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Carriage of *Escherichia coli* Producing CTX-M-Type Extended-Spectrum β-Lactamase in Healthy Vietnamese Individuals

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Healthy carriage of CTX-M-type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* was examined by thrice collecting fecal samples from the same 199 healthy Vietnamese subjects every 6 months. Using pulsed-field gel electrophoresis (PFGE), identical PFGE patterns throughout the three samplings were not observed, although prevalence of *E. coli* in the subjects was around 50% in the three samplings. Our results suggested a short carriage period of the CTX-M-type ESBL-producing *E. coli* in healthy Vietnamese subjects.

A high prevalence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* has been reported in Southeast and South Asian countries (1–5). Southeast and South Asian countries are regarded as one of the epicenters of antibiotic-resistant bacteria (6). The emerging ESBL have been classified by their amino acid sequences into more than 10 families, including CTX-M, SHV, and TEM types (7, 8). Antibiotic resistance genes, such as $bla_{\text{CTX-M}}$, are horizontally transferred among bacterial cells via conjugative antibiotic-resistant plasmids, even beyond bacterial species (9). Nosocomial and community-acquired infections of CTX-M-type ESBL-producing *Enterobacteriaceae* have been distributed worldwide (9, 10).

The pathway of the ESBL-producing *Enterobacteriaceae* distribution is consistent with environmental water and soil (sewage), wild animals, livestock, and human communities, including hospitals and nursing homes (10, 11). Epidemiological studies have been performed to determine its prevalence in various populations, and the distribution of antibiotic-resistant bacteria has been analyzed (10, 11). However, the nature and role of healthy individuals in the transfer and distribution of ESBL-producing *Enterobacteriaceae* are not well understood.

The study setting and population in the Bavi district of Hanoi, Vietnam was described previously (12). Stool specimens from the study population were collected three times every 6 months from June 2013 to June 2014. Vietnamese individuals who were under clinical treatments within a week before each sampling began were excluded from this study. Finally, 199 healthy Vietnamese individuals were enrolled, and a total of 597 stool specimens were collected. The mean age of the participants at the last sampling was 40.5 years (standard deviation [SD] = 23.1), and the age range was between 1 and 92 years. The study population consisted of 93 men (46.7%) and 106 women (53.3%). Informed consent was obtained from all participants. The study protocol was reviewed and approved by the participating institutes. Statistical significance was evaluated with the χ^2 test using IBM SPSS Statistics software version 20 (International Business Machines Corporation, Armonk, NY).

The collected specimens were subjected to microbiological experiments to isolate ESBL-producing *Escherichia coli* within a sampling day. ESBL-producing *E. coli* screening, confirmation of ESBL phenotype, and bacterial species determination were performed as described previously (12). The prevalence of ESBL-producing *E. coli* in the study population of each sampling was 46.7% in June 2013, 52.8% in November 2013, and 46.2% in June 2014. There was no significant difference among the samplings. Antibiotic resistance profiles of the ESBL-producing *E. coli* in this study were observed by a standard disc diffusion test described previously (12). Overall, resistance against ampicillin (AMP) (97.9%), streptomycin (STR) (63.0%), tetracycline (TET) (74.1%), trimethoprim-sulfamethoxazole (SXT) (74.6%), and nalidixic acid (NAL) (42.4%) were evident. The ESBL-producing *E. coli* in this study was completely susceptible to meropenem (MEM).

Phylogenetic groups of the ESBL-producing *E. coli* were observed by conventional phylogenetic grouping protocol (13). Although phylogenetic group A was prevalent in the June 2013 (37.6%) and June 2014 (40.2%) samplings, phylogenetic group D was the predominant type (53.3%) in November 2013, and its detection rate was significantly different from the detection rate of phylogenetic group D in the other samplings. In this study population, phylogenetic group B2 was a minority (15.1% in June 2013, 4.8% in November 2013, and 12.0% in June 2014).

