Fecal Carriage of Antimicrobial-Resistant Enterobacteriaceae in Healthy Korean Adults

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The spread of antibiotic-resistant Enterobacteriaceae in the community is one of the main challenges for antibiotic treatment of community-onset infections. We evaluated the microbiological and molecular characteristics of stool samples from adults with comprehensive health examinations. Of 109 fecal samples, bacterial growth was observed in 86 samples and 61 gram-negative bacterial isolates were identified, of which 45 were *Escherichia coli* isolates (73.8%). Two isolates of *Raoultella* showed imipenem resistance, and both *E. coli* and *Citrobacter freundii* showed intermediate resistance to imipenem. Colistin resistance was identified in isolates of *Klebsiella variicola* and *Salmonella subterranean*, but no isolates carried *mcr-1*. As for *E. coli* genotypes, 35 sequence types were identified. *bla*_{TEM-1}, *bla*_{TEM-30}, and *bla*_{CTX-M} were identified in 15, 1, and 4 *E. coli* isolates, respectively. In addition, all four *Klebsiella pneumoniae* isolates carried *bla*_{SHV}. Many genotypes that have been identified in isolates causing human infections were found in isolates in this study. There is a need to control the rise and spread of antibiotic-resistant pathogens by fecal carriage.

Keywords: Enterobacteriaceae, extended-spectrum β -lactamase (ESBL), CTX-M

Introduction

The growing trend of antimicrobial resistance is a major concern in Asian countries, including Korea. An extended-spectrum β -lactamase (ESBL) enzyme pandemic emerged in the community in the early 2000s and was presented as asymptomatic fecal carriage of ESBL-producing Enterobacteriaceae (ESBL-E), posing a major health concern worldwide; thus, the spread of ESBL-E in the community has become one of the main challenges for antibiotic treatment of community-onset infections [1]. In Korea, prior investigation had shown a high prevalence (20%) of fecal carriage of ESBL-E in the community [2]. Although specific clones such as ST131 may play a role in the pandemic [3], the molecular epidemiology of ESBL-E in the community has rarely been scrutinized in Korea.

Carbapenems are antibiotics used for the treatment of

infections known or suspected to be caused by ESBL-E in the community. The widespread use of carbapenems, however, subsequently resulted in the emergence of communitywide carriage of imipenem-resistant gram-negative bacteria [4] and the spread of carbapenemase-producing Enterobacteriaceae [5]. Furthermore, the spread of carbapenem-resistant pathogens in the community led to an increase in the use of colistin-methanesulfonate. Polymyxin antibiotics including colistin are antimicrobial polypeptides, mediating bactericidal activity by interacting with the lipid A component of the lipopolysaccharide (LPS) present on gram-negative pathogens. Following colistin exposure, colistin-resistant *Klebsiella pneumoniae* in fecal samples of healthy individuals has been emerged worldwide [6]. Nowadays, a new plasmid-mediated colistin resistance in Enterobacteriaceae involving the *mcr-1* gene has been reported in animals and humans [7]. In Korea, there is no

report describing imipenem- and colistin-resistant Enterobacteriaceae in stool samples. Therefore, we intended to evaluate the prevalence of fecal carriage of antibiotic-resistant Enterobacteriaceae in healthy Korean adults who underwent medical examinations. In addition, we analyzed the molecular characteristics of ESBL-E and gram-negative bacteria with reduced susceptibility to imipenem and colistin. There is a need for effective infection prevention and control measures and strict use of antibiotics to control the rise and spread of fecal carriage of antibiotic-resistant pathogens.

Materials and Methods

Collection of Stool Samples

Our study population included participants, 18 years or older, who underwent comprehensive health examinations at Kangbuk Samsung Hospital in Seoul, Korea. The current analysis included subjects who consented to donate their stool samples between June and September 2014. Stool donors included in the study were selected after applying the following exclusion criteria: (i) use of antibiotics within 6 weeks and (ii) intake of probiotics within 4 weeks that might have influenced the gut environment.

This study was approved by the Institutional Review Board at Kangbuk Samsung Hospital (IRB No. 2014-08-035) and the requirement for informed consent was waived as we used remaining stool samples without any personal data.

