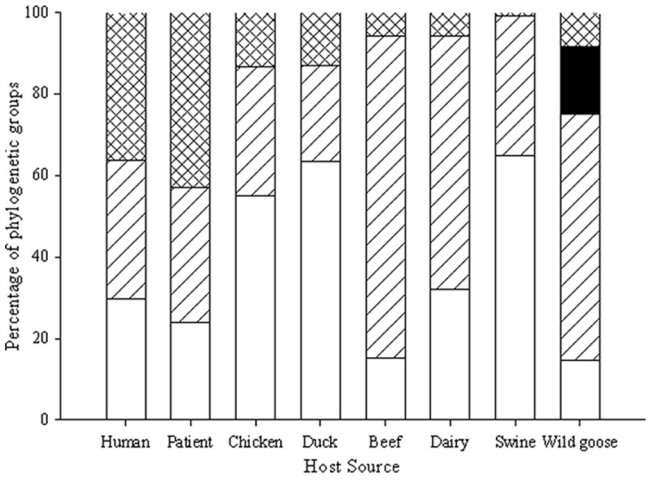


FIG. 1. Distribution of phylogenetic groups among *E. coli* isolates obtained from humans and domesticated animals: open bar, group A; diagonally hatched bar, group B1; black bar, group B2; cross-hatched bar, group D.

multiplex PCR as previously described (44). Genomic DNA from strains was extracted from cells as described above, diluted 10-fold in Tris-EDTA buffer, and 1 μl was used as template for multiplex PCR using a Labcycler (SensoQuest, City, Germany) instrument.

RESULTS AND DISCUSSION

Phylogenetic grouping patterns. A total of 1,585 *E. coli* isolates obtained from humans and domesticated animals were examined for their genetic relatedness by using HFERP DNA fingerprinting as described by Johnson et al. (29). Strains sharing the same individual host and having a genetic similarity of ≥92% in HFERP banding patterns were considered to be clones (29) and were removed from further analyses. Based on this definition, 659 strains were considered to be unique and were subjected to further phylogenetic grouping and virulence gene analyses. The *E. coli* isolates from migrating wild geese and the Yeongsan River, however, were not subjected to HFERP analysis to remove clones.

The distribution of phylogenetic groups among genomically unique *E. coli* isolates obtained from humans and animals is summarized in Fig. 1. The *E. coli* strains from each host source showed a different distribution pattern of phylogenetic groups. The *E. coli* strains from healthy humans were nearly equally represented in each phylogenetic group, with 29, 34, and 36% of the strains in phylogenetic groups A, B1, and D, respectively. There was a slightly greater number of isolates in phylogenetic group D (42.9%) from human patients than from the other phylogenetic groups (A, 23.8%; B1, 33.3%). The majority of *E. coli* isolates from chickens were localized to phylogenetic group A (55%), followed by strains in groups B1 (31.7%) and D (13.3%). A similar pattern of distribution was also found among isolates from domesticated ducks, where about 63, 24, and 13% of strains were in phylogenetic groups A, B1, and D, respectively. In contrast, *E. coli* isolates from beef cattle had the greatest percentage of group B1 strains (79.2%) among all sources and fewer isolates belonging to groups A (15.1%) and D (5.7%). A similar trend was observed among *E. coli* isolates from dairy cattle, where 62% of the isolates belonged to group B1 and a smaller percentage belonged to groups A (32.0%) and D (5.7%). Swine isolates showed a unique phylogenetic group distribution, with an extremely low percentage of group D (0.7%) strains, a relatively high percentage of group A (64.7%) strains, and a moderate percentage of group B1 (34.5%) strains. The phylogenetic group distribution of isolates from migrating wild geese was the most distinctive; the majority of isolates (60.4%) were in phylogenetic group B1, and 16.7, 14.6 and 8.3% of the remaining isolates were in phylogenetic groups B2, A, and D, respectively. It should be noted that the phylogenetic group distribution pattern seen among *E. coli* isolates from migrating wild geese was significantly different from that seen among isolates from domesticated chicken and duck, although the chicken and duck isolates showed similar phylogenetic distribution patterns. Taken together, results of these studies indicate that *E. coli* isolates belonging to phylogenetic group A were more frequently found in chickens, ducks, and swine, whereas those belonging to phylogenetic group B1 were predominantly found in isolates obtained from beef and dairy cattle. Results in Fig. 1 also show that there was a different distribution pattern of *E. coli* phylogenetic group D strains among humans and animals. While the majority of strains from healthy humans (36%) and patients (42.9%) belonged to phylogenetic group D, strains in this phylogenetic group generally comprised a small number of isolates obtained from all the animals, including wild geese.

Among the 659 genomically unique strains examined, 15 isolates (11, 2, 1, and 1 from healthy humans, ducks, chickens, and human patients, respectively) were found to be members of Clermont phylogenetic group D and carried the *chu*A$^+$, *yja*A mutant, and TSPE4.C2$^+$ genes. Recently, Gordon et al. (15) reported that strains having this genotype and containing the *ibeA* gene are likely members of phylogenetic group B2. PCR analyses done here indicated that 5 of the 15 isolates (3, 1, and 1 from healthy humans, a chicken, and a human patient, respectively) contained the *ibeA* gene. While this result suggested that these strains may possibly belong to phylogenetic group B2 as redefined by Gordon et al. (16), we propose to assign these five strains to phylogenetic group D until the method proposed by Gordon et al. is evaluated by other investigators using a larger number of geographically diverse isolates and becomes a more established method for the assignment of *E. coli* strains to phylogenetic groups.