Then, genotyping of $bla_{\text{CTX-M}}$ was performed with TaKaRa EX *Taq* DNA polymerase (TaKaRa Bio Inc., Shiga, Japan) and primers specific to the $bla_{\text{CTX-M-1}}$ group (5′-TAWTTCGTMTCTTCC

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TABLE 1 Genotyping of *E. coli* isolates possessing bla_{CTX-M} among the three samplings[a]

Detected sampling(s) of *E. coli* possessing bla_{CTX-M}			Detected bla_{CTX-M} group										Detected phylogenetic group							
			Any CTX-M		CTX-M-1		CTX-M-2		CTX-M-9		CTX-M-25		A		B1		B2		D	
June 2013	November 2013	June 2014	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
+			25	12.6	28	14.1	1	0.5	27	13.6	2	1.0	25	12.6	17	8.5	12	6.0	17	8.5
	+		36	18.1	14	7.0	0	0.0	35	17.6	0	0.0	29	14.6	12	6.0	3	1.5	45	22.6
		+	28	14.1	14	7.0	0	0.0	30	15.1	1	0.5	29	14.6	19	9.5	11	5.5	14	7.0
+	+		22	11.1	4	2.0	0	0.0	15	7.5	0	0.0	2	1.0	0	0.0	2	1.0	4	2.0
+		+	17	8.5	2	1.0	0	0.0	8	4.0	0	0.0	7	3.5	2	1.0	0	0.0	2	1.0
	+	+	18	9.0	0	0.0	0	0.0	25	12.6	0	0.0	0	0.0	0	0.0	0	0.0	5	2.5
+	+	+	29	14.6	0	0.0	0	0.0	16	8.0	0	0.0	1	0.5	0	0.0	0	0.0	2	1.0

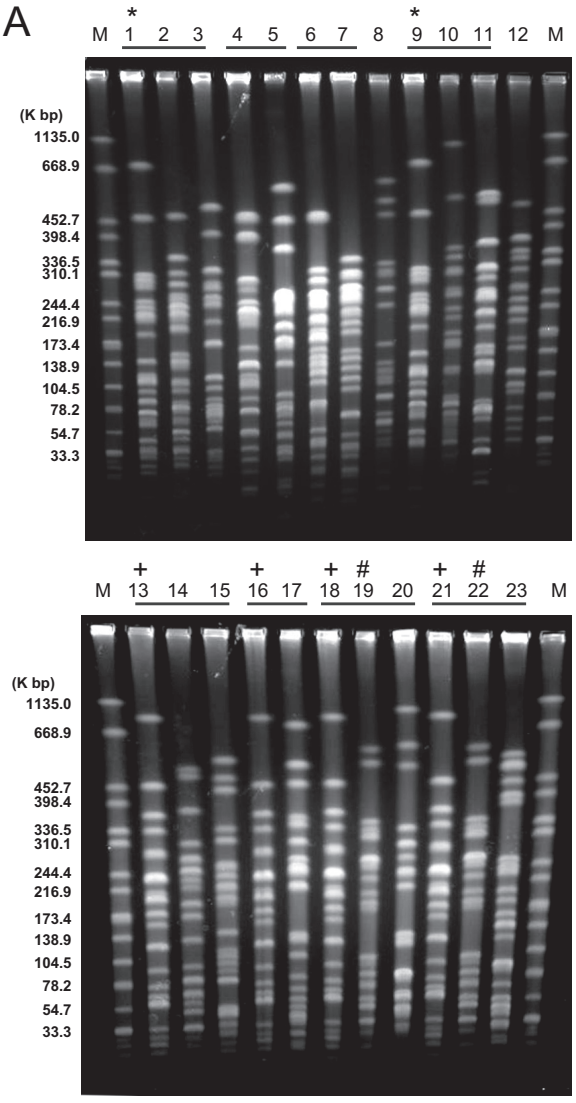
[a] Detection rates of bla_{CTX-M} and phylogenetic group are indicated as percentages of the 199 participants.

AGAATAAGG-3′ and 5′-ATGAGTTTCCCCATTCCGTTTCC-3′), $bla_{CTX-M-2}$ group (5′-ATGATGACTCAGAGCATTCGC-3′ and 5′-TCAGAAACCGTGGGTTACGAT-3′), $bla_{CTX-M-9}$ group (5′-TGTAACACGGATTGACCGTAT-3′ and (5′-ACTCAGCAA AAGTTCGATTTATTC-3′), and $bla_{CTX-M-25}$ group (5′-ATGATG AGAAAAAGCGTAAGG-3′ and 5′-TTAATAACCGTCGGTGAC AAT-3′). PCR conditions, except for the $bla_{CTX-M-9}$ group, were 5 min of initial denaturation at 98°C followed by 35 cycles at 98°C for 10 s, 55°C for 30 s, and 72°C for 1 min. PCR conditions for the $bla_{CTX-M-9}$ group were the same as the above PCR conditions except for the annealing temperature, which was 58°C. Consequently, the $bla_{CTX-M-9}$ group was the predominant type in the population throughout the sampling period, and the detection rates of the $bla_{CTX-M-9}$ group in November 2013 (86.7%) and June 2014 (85.9%) were significantly higher than that in June 2013 (71.0%). The predominance of the $bla_{CTX-M-9}$ group was also observed in healthy Thai individuals in our previous study (1). Therefore, endemic ESBL-producing *E. coli* in Southeast Asian countries might predominantly possess $bla_{CTX-M-9}$ group.