Gram-Negative Rod Isolates

Stool samples were placed in 1 ml of sterile phosphate-buffered saline and vortexed. Then, one loopful of the suspension was plated on Luria-Bertani (LB) agar medium. The plates were incubated at 37°C, and surviving colonies were plated on MacConkey agar medium to select gram-negative organisms. To identify species that formed colonies on MacConkey agar medium, we performed 16S rRNA gene sequence analysis using the primer set fD1/rp2 [8]. When necessary, *rpoB* gene sequences were determined and utilized for species identification [9].

Antimicrobial Susceptibility Testing of Imipenem and Colistin

Antimicrobial susceptibility was determined using the modified broth microdilution method for seven antimicrobial agents (imipenem, colistin, cefepime, gentamicin, ciprofloxacin, tetracycline, and rifampin), and the results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institutes (CLSI) [10]. Susceptibility was defined according to CLSI breakpoints. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as control strains. The isolates were screened for the production of carbapenemases and ESBLs using a carbapenemase-inhibition test with EDTA and a double-disc synergy test with BD BBL Sensi-Disks (Becton, Dickinson and Company, USA), respectively.

Screening of β -Lactamase Genes and Genotyping

All of the imipenem-resistant isolates were screened by PCR for *bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM}, *bla*_{GIM}, *bla*_{SIM}, *bla*_{NDM}, and *bla*_{KPC} [11]. The presence of genes responsible for ESBL activity, *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} was also screened by PCR and the genes were sequenced [12]. For imipenem-resistant *Raoultella* spp. isolates, *bla* genes were screened. For all *E. coli* and *K. pneumoniae* isolates, we performed multilocus sequencing typing (MLST), as described previously [13, 14]. If the isolates from the same sample showed the same sequence type (ST), we considered them as a single isolate. For 45 *E. coli* isolates, we also performed pulsed-field gel electrophoresis (PFGE) as previously described [15]. A dendrogram was constructed based on the Dice similarity coefficient and unweighted-pair group method with arithmetic averages (UPGMA), using BioNumerics ver. 5.1 (Applied Maths, USA). Three isolates (477-2, 482-2, and 753-2) could not be analyzed, because they were not revived.

Results

Isolation of Gram-Negative Bacteria

A total of 1,417 fecal samples were collected during the study period. Of these, we randomly selected 109 samples for bacterial isolation and tried to incubate them on LB agar medium. Bacterial growth was observed in 86 samples (78.9%) and we isolated a total of 125 different colonies. For selection of gram-negative bacterial isolates, the colonies were plated on MacConkey agar medium and 64 gram-negative bacterial isolates were obtained.

Species Identification

As a result of species identification, 48 isolates were identified as *E. coli* (75%) (Table 1). Nine *Klebsiella* spp. isolates (four *K. pneumoniae*, three *K. oxytoca*, and two *K. variicola*) were identified. Two non-duplicate *Enterobacter cloacae* isolates were recovered, and isolates of *Escherichia hermannii*, *Citrobacter freundii*, *Raoultella planticola*, *Raoultella ornithinolytica*, and *Salmonella subterranean* were identified.

From sample #1, one *K. pneumoniae* and two *E. coli* isolates were recovered (Table 1). *E. coli* and *K. pneumoniae* isolates were also identified simultaneously from another sample (#54). From sample #412, both *E. coli* and *K. oxytoca* were recovered, and *C. freundii* and *R. planticola* were identified from sample #458. *E. hermannii* and *S. subterranean* were recovered simultaneously from sample #754. In addition, different strains of *E. coli* were identified in #7, #82, #419, and #486.

Prevalence of β -Lactamase-Producing Isolates

*bla*_{TEM-1} and *bla*_{TEM-30} were identified in 15 and 1 (isolate

Table 1. Antimicrobial resistance, β -lactamase gene, and genotype of *E. coli* isolates.