In the study reported here, no *E. coli* Clermont phylogenetic group B2 strains, using the classical, accepted definition, were found among the isolates we obtained from humans or domesticated animals in South Korea (Fig. 1). This is not likely a methodological issue as the multiplex (triplex) PCR method used in this study was previously shown to correctly assign 95% of strains to phylogenetic groups B1 and B2, compared to the multilocus sequence typing method (16). Moreover, since phylogenetic group B2 strains were identified among the isolates examined from river water and migrating geese, this indicates that the methods used was sufficiently robust to detect strains in the group. Previously, Escobar-Páramo et al. (12) reported that the prevalence of phylogenetic group B2 isolates among individuals in temperate regions of mainland France, Michigan, and Tokyo was greater than that found among people in tropical populations from Bogotá (Columbia), Cotonou (Benin), and French Guyana. In contrast, our data show that the

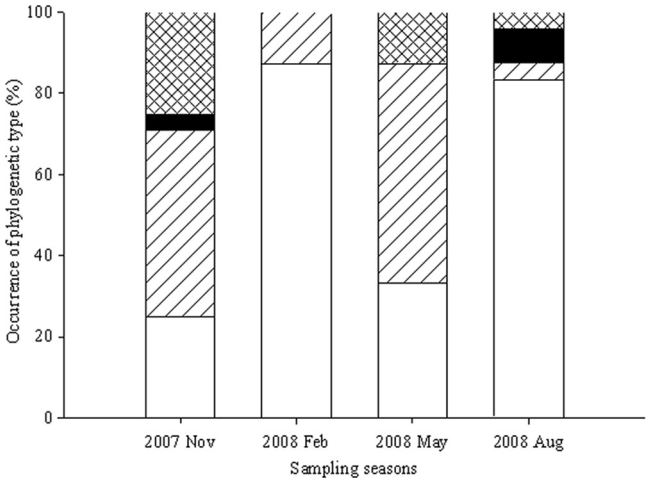


FIG. 2. Seasonal variations in phylogenetic group distribution among *E. coli* isolates obtained from the Yeongsan River, Jeonnam province, South Korea: open bar, group A; diagonally hatched bar, group B1; black bar, group B2; cross-hatched bar, group D.

phylogenetic group distribution for human isolates from Jeonnam Province, South Korea, was nearly equally divided among phylogenetic groups A (30%), B1 (34%), and D (36.2%). Interestingly, it was also previously reported that *E. coli* strains from humans in Tokyo were predominantly in phylogenetic group B2, and no B1 strains were present (40). The different phylogenetic group distributions among *E. coli* strains from Japan and Korea may be due to differences in dietary habits. Moreover, distributional differences among phylogenetic groups of human *E. coli* isolates are not static and were shown to change in response to geographic shifts in populations, which typically result in subsequent alterations to diet (49). For example, shifts in *E. coli* phylogenetic groups were found among 25 humans who expatriated from metropolitan France to French Guyana (50). These data suggest that there is a strong environmental influence on the phylogenetic group distribution of intestinal *E. coli* isolates in humans.

The phylogenetic distribution of human *E. coli* isolates may also be impacted by the use of antibiotics. It was previously reported that *E. coli* strains belonging to phylogenetic group B2 were less likely to be resistant to antibiotics than non-B2 group strains (24, 51). Skurnik et al. (51) reported that only 3.7% of group B2 strains carried integrons, whereas greater than 16% of strains from other phylogenetic groups did. Compared to other industrialized countries, the use of antibiotics in South Korea is quite extensive, with 33.2 defined daily doses/1,000 inhabitants/day. In contrast, the rate in countries belonging to the Organisation for Economic Co-operation and Development averaged 21.3 defined daily doses/1,000 inhabitants/day (34). Moreover, *E. coli* strains resistant to multiple antimicrobial substances are frequently observed in South Korea (4, 5, 36). Taken together, these factors may contribute to the absence of phylogenetic group B2 strains among the Korean human populations we examined.