At the individual level, ESBL-producing *E. coli*, regardless of bla_{CTX-M} group, was detected at least one time in 175 (87.9%) of the 199 participants during the sampling period. The number of participants who harbored ESBL-producing *E. coli* possessing any kind of bla_{CTX-M} throughout the sampling period was 29 (14.6%). Among those, only three participants (1.5%), who belonged to different household, carried ESBL-producing *E. coli* isolates that belonged to the same phylogenetic group in addition to the commonly detected $bla_{CTX-M-9}$ throughout the sampling period. Carriage numbers and their ratios of bla_{CTX-M} groups and phylogenetic groups (Table 1) of the ESBL-producing *E. coli* are summarized by the sampling periods. The ability to properly classify *E. coli* isolates by their phylogenetic group has been evaluated using multilocus sequence typing classification in our previous study (12), and the results of our bla_{CTX-M} grouping was completely consistent with the sequence analysis interpretation (T. K. N. Bui, unpublished data). Therefore, the frequent alteration in the healthy carriage of ESBL-producing *E. coli* was not simply due to the inaccuracy of genetic classifications.

To explain genetic relatedness among the CTX-M-type ESBL-producing *E. coli*, pulsed-field gel electrophoresis (PFGE) was performed as described in our previous study (14). In the case that number of the CTX-M-type ESBL-producing *E. coli* isolates detected in each household among the three samplings was at least four, the households' isolates were examined. In this study, 261 of the 290 CTX-M-type ESBL-producing *E. coli* isolates, which belonged to 36 of the 47 households, were analyzed (Fig. 1A). Various PFGE patterns were observed from the examined CTX-M-type ESBL-producing *E. coli* isolates. Discrepancy between phylogenetic groups of certain CTX-M-type ESBL-producing *E. coli* isolates and similarity of their PFGE patterns (e.g., lanes 1 and 9 in Fig. 1A) were observed in 12 (4.6%) of the 261 examined *E. coli* isolates. In this study, we used PCR-based phylogenetic grouping (13). Generally, mutation in a primer target sequence gives rise to recognition error of the primer. Therefore, the discrepancy in our study might be a consequence of certain mutations on one of the three genetic markers for determination of the phylogenetic grouping. We compared the PFGE patterns obtained from the examined *E. coli* isolates, and the case of exactly identical PFGE patterns, suggesting clonal distribution, was observed in at least two *E. coli* isolates. There were two types of clonal distribution, i.e., intrahousehold and interhousehold distributions. Eighteen intrahousehold clonal distributions were observed in 16 of the 36 households (44.4%), and one or two PFGE patterns were observed in two to four CTX-M-type ESBL-producing *E. coli* isolates obtained from the same household. As interhousehold clonal distributions, seven cases were confirmed. The interhousehold distributions were detected in 15 of the 36 households (41.7%), and two to six CTX-M-type ESBL-producing *E. coli* isolates shared one of the seven PFGE patterns. However, an identical PFGE pattern was not observed from the CTX-M-type ESBL-producing *E. coli* isolates in plural sampling periods. Taken together, these results suggested that transient clonal intrafamily and interfamily clonal distributions of certain CTX-M-type ESBL-producing *E. coli* clones occurred at each sampling period. At the individual level, observed PFGE patterns again varied among the three samplings (Fig. 1B), implying shorter carriage period (less than 6 months) of the CTX-M-type ESBL-producing *E. coli* in the study subjects. Considering the *E. coli* in intestinal microflora, commensal *E. coli* colonizes over longer periods, such as months or years. In contrast, extraintestinal *E. coli*, such as pathogenic *E. coli*, does not colonize for longer periods; rather, it colonizes transiently in the gastrointestinal tract (15). Escobar-Paramo et al. reported that the phylogenetic group ratio of commensal *E. coli* in humans was different from that of other mammals and that the phylogenetic group ratio of other mammals altered with the sampling year, habitat, diet, and climate (16). Therefore, infection of