Species (isolate #)	MIC (mg/l) ^a							β -Lactamase gene	ST (allele #) ^b	Clonal complex
	IMI	COL	CPM	GEN	CIP	TET	RIF			
62-1	1	2	≤ 0.06	1	≤ 0.06	>64 (R)	16 (R)		10 (10-11-4-8-8-8-2)	
82-2	2 (I)	1	≤ 0.06	64 (R)	0.25	1	16 (R)	TEM-1, CTX-M-14	10 (10-11-4-8-8-8-2)	
477-1	0.25	1	≤ 0.06	2	>64 (R)	64 (R)	16 (R)	TEM-1	10 (10-11-4-8-8-8-2)	
477-2	0.25	1	≤ 0.06	2	0.5	0.5	8 (R)	TEM-1	10 (10-11-4-8-8-8-2)	
486-1	0.5	1	≤ 0.06	2	≤ 0.06	0.5	16 (R)		10 (10-11-4-8-8-8-2)	CC10
716-1	0.5	1	≤ 0.06	2	0.5	0.5	8 (R)		10 (10-11-4-8-8-8-2)	
442-1	1	1	≤ 0.06	>64 (R)	32 (R)	>64 (R)	32 (R)	TEM-1	744 (10-11-135-8-8-8-2)	
54-2	0.5	1	≤ 0.06	>64 (R)	0.5	1	8 (R)		Novel ^c (10-11-522-1-8-8-2)	
445-2	0.25	1	16 (R)	16 (R)	1	64 (R)	8 (R)		58 (6-4-4-16-24-8-14)	
1-2	1	1	≤ 0.06	1	≤ 0.06	1	8 (R)		971 (6-4-4-16-24-8-121)	
419-1	1	2	≤ 0.06	2	0.5	>64 (R)	16 (R)		767 (6-4-4-16-24-8-104)	CC58
435-2	0.5	2	≤ 0.06	1	≤ 0.06	1	16 (R)		155 (6-4-14-16-24-8-14)	
70-1	0.5	1	≤ 0.06	4	≤ 0.06	1	16 (R)		409 (10-11-1-1-71-8-6)	
482-1	0.5	1	≤ 0.06	2	≤ 0.06	1	16 (R)		1316 (10-214-1-1-71-8-6)	CC 409-1316
482-2	0.5	1	≤ 0.06	2	≤ 0.06	1	16 (R)		1316 (10-214-1-1-71-8-6)	
18-1	0.5	2	≤ 0.06	8 (I)	≤ 0.06	1	16 (R)	TEM-1	14 (14-14-10-14-17-7-10)	
1-3	1	1	≤ 0.06	1	≤ 0.06	1	8 (R)		1193 (14-14-10-200-17-7-10)	
97-1	1	1	>64 (R)	2	≤ 0.06	1	16 (R)	TEM-1, CTX-M-15	1193 (14-14-10-200-17-7-10)	CC 14-1193
424-1	1	1	≤ 0.06	2	≤ 0.06	1	16 (R)	TEM-1	1193 (14-14-10-200-17-7-10)	
448-1	1	2	≤ 0.06	2	>64 (R)	64 (R)	16 (R)	TEM-1	1193 (14-14-10-200-17-7-10)	
17-1	1	0.5	≤ 0.06	1	≤ 0.06	1	16 (R)		38 (4-26-2-25-5-5-19)	
82-1	1	1	16 (R)	32 (R)	2 (I)	>64 (R)	16 (R)	TEM-1	38 (4-26-2-25-5-5-19)	
86-2	1	1	>64 (R)	1	64 (R)	1	16 (R)	CTX-M-14	38 (4-26-2-25-5-5-19)	
20-1	0.5	2	≤ 0.06	2	0.5	64 (R)	16 (R)	TEM-1	69 (21-35-27-6-5-5-4)	
419-2	1	1	≤ 0.06	2	32 (R)	1	8 (R)	TEM-1	69 (21-35-27-6-5-5-4)	
753-1	1	1	≤ 0.06	2	64 (R)	1	16 (R)	TEM-30	95 (37-38-19-37-17-11-26)	
753-2	1	1	≤ 0.06	2	0.5	2	8 (R)	TEM-30	95 (37-38-19-37-17-11-26)	
417-1	1	1	64 (R)	2	64 (R)	>64 (R)	16 (R)	CTX-M-174	131 (53-40-47-13-36-28-29)	
35-1	0.25	2	≤ 0.06	2	≤ 0.06	>64 (R)	8 (R)		206 (6-7-5-1-8-18-2)	
31-2	0.5	0.5	≤ 0.06	4	≤ 0.06	>64 (R)	8 (R)		405 (35-37-29-25-4-5-73)	
5-1	1	1	≤ 0.06	2	≤ 0.06	>64 (R)	16 (R)		457 (101-88-97-108-26-79-2)	
7-1	0.5	2	≤ 0.06	2	0.5	2	8 (R)		710 (6-153-4-91-7-8-6)	Singleton
35-2	0.5	2	≤ 0.06	1	≤ 0.06	1	16 (R)		1072 (64-4-5-83-8-8-6)	
12-2	1	1	≤ 0.06	1	0.5	1	4 (R)	TEM-1	3570 (6-4-12-1-9-281-7)	
3-2	1	2	≤ 0.06	>64 (R)	≤ 0.06	>64 (R)	16 (R)	TEM-1	5148 (83-627-245-404-91-17-181)	
7-3	1	2	≤ 0.06	1	≤ 0.06	1	16 (R)		Novel ^c (6-23-12-1-466-7-7)	
52-2	0.5	1	0.12	4	0.5	>64 (R)	16 (R)	TEM-1	517 (109-65-5-1-9-13-14)	
58-1	1	1	≤ 0.06	1	64 (R)	1	16 (R)	TEM-1	Novel ^c (N ^c -14-526-200-17-7-10)	
66-2	0.5	1	≤ 0.06	1	≤ 0.06	1	16 (R)	TEM-1	174 (20-67-56-64-5-50-45)	
78-1	0.5	1	≤ 0.06	4	≤ 0.06	1	16 (R)		3075 (10-23-109-8-270-8-2)	
84-3	0.5	2	≤ 0.06	2	≤ 0.06	0.5	8 (R)		Novel ^c (6-4-1-95-69-N ^c -20)	
412-1	0.25	2	≤ 0.06	2	≤ 0.06	0.5	8 (R)		Novel ^c (479-4-1-95-69-8-20)	