In addition to animals and humans, the phylogenetic distribution of environmental *E. coli* isolates from the Yeongsan River, Jeonnam province, South Korea, was also examined during the four seasons of an entire year. The Yeongsan River water contained, on average, greater than 200 CFU of *E. coli* per ml in all seasons (data not shown). Results in Fig. 2 show the seasonal variation in the phylogenetic group distribution of *E. coli* strains in the Yeongsan River. Similar distribution patterns were seen in November 2007 and May 2008 samples. A high percentage of group B1 strains was found in both the November 2007 and May 2008 samples (45.8% and 54.2%, respectively) while a smaller percentage of strains was shown

TABLE 2. The occurrence of *E. coli* strains with virulence genes and phylogenetic groups

Source	Phylogenetic group	Unique strain data			
		No. of strains	No. of strains with virulence genes	% Strains with virulence genes (relative to the total for the source)	% Strains with virulence genes
Healthy human	A	42	0	0	0
	B1	48	2	1.4	4.2
	D	51	1	0.7	2.0
Patient	A	5	0	0	0
	B1	7	1	4.8	14.3
	D	9	0	0	0
Chicken	A	33	1	1.7	3.0
	B1	19	0	0	0
	D	8	0	0	0
Duck	A	88	0	0	0
	B1	33	0	0	0
	D	18	0	0	0
Beef cattle	A	16	2	1.9	12.5
	B1	84	17	15.7	20.2
	D	6	1	0.9	16.7
Dairy cattle	A	17	4	7.6	23.5
	B1	33	5	9.4	15.2
	D	3	0	0	0
Swine	A	90	2	1.4	2.2
	B1	48	2	1.4	4.2
	D	1	0	0	0

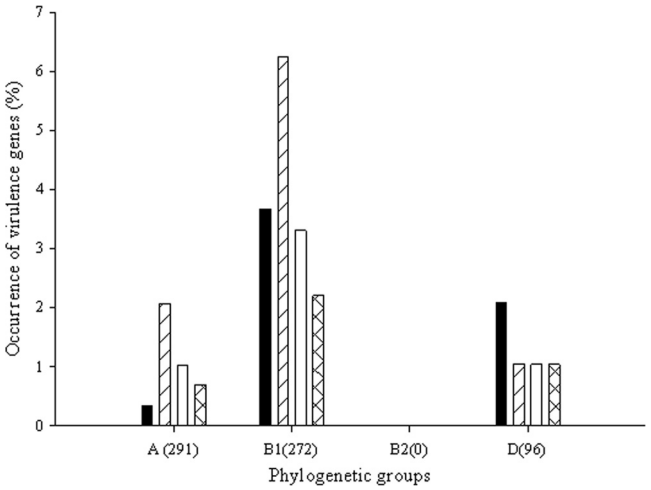


FIG. 3. Distribution of virulence genes among phylogenetic groups of *E. coli* obtained from humans and domesticated animals: black bar, *eaeA*; diagonally hatched bar, *hlyA*; open bar, stx_1; cross-hatched bar, stx_2. Numbers of isolates are given in parentheses.

to comprise phylogenetic groups A (25.0% and 33.3%, respectively) and D (25.0% and 12.5%, respectively). In contrast, the February 2008 and August 2008 samples contained a high percentage of group A strains (87.5% and 83.3%, respectively). In contrast to what was found with *E. coli* isolates from humans and domesticated animals, *E. coli* strains in phylogenetic group B2 were detected in the November 2007 (4.2%) and August 2008 (8.3%) water samples. However, these strains were only infrequently isolated. Results in Fig. 1 also show that a greater percentage of *E. coli* strains obtained from chickens, ducks, and swine were in phylogenetic group A, whereas a high percentage of strains in group B1 were observed among *E. coli* isolates obtained from beef and dairy cattle and wild geese.

Virulence gene distribution. The occurrence and distribution patterns of virulence genes among the phylogenetic groups of unique *E. coli* isolates obtained from the various human and animal hosts is shown in Table 2. Of the 659 unique strains and the 48 wild goose and 96 freshwater isolates examined, only 38 strains (4.7%) from healthy humans, human patients, chickens, beef cattle, dairy cattle, and swine were found to contain virulence genes. Approximately 20% of the beef cattle isolates in phylogenetic group B1 (17 of 84 strains) were found to carry virulence genes, and 16.7 and 12.5% of the strains were in phylogenetic groups D and A, respectively. The pattern of distribution of virulence genes in dairy cattle differed from that of beef cattle. While 23.5% of dairy cattle strains containing virulence genes were in phylogenetic group A (4 out of 17 strains), 15.2% of the strains were in group B1 (5 out of 33 strains). None of the dairy cattle strains in phylogenetic group D contained virulence genes. Taken together, our results indicated that the percentage of *E. coli* strains carrying virulence genes was unequally distributed among sources and depended on both on the host source and the prevalence of strains in each phylogenetic group. For example, phylogenetic group B1 strains from each host source generally had a great percentage of strains carrying virulence genes, and those in group D had a lesser number.