A



Lane #	ID #	CTX-M group	Phy.G
1	36111.Jun-13*	9	B2
2	36111.Non-13	9	A
3	36111.Jun-14	9	D
4	36122.Jun-13	9	B1
5	36122.Jun-14	9	B1
6	36211.Nov-13	9	D
7	36211.Jun-14	9	A
8	36222.Jun-13	1	D
9	36313.Jun-13*	9	A
10	36313.Nov-13	9	D
11	36313.Jun-14	1 + 9	A
12	36321.Nov-13	1 + 9	A
13	30111.Jun-13+	1	B2
14	30111.Nov-13	1	D
15	30111.Jun-14	9	A
16	30122.Jun-13+	1	B2
17	30122.Nov-13	1 + 9	B1
18	30211.Jun-13+	1	B2
19	30211.Nov-13#	9	B2
20	30211.Jun-14	9	D
21	30222.Jun-13+	1	B2
22	30222.Nov-13#	9	B2
23	30222.Jun-14	9	A

B

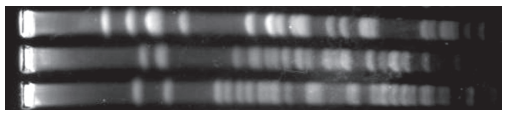
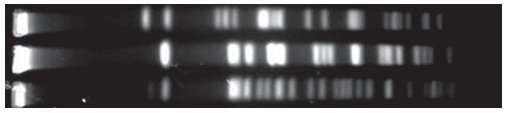
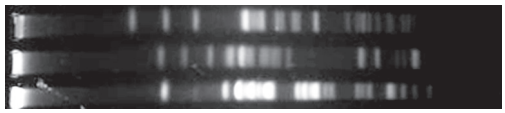
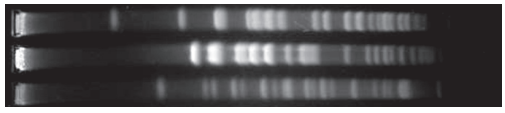
Samp.	CTX-M group	Phy.G	PFGE pattern	ID#
Jun-14	9	D		5323
Nov-13	9	D		
Jun-13	9	D		
Jun-14	9	A		37122
Nov-13	9	A		
Jun-13	1 + 9	A		
Jun-14	9	A		38313
Nov-13	9	D		
Jun-13	9	A		
Jun-14	9	D		48122
Nov-13	9	B1		
Jun-13	1	A		

FIG 1 Pulsed-field gel electrophoresis profiles of the *E. coli* possessing bla_{CTX-M} in Vietnamese healthy individuals. Representative PFGE results of the CTX-M-type ESBL-producing *E. coli* isolates at household level (A) and individual level (B) are shown. The *E. coli* isolates indicating identical PFGE patterns are indicated by *, +, and #. CTX-M, detected group(s) of bla_{CTX-M}; Phy.G, detected phylogenetic group of the *E. coli* isolates.

certain extraintestinal *E. coli* isolates possessing certain types of bla_{CTX-M} might result in the shorter carriage period and the frequent alteration of the participants' carriage of the CTX-M-type ESBL-producing *E. coli*. In addition to clonal distribution of bacteria, bla_{CTX-M} is horizontally transferred through antibiotic-resistant plasmids among *Enterobacteriaceae* (17), and this *in vivo* horizontal transfer has been observed in humans (18, 19). Although there was no supporting data obtained in this study, we did not exclude this possibility.

Although our results indicate that the CTX-M-type ESBL-producing *E. coli* was carried less than 6 month in healthy individuals living in a rural area of Vietnam, many aspects of the epidemiology remain to be explained. Those include the source of certain extraintestinal ESBL-producing *Enterobacteriaceae*, the reason why regional difference in phylogenetic group and bla_{CTX-M} group are observed, and the different distribution from clinic-oriented isolates such as the *E. coli* O25b-B2-ST131 clone. Further studies are needed to identify factors contributing to the behavior of the CTX-M-type ESBL-producing *E. coli* and to determine how to contain this ESBL-producing bacteria.

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