Table 1. Continued.

Species (isolate #)	MIC (mg/l) ^a							β-Lactamase gene	ST (allele #) ^b	Clonal complex
	IMI	COL	CPM	GEN	CIP	TET	RIF			
426-3	1	1	≤0.06	2	≤0.06	1	8 (R)		1726 (6-23-4-1-9-26-2)	Singleton
447-1	0.5	1	≤0.06	2	≤0.06	1	8 (R)		162 (9-65-5-1-9-13-6)	
459-1	0.5	1	≤0.06	2	≤0.06	0.5	16 (R)		1727 (6-19-3-16-11-8-6)	
472-1	0.5	1	≤0.06	1	≤0.06	1	16 (R)		Novel ^c (10-11-N-8-8-8-2)	
486-2	0.5	1	≤0.06	1	≤0.06	0.5	16 (R)		1571 (6-25-7-140-1-175-2)	
721-2	0.5	1	≤0.06	2	≤0.06	0.5	8 (R)		216 (10-11-57-8-7-18-6)	

^aIMI, imipenem; COL, colistin; CPM, cefepime; GEN, gentamicin; CIP, ciprofloxacin; TET, tetracycline; RIF, rifampin. R and I in parenthesis, resistance and intermediate, respectively.

^b*adk-fumC-gyrB-icd-purA-recA*

^cNovel sequence type (ST) or novel allele; we were not able to obtain novel ST and allele numbers because only new alleles or STs defined by NGS short reads are accepted in the new MLST website for *E. coli* (<http://enterobase.warwick.ac.uk/species/index/ecoli>).

#753-1) *E. coli* isolates, respectively (Table 1). *bla*_{CTX-M} was identified in four *E. coli* isolates, with two containing *bla*_{CTX-M-14}, one *bla*_{CTX-M-15}, and one *bla*_{CTX-M-174}. Two *E. coli* isolates (#82-2 and #97-1) contained both *bla*_{TEM-1} and *bla*_{CTX-M}. *bla*_{CTX-M-174} was identified in an ST131 isolate. *bla*_{SHV} was identified in all four *K. pneumoniae* isolates, but the kinds of *bla*_{SHV} were different among the isolates (Table 2). We did not identify any *bla* genes in the imipenem-resistant *Raoultella* spp. isolates.

Reduced Susceptibility to Imipenem and Colistin

Among the 64 gram-negative bacterial isolates, reduced susceptibility to imipenem was observed in four isolates, with two isolates of *Raoultella* showing imipenem resistance (MICs, 4 mg/l), and one isolate each of *E. coli* (isolate 82-2) and *C. freundii* showing intermediate resistance to imipenem (MICs, 2 mg/l) (Table 1). Only two isolates (one *K. variicola* and one *S. subterranean*) were resistant to colistin (MICs, 32 and 8 mg/l, respectively), and one *R. planticola* isolate had intermediate resistance to colistin (MIC, 4 mg/l). No isolates carried *mcr-1*.