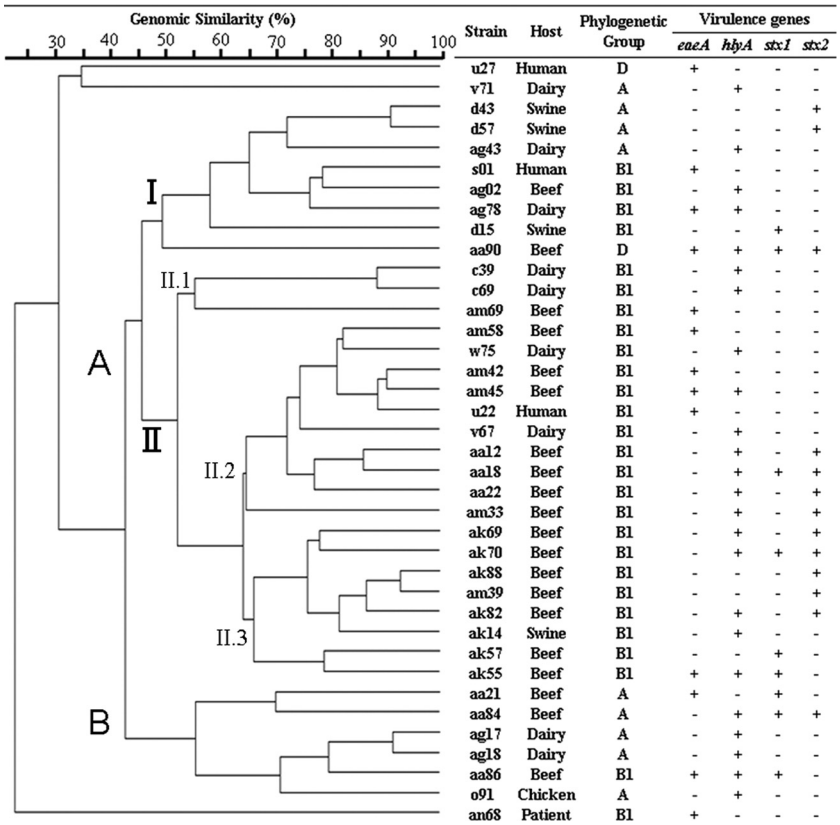
The greatest number of strains carrying virulence genes was found in phylogenetic group B1 strains obtained from beef cattle (15.7%), followed by group B1 strains from dairy cattle (9.4%). Ishii et al. (23) and Girardeau et al. (15) reported that Shiga-like toxin-producing *E. coli* strains segregated mainly into phylogenetic group B1. This is similar to the results we report here for isolates obtained from Jeonnam Province, South Korea. No strains from ducks or wild geese were found to contain virulence genes.

The distributional pattern of virulence genes tested in this study is shown in Fig. 3. The *eaeA* (encoding an attaching and effacing [A/E] protein, intimin, responsible for pathogenicity) was detected less in phylogenetic group A strains than in strains of the other groups (A, 0.34%; B1, 3.68%; and D, 2.08%), which is in agreement with results from a previous report (15). The intimin protein has been shown to be important for enterohemorrhagic infection of *E. coli* (1, 3, 19, 32, 33).

TABLE 3. Comparison of the virulence gene patterns in unique *E. coli* strains obtained from different human and domesticated animal sources

eaeA virulence pattern	Virulence gene pattern			No. of unique strains with virulence gene pattern found in the indicated source[a]								No. of strains with virulence gene profile (n = 705)[c]
	hlyA	stx_1	stx_2	Healthy humans (3/141)	Patients (1/21)	Chickens (1/60)	Ducks (0/139)	Beef cattle (20/104)	Dairy cattle (9/53)	Swine (4/139)	Wild geese (0/48)[b]	
+	+	+	+	0	0	0	0	1	0	0	0	1
+	+	+	−	0	0	0	0	2	0	0	0	2
+	+	−	−	0	0	0	0	1	1	0	0	2
+	−	+	−	0	0	0	0	1	0	0	0	1
+	−	−	−	3	1	0	0	3	0	0	0	7
−	+	+	+	0	0	0	0	3	0	0	0	3
−	+	−	−	0	0	1	0	1	8	1	0	11
−	+	−	+	0	0	0	0	5	0	0	0	5
−	−	+	−	0	0	0	0	1	0	1	0	2
−	−	−	+	0	0	0	0	2	0	2	0	4
−	−	−	−	138	20	59	139	84	44	135	48	667

[a] Values in parentheses represent the number of strains with virulence genes/number of unique strains tested.
[b] Clonal isolates not removed.
[c] n, total number of strains with virulence gene profiles.



Strain	Host	Phylogenetic Group	Virulence genes			
			eaeA	*hlyA*	*stx1*	*stx2*
u27	Human	D	+	-	-	-
v71	Dairy	A	-	+	-	-
d43	Swine	A	-	-	-	+
d57	Swine	A	-	-	-	+
ag43	Dairy	A	-	+	-	-
s01	Human	B1	+	-	-	-
ag02	Beef	B1	-	+	-	-
ag78	Dairy	B1	+	+	-	-
d15	Swine	B1	-	-	+	-
aa90	Beef	D	+	+	+	+
c39	Dairy	B1	-	+	-	-
c69	Dairy	B1	-	+	-	-
am69	Beef	B1	+	-	-	-
am58	Beef	B1	+	-	-	-
w75	Dairy	B1	-	+	-	-
am42	Beef	B1	+	-	-	-
am45	Beef	B1	+	+	-	-
u22	Human	B1	+	-	-	-
v67	Dairy	B1	-	+	-	-
aa12	Beef	B1	-	+	-	+
aa18	Beef	B1	-	+	+	+
aa22	Beef	B1	-	+	-	+
am33	Beef	B1	-	+	-	+
ak69	Beef	B1	-	+	-	+
ak70	Beef	B1	-	+	+	+
ak88	Beef	B1	-	-	-	+
am39	Beef	B1	-	-	-	+
ak82	Beef	B1	-	+	-	+
ak14	Swine	B1	-	+	-	-
ak57	Beef	B1	-	-	+	-
ak55	Beef	B1	+	+	+	-
aa21	Beef	A	+	-	+	-
aa84	Beef	A	-	+	+	+
ag17	Dairy	A	-	+	-	-
ag18	Dairy	A	-	+	-	-
aa86	Beef	B1	+	+	+	-
o91	Chicken	A	-	+	-	-
an68	Patient	B1	+	-	-	-

FIG. 4. Genetic relatedness of *E. coli* strains possessing virulence genes. The dendrogram was generated from HFERP DNA fingerprints using Pearson's product-moment correlation coefficient and the unweighted pair group method with arithmetic means clustering method.

The *eaeA* gene has also been used to detect a chromosomally localized pathogenicity island, referred to as the locus of enterocyte effacement, and strains containing *eaeA* and lacking stx_1 and stx_2 are referred to as EPEC (21). In our studies, potential EPEC strains were detected in 2.2, 4.7, and 2.8% of isolates from healthy humans, human patients, and beef cattle, respectively, while potential EHEC strains ($eaeA^+$ stx^+) were detected in 4.7 and 1.9% of strains from beef and dairy cattle, respectively (Table 3). By far, the greatest percentage of strains containing *eaeA*, *hlyA*, stx_1, and stx_2 belonged to phylogenetic group B1 (Fig. 3). Genes encoding stx_1 and stx_2 were found in 2.5% (18 out of 707) of the strains examined, and the greatest number of *E. coli* strains carrying virulence genes were seen in the beef (18.9%; 20 out of 104 strains) and dairy (17.0%; 9 out of 53) cattle isolates (Table 3). Similar percentages of Shiga-like toxin-producing *E. coli* strains were reported to be present among *E. coli* isolates obtained from cattle fecal material in Germany (56) and Australia (7).

Population structure of *E. coli* strains obtained from human and domesticated animal hosts. The genetic relatedness of the unique *E. coli* strains containing virulence genes is shown in Fig. 4. Generally speaking, the strains could be divided into two major groups (A and B) at the 45% similarity level. The group A strains could be further subdivided into two subclusters, I and II. Subcluster II strains were further subdivided into three subgroups (II.1, II.2, and II.3). The subgroup II.2 and II.3 strains were separated at the 63% similarity level and comprised 47% (18 out of 38) of the analyzed strains. Regardless of host and virulence profiles, 47% of the strains (18 of 38) were related to each other at a level of ≥80% similarity. Moreover, 22% (6 out of 27) of the phylogenetic group B1 strains were clustered at a similarity level of ≥88%. The majority (71.1%) of strains carrying virulence genes belonged to phylogenetic group B1, and 71% (15 out of 21) of the cluster A, subgroup II, strains were from beef cattle.

The patterns of virulence gene profiles were not uniformly distributed among the strains examined by HFERP analysis. For example, while strains aa18, ak70, and aa84 shared the same virulence gene profile (*hlyA*, stx_1, and stx_2 positive), they were only distantly genetically related, with less than 70% similarity. It should also be noted that one phylogenetic group D strain carried all four virulence genes tested and was not genetically related to any of the strains carrying similar virulence genes.

TABLE 4. Assignment of unique strains to phylogenetic groups by using HFERP DNA fingerprint and Jackknife analyses

Assigned phylogenetic group	% *E. coli* isolates by group		
	A	B1	D
A	73.2	18.4	22.9
B1	19.2	75.7	19.8
D	7.6	5.9	57.3

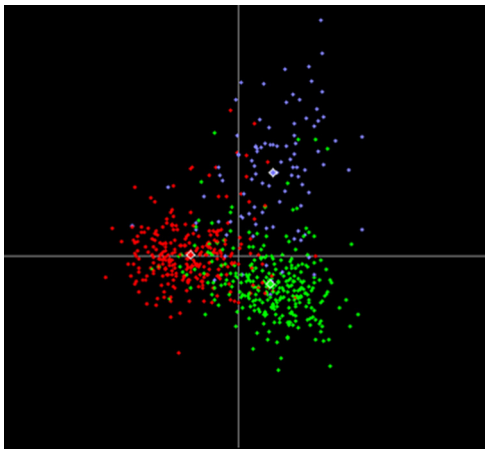


FIG. 5. Phylogenetic grouping analysis of HFERP DNA fingerprints using MANOVA: green, group A; red, group B1; purple, group D HFERP DNA fingerprints from *E. coli* strains obtained from animal and human sources were numerically converted to binary band-matching character tables and analyzed by MANOVA accounting for the covariance structure.

Multivariate analysis of variance (MANOVA) was used to determine if the HFERP DNA fingerprint patterns of strains could be used to differentiate phylogenetic groups. The percentage of strains correctly classified into each group was determined by using Jackknife analysis (Table 4). Results shown in Fig. 5 and Table 4 indicate that cluster analysis separated the strains into three groups which did not correlate well with phylogenetic groupings. Approximately 70 to 75% of group A and B1 strains were correctly assigned to their respective phylogenetic groups, whereas about 20% of these strains were misclassified. The phylogenetic group D strains showed the lowest percentage of correct assignment (57.3%).