Cefepime and gentamicin resistance was found only in *E. coli* isolates; five for cefepime and six for gentamicin (Table 1). Eight *E. coli* isolates and one *C. freundii* isolate were resistant to ciprofloxacin, and 13 *E. coli* isolates and one *E. hermannii* isolate were resistant to tetracycline (Tables 1 and 3). All four *K. pneumoniae* isolates were susceptible to all antimicrobials tested except rifampin (Table 2). All isolates recovered in this study showed rifampin resistance.

Genotypes

For *E. coli* and *K. pneumoniae* isolates, MLST was performed. For 45 *E. coli* isolates, 35 STs were identified (Table 1). Only five STs (ST10, ST1316, ST1193, ST38, and ST69) included multiple *E. coli* isolates. Four clonal complexes were identified. In the *E. coli* isolates, six STs were newly found in this study. PFGE results also showed very diverse genotypes (Fig. 1). Even isolates belonging to the same STs showed different PFGE bands. For example, five isolates of ST10 exhibited different PFGE patterns, as in ST38, ST69, and ST1193. In the four *K. pneumoniae* isolates, four different

Table 2. Antimicrobial resistance, β-lactamase gene, and genotype of *K. pneumoniae* isolates.

Species (isolate #)	MIC (mg/l) ^a							β-Lactamase gene	ST (allele #) ^b
	IMI	COL	CPM	GEN	CIP	TET	RIF		
1-1	0.25	1	≤0.06	0.5	≤0.06	1	32 (R)	SHV-37	188(3-1-1-3-4-28-39)
54-1	0.25	2	≤0.06	0.5	≤0.06	1	32 (R)	SHV-1	70 (2-6-17-1-20-10-25)
93-3	1	1	≤0.06	0.5	≤0.06	1	32 (R)	SHV-32	1966 (2-1-2-1-10-4-42)
440-1	0.5	1	≤0.06	0.5	≤0.06	2	32 (R)	SHV-27	2885 (2-6-1-1-1-4-4)

^aIMI, imipenem; COL, colistin; CPM, cefepime; GEN, gentamicin; CIP, ciprofloxacin; TET, tetracycline; RIF, rifampin. R and I in parenthesis, resistance and intermediate, respectively.

^b*gapA-infA-mdh-pgi-phoE-rpoB-tonB*

Table 3. Antimicrobial resistance and β -lactamase gene of isolates except *E. coli* and *K. pneumoniae*.

Species (isolate #)	MIC (mg/l) ^a						
	IMI	COL	CPM	GEN	CIP	TET	RIF
<i>Klebsiella oxytoca</i>							
63-1	0.5	2	≤0.06	1	≤0.06	0.5	16 (R)
412-2	0.25	2	≤0.06	1	≤0.06	1	16 (R)
469-1	0.5	2	≤0.06	1	0.12	1	16 (R)
<i>Klebsiella variicola</i>							
464-1	0.5	32 (R)	≤0.06	0.5	≤0.06	1	16 (R)
475-3	0.5	1	0.12	0.5	≤0.06	1	32 (R)
<i>Enterobacter cloacae</i>							
461-1	0.5	2	2	1	≤0.06	2	16 (R)
479-2	1	2	0.12	0.5	≤0.06	2	32 (R)
<i>Escherichia hermannii</i>							
754-3	1	2	0.12	1	0.25	>64 (R)	16 (R)
<i>Citrobacter freundii</i>							
458-2	2 (I)	1	≤0.06	1	4 (R)	1	32 (R)
<i>Raoultella ornithinolytica</i>							
488-1	4 (R)	1	≤0.06	1	≤0.06	1	16 (R)
<i>Raoultella planticola</i>							
458-1	4 (R)	4 (I)	≤0.06	1	≤0.06	1	32 (R)
<i>Salmonella subterranean</i>							
754-1	0.25	8 (R)	≤0.06	0.25	≤0.06	0.5	9 (R)

^aIMI, imipenem; COL, colistin; CPM, cefepime; GEN, gentamicin; CIP, ciprofloxacin; TET, tetracycline; RIF, rifampin. R and I in parenthesis, resistance and intermediate, respectively.

STs were identified (Table 2).