Conclusions. A total of 659 genomically unique *E. coli* isolates obtained from domesticated animals and humans were subjected to phylogenetic grouping analysis using multiplex PCR. Of the strains examined, 291, 272, and 96 isolates were assigned to phylogenetic groups A, B1, and D, respectively. No group B2 strains were found among *E. coli* isolated from feces of any of domesticated animals and or from humans from Jeonnam Province, South Korea. However, strains in phylogenetic group B2 were found in the isolates obtained from wild geese and Yeongsan River water. The clustering of strains by HFERP DNA fingerprint analysis did not correlate well with phylogenetic group designations made based on PCR analyses, and the method misclassified about 20% of group A and B1 strains and about 40% of group D strains. While it was also previously reported that BOX-PCR DNA fingerprinting may not be useful for differentiating strains within *E. coli* phylogenetic groups (26), the method has proven to be useful for strain-level discrimination, to cluster genetically similar *E. coli* ecotypes, and to differentiate sources and virotypes of *E. coli* (9, 23, 29).

The distribution of *E. coli* strains in the three phylogenetic groups varied depending on the animal host/source of the isolates; beef and dairy cattle isolates showed relatively similar distributional patterns of phylogenetic groups as did the duck and chicken isolates. Our data also support previous suggestions that diet and antibiotic usage may strongly influence the phylogenetic group distribution of *E. coli* strains (17, 18, 24, 51). Moreover, results from these studies indicate that the distribution of *E. coli* strains in phylogenetic groups may be strongly influenced by geographical boundaries. Therefore, further physiological and epidemiological studies are needed to clarify why phylogenetic group B2 strains are rare in South Korea.

More virulence genes were found in the South Korean phylogenetic group B1 strains we examined than in strains from the other phylogenetic groups. This suggests that these strains may either share a common ancestor or are subjected to intensive horizontal gene transfer and recombination events. The relatively frequent occurrence of *eaeA*-positive strains among beef cattle isolates suggests that further surveillance studies are required in order to properly assess risks associated with *E. coli* from different animal sources in Korea.

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REFERENCES

1. **Boerlin, P., S. Chen, J. K. Colbourne, R. Johnson, S. De Grandis, and C. Gyles.** 1998. Evolution of enterohemorrhagic *Escherichia coli* hemolysin plasmids and the locus for enterocyte effacement in Shiga toxin-producing *E. coli*. Infect. Immun. **66:**2553–2561.
2. **Boyd, E. F., and D. L. Hartl.** 1998. Chromosomal regions specific to pathogenic isolates of *Escherichia coli* have a phylogenetically clustered distribution. J. Bacteriol. **180:**1159–1165.
3. **China, B., E. Jacquemin, A. C. Devrin, V. Pirson, and J. Mainil.** 1999. Heterogeneity of the *eae* genes in attaching/effacing *Escherichia coli* from cattle: comparison with human strains. Res. Microbiol. **150:**323–332.
4. **Choi, C., H. J. Ham, D. Kwon, J. Kim, D. S. Cheon, K. Min, W. S. Cho, H. K. Chung, T. Jung, K. Jung, and C. Chae.** 2002. Antimicrobial susceptibility of pathogenic *Escherichia coli* isolated from pigs in Korea. J. Vet. Med. Sci. **64:**71–73.
5. **Chong, Y., and K. Lee.** 2000. Present situation of antimicrobial resistance in Korea. J. Infect. Chemother. **6:**189–195.
6. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
7. **Cobbold, R., and P. Desmarchelier.** 2000. A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian diary herds. Vet. Microbiol. **71:**125–137.
8. **Cocolin, L., M. Manzano, C. Cantoni, and G. Comi.** 2000. A multiplex-PCR method to detect enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* in artificially contaminated foods. Int. J. Hyg. Environ. Health **203:**159–164.
9. **Dombek, P. E., L. K. Johnson, S. T. Zimmerley, and M. J. Sadowsky.** 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. **66:**2572–2577.
10. **Duriez, P., O. Clermont, S. Bonacorsi, E. Bingen, A. Chaventre, J. Elion, B. Picard, and E. Denamur.** 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology **147:**1671–1676.
11. **Escobar-Paramo, P., O. Clermont, A. B. Blanc-Potard, H. Bui, C. Le Bouguenec, and E. Denamur.** 2004. A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. Mol. Biol. Evol. **21:**1085–1094.
12. **Escobar-Paramo, P., K. Grenet, A. Le Menac'h, L. Rode, E. Salgado, C. Amorin, S. Gouriou, B. Picard, M. C. Rahimy, A. Andremont, E. Denamur, and R. Ruimy.** 2004. Large-scale population structure of human commensal *Escherichia coli* isolates. Appl. Environ. Microbiol. **70:**5698–5700.
13. **Fagan, P. K., M. A. Hornitzky, K. A. Bettelheim, and S. P. Djordjevic.** 1999. Detection of Shiga-like toxin (stx_1 and stx_2), intimin (*eaeA*), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. Appl. Environ. Microbiol. **65:**868–872.

14. **Germon, P., Y. H. Chen, L. He, J. E. Blanco, A. Bree, C. Schouler, S. H. Huang, and M. Moulin-Schouleur.** 2005. *ibeA*, a virulence factor of avian pathogenic *Escherichia coli*. Microbiology **151:**1179–1186.
15. **Girardeau, J. P., A. Dalmasso, Y. Bertin, C. Ducrot, S. Bord, V. Livrelli, C. Vernozy-Rozand, and C. Martin.** 2005. Association of virulence genotype with phylogenetic background in comparison to different seropathotypes of Shiga toxin-producing *Escherichia coli* isolates. J. Clin. Microbiol. **43:**6098–6107.
16. **Gordon, D. M., O. Clermont, H. Tolley, and E. Denamur.** 2008. Assigning *Escherichia coli* strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. Environ. Microbiol. **10:**2484–2496.
17. **Gordon, D. M., and A. Cowling.** 2003. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiology **149:**3575–3586.
18. **Gordon, D. M., S. E. Stern, and P. J. Collignon.** 2005. Influence of the age and sex of human hosts on the distribution of *Escherichia coli* ECOR groups and virulence traits. Microbiology **151:**15–23.
19. **Gyles, C., R. Johnson, A. Gao, K. Ziebell, D. Pierard, S. Aleksic, and P. Boerlin.** 1998. Association of enterohemorrhagic *Escherichia coli* hemolysin with serotypes of Shiga-like toxin-producing *Escherichia coli* of human and bovine origins. Appl. Environ. Microbiol. **64:**4134–4141.
20. **Herzer, P. J., S. Inouye, M. Inouye, and T. S. Whittam.** 1990. Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. J. Bacteriol. **172:**6175–6181.
21. **Hornitzky, M. A., K. Mercieca, K. A. Bettelheim, and S. P. Djordjevic.** 2005. Bovine feces from animals with gastrointestinal infections are a source of serologically diverse atypical enteropathogenic *Escherichia coli* and Shiga toxin-producing *E. coli* strains that commonly possess intimin. Appl. Environ. Microbiol. **71:**3405–3412.
22. **Ishii, S., W. B. Ksoll, R. E. Hicks, and M. J. Sadowsky.** 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. Appl. Environ. Microbiol. **72:**612–621.
23. **Ishii, S., K. P. Meyer, and M. J. Sadowsky.** 2007. Relationship between phylogenetic groups, genotypic clusters, and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. Appl. Environ. Microbiol. **73:**5703–5710.
24. **Johnson, J. R., P. Goullet, B. Picard, S. L. Moseley, P. L. Roberts, and W. E. Stamm.** 1991. Association of carboxylesterase B electrophoretic pattern with presence and expression of urovirulence factor determinants and antimicrobial resistance among strains of *Escherichia coli* that cause urosepsis. Infect. Immun. **59:**2311–2315.
25. **Johnson, J. R., and M. Kuskowski.** 2000. Clonal origin, virulence factors, and virulence. Infect. Immun. **68:**424–425.
26. **Johnson, J. R., and T. T. O'Bryan.** 2000. Improved repetitive-element PCR fingerprinting for resolving pathogenic and nonpathogenic phylogenetic groups within *Escherichia coli*. Clin. Diagn. Lab. Immunol. **7:**265–273.
27. **Johnson, J. R., E. Oswald, T. T. O'Bryan, M. A. Kuskowski, and L. Spanjaard.** 2002. Phylogenetic distribution of virulence-associated genes among *Escherichia coli* isolates associated with neonatal bacterial meningitis in the Netherlands. J. Infect. Dis. **185:**774–784.
28. **Johnson, J. R., and A. L. Stell.** 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.
29. **Johnson, L. K., M. B. Brown, E. A. Carruthers, J. A. Ferguson, P. E. Dombek, and M. J. Sadowsky.** 2004. Sample size, library composition, and genotypic diversity among natural populations of *Escherichia coli* from different animals influence accuracy of determining sources of fecal pollution. Appl. Environ. Microbiol. **70:**4478–4485.
30. **Kleanthous, H., H. R. Smith, S. M. Scotland, R. J. Gross, B. Rowe, C. M. Taylor, and D. V. Milford.** 1990. Haemolytic uraemic syndromes in the British Isles, 1985-8: association with verocytotoxin producing *Escherichia coli*. Part 2: Microbiological aspects. Arch. Dis. Child. **65:**722–727.
31. **Korea Food and Drug Administration.** 2003. Report on national antimicrobial resistance control. South Korea Food and Drug Administration, Seoul, South Korea.
32. **Kudva, I. T., K. Blanch, and C. J. Hovde.** 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl. Environ. Microbiol. **64:**3166–3174.
33. **Kudva, I. T., P. G. Hatfield, and C. J. Hovde.** 1997. Characterization of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* serotypes isolated from sheep. J. Clin. Microbiol. **35:**892–899.
34. **Lee, E., J. Bae, and K. Park.** 2000. Drug use evaluation. Korea Institute for Health and Social Affairs, Seoul, South Korea. (In Korean.)