Discussion

In this study, 64 gram-negative bacterial isolates were identified in 109 stool samples obtained from comprehensive health examinees in Korea. A total of 24 isolates were ESBL producers and cultivated from 22 participants; this accounted for 20% of fecal carriage rates of ESBL-E. Reduced susceptibility to imipenem was noted in *C. freundii* and *Raoultella* species. Colistin resistance was found in two isolates of *K. variicola* and *S. subterranean*, but there is no carriage of *mcr-1*. Colistin resistance in gram-negative bacteria is known to occur mainly by the addition of a cationic group, such as 4-amino-4-deoxy-L-arabinose or phosphoethanolamine to the lipid A moiety of LPS, which results in a decrease in the net negative charge of the bacterial outer membrane [16]. However, no colistin resistance mechanism was known in these two species.

The reported asymptomatic carriage rates of ESBL-E in the feces of community dwellers varies in Asian countries: 5.0% in Japan [17], 40% in Thailand [18], and 50.6% in China [19]. Most carriers were positive for CTX-M-type ESBL, which has nearly displaced other ESBL enzymes in Enterobacteriaceae, including TEM and SHV ESBL variants. In Korea, the prevalence of ESBL-E carriage was 20.3% [4], but molecular typing was not further performed in these isolates. Although a limited number of samples was investigated, our study showed that not a few ESBL- or β -lactamase-producing *E. coli* and *K. pneumoniae* isolates were carried in healthy persons. The result that clones identified as causes of infection in previous studies were found in stool samples in our study suggests that continuous monitoring is required. In this study, no imipenem-resistant isolates were identified in *E. coli* and *K. pneumoniae*. Instead, two *Raoultella* spp. isolates were found to be resistant to imipenem. Although no MBL genes were identified in them, we focused on the imipenem-resistant *Raoultella* spp. because of the increase in clinical significance of both *R. planticola* and *R. ornithinolytica* [20].

In the molecular analysis of *E. coli* isolates, diverse genotypes were identified, including newly identified STs. ST131, in particular, is known as the most prevalent CTX-M-producing *E. coli* clone in the world [21]. In this study, ST131 was identified in only one isolate and produced CTX-M-174, which is a variant of CTX-M-14 differing in two amino acids (Glu7Leu and Asp242Gly). Four *E. coli* isolates were identified as CTX-M producers, with one CTX-M-174, two CTX-M-14, and one CTX-M-15; these belonged to ST10, ST1193, and ST38. In the literature, ST10 was reported as one of the major genotypes of CTX-M-producing *E. coli* isolates from South Korea [22]. ST1193 was most commonly identified among ciprofloxacin-nonsusceptible *E. coli* bloodstream isolates from South Korea [15]. ST14, a single locus variant of ST1193, was found in outbreak-related isolates producing OXA-232 in South Korea [23]. It indicates that CTX-M-producing *E. coli* isolates may be colonized in healthy persons and infect immune-compromised patients. In addition to *bla*_{TEM-1} being identified in 14 *E. coli* isolates, *bla*_{TEM-30} was found in one isolate. TEM-30 is a derivative of TEM-1, differing in a single amino acid (R241S). It should be kept in mind that tazobactam was potent against TEM-30 β -lactamase [24], because ST95 was the most common clone in uropathogenic *E. coli* from Korean children with urinary tract infection [25].

It is worthy to note that all four *K. pneumoniae* isolates carried *bla*_{SHV}. Whereas ST1966 was found in *K. pneumoniae* isolates from chicken, ST70 and ST2885 had been identified

Fig. 1. Pulsed-field gel electrophoresis patterns of 45 *E. coli* isolates.

The sequence type (ST) of isolates are indicated at the right. The dendrogram was constructed by Dice similarity coefficient and UPGMA. The scale bar at the top indicates the similarity coefficient (%). M, size marker.

in human isolates causing human infections (<http://bigsd.bpasteur.fr/>). In addition, ST188 had been identified in an isolate causing invasive disease. These results may suggest the possible association between infection and fecal carriage of ESBL-producing *K. pneumoniae* isolates, although the genotypes have not been identified in isolates from South Korea.

In summary, we were able to isolate gram-negative bacteria in about half of the 109 stool samples from adults that underwent medical examination. Although diverse genotypes were identified among *E. coli* isolates, several STs included multiple isolates, and many STs had been

reported to cause infections in previous studies. Several ESBL-producing *E. coli* and *K. pneumoniae* isolates were found, and imipenem-resistant *Raoultella* spp. isolates were also identified. There is a need for effective infection prevention and control measures and strict use of antibiotics to control the rise and spread of fecal carriage of antibiotic-resistant pathogens.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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