35. **Le Gall, T., O. Clermont, S. Gouriou, B. Picard, X. Nassif, E. Denamur, and O. Tenaillon.** 2007. Extraintestinal virulence is a coincidental by-product of commensalism in B2 phylogenetic group *Escherichia coli* strains. Mol. Biol. Evol. **24:**2373–2384.
36. **Lim, S. K., H. S. Lee, H. M. Nam, Y. S. Cho, J. M. Kim, S. W. Song, Y. H. Park, and S. C. Jung.** 2007. Antimicrobial resistance observed in *Escherichia coli* strains isolated from fecal samples of cattle and pigs in Korea during 2003–2004. Int. J. Food Microbiol. **116:**283–286.
37. **Moreno, E., I. Planells, G. Prats, A. M. Planes, G. Moreno, and A. Andreu.** 2005. Comparative study of *Escherichia coli* virulence determinants in strains causing urinary tract bacteremia versus strains causing pyelonephritis and other sources of bacteremia. Diagn. Microbiol. Infect. Dis. **53:**93–99.
38. **Nowrouzian, F. L., I. Adlerberth, and A. E. Wold.** 2006. Enhanced persistence in the colonic microbiota of *Escherichia coli* strains belonging to phylogenetic group B2: role of virulence factors and adherence to colonic cells. Microbes Infect. **8:**834–840.
39. **Nowrouzian, F. L., A. E. Wold, and I. Adlerberth.** 2005. *Escherichia coli* strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. J. Infect. Dis. **191:**1078–1083.
40. **Obata-Yasuoka, M., W. Ba-Thein, T. Tsukamoto, H. Yoshikawa, and H. Hayashi.** 2002. Vaginal *Escherichia coli* share common virulence factor profiles, serotypes and phylogeny with other extraintestinal *E. coli*. Microbiology **148:**2745–2752.
41. **Ochman, H., and R. K. Selander.** 1984. Standard reference strains of *Escherichia coli* from natural populations. J. Bacteriol. **157:**690–693.
42. **Orskov, F., and I. Orskov.** 1992. *Escherichia coli* serotyping and disease in man and animals. Can. J. Microbiol. **38:**699–704.
43. **Ostroff, S. M., P. I. Tarr, M. A. Neill, J. H. Lewis, N. Hargrett-Bean, and J. M. Kobayashi.** 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J. Infect. Dis. **160:**994–998.
44. **Paton, A. W., and J. C. Paton.** 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{O111}, and rfb_{O157}. J. Clin. Microbiol. **36:**598–602.
45. **Picard, B., J. S. Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen, J. Elion, and E. Denamur.** 1999. The link between phylogeny and virulence in Escherichia coli extraintestinal infection. Infect. Immun. **67:**546–553.
46. **Proulx, F., E. G. Seidman, and D. Karpman.** 2001. Pathogenesis of Shiga toxin-associated hemolytic uremic syndrome. Pediatr. Res. **50:**163–171.
47. **Sambrook, J., and W. D. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
48. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51:**873–884.
49. **Siegler, R. L., T. G. Obrig, T. J. Pysher, V. L. Tesh, N. D. Denkers, and F. B. Taylor.** 2003. Response to Shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. Pediatr. Nephrol. **18:**92–96.
50. **Skurnik, D., D. Bonnet, C. Bernede-Bauduin, R. Michel, C. Guette, J. M. Becker, C. Balaire, F. Chau, J. Mohler, V. Jarlier, J. P. Boutin, B. Moreau, D. Guillemot, E. Denamur, A. Andremont, and R. Ruimy.** 2008. Characteristics of human intestinal *Escherichia coli* with changing environments. Environ. Microbiol. **10:**2132–2137.
51. **Skurnik, D., A. Le Menac'h, D. Zurakowski, D. Mazel, P. Courvalin, E. Denamur, A. Andremont, and R. Ruimy.** 2005. Integron-associated antibiotic resistance and phylogenetic grouping of *Escherichia coli* isolates from healthy subjects free of recent antibiotic exposure. Antimicrob. Agents Chemother. **49:**3062–3065.
52. **U.S. Environmental Protection Agency.** 2002. Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using membrane-thermotolerant *Escherichia coli* agar (modified mTEC). EPA-821-R-02-023. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
53. **Wang, G., C. G. Clark, and F. G. Rodgers.** 2002. Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. J. Clin. Microbiol. **40:**3613–3619.
54. **Watt, S., P. Lanotte, L. Mereghetti, M. Moulin-Schouleur, B. Picard, and R. Quentin.** 2003. *Escherichia coli* strains from pregnant women and neonates: intraspecies genetic distribution and prevalence of virulence factors. J. Clin. Microbiol. **41:**1929–1935.
55. **Zhang, L., B. Foxman, and C. Marrs.** 2002. Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. J. Clin. Microbiol. **40:**3951–3955.
56. **Zschock, M., H. P. Hamann, B. Kloppert, and W. Wolter.** 2000. Shiga toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. Lett. Appl. Microbiol. **31:**203–208.