

## Prevalence of fecal carriage of extended-spectrum $\beta$ -lactamase-producing *Enterobacteriaceae* among healthy adult people in Japan

Ulzii-Orshikh Luvsansharav · Itaru Hirai · Marie Niki · Arisa Nakata · Aya Yoshinaga · Toshiki Moriyama · Yoshimasa Yamamoto

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**Abstract** In Japan, the prevalence of extended-spectrum  $\beta$ -lactamase (ESBL) producers in hospital settings has shown an increasing trend. In the present study, we investigated the prevalence of the fecal carriage of ESBL-producing *Enterobacteriaceae* among healthy adults. Stool samples were collected for the phenotypic and genotypic identification of ESBL-producing *Enterobacteriaceae*. We found the prevalence of ESBL producers to be 6.4% by phenotypic identification, and 92.9% of them possessed the *bla*<sub>CTX-M</sub> gene. Among the CTX-M ESBL-producing *Enterobacteriaceae*, we identified 11 *Escherichia coli* and 2 *Klebsiella pneumoniae* strains. The findings suggest that the fecal carriage of CTX-M-type ESBL producers by healthy people is rapidly increasing in Japan. This may be one of the causes of the increased spread of ESBL-producing bacteria in hospitals.

**Keywords** *Enterobacteriaceae* · Extended-spectrum beta-lactamase (ESBL) · Japanese

The genes for extended-spectrum  $\beta$ -lactamases (ESBLs), which hydrolyse extended-spectrum cephalosporins and are inhibited by clavulanic acid, are spreading among *Enterobacteriaceae* [1]. In particular, *Escherichia coli* strains that produce CTX-M-type  $\beta$ -lactamases have become widely

prevalent in the general population and are probably being imported into the hospital community [2].

In Japan, the prevalence of ESBL producers among clinical isolates has shown an increasing trend. In 2000, Komatsu et al. [3] reported a 0.5% prevalence of ESBL producers in the feces of diarrhea patients. Another review reported prevalences of 0.6, 7.2, 10.0, and 14% in clinical isolates of *E. coli* and *Klebsiella pneumoniae* in Japan in 1990, 1995, 2000, and 2003, respectively [4]. A hospital study in southwestern Japan showed that the prevalence of ESBL-producing bacteria increased from 3.6 to 15.2% between 2003 and 2009 [5]. However, little is known about the frequency of ESBL-producing bacteria in healthy Japanese people. Therefore, the aim of this study was to investigate the prevalence of the fecal carriage of ESBL-producing *Enterobacteriaceae* among asymptomatic Japanese adults.

Stool samples from adult volunteers in Osaka, Japan, were collected from July 2009 to June 2010. Written informed consent was obtained from each individual who volunteered to participate in the study. We analyzed a total of 218 samples, 1 from each participant.

All participants were asked to fill out a standardized questionnaire including general demographic circumstances, antibiotic usage within 3 months prior to sample collection, and lifetime history of hospitalization.

Stool samples were assessed for the presence of ESBL-producing *Enterobacteriaceae* by phenotypic and genotypic methods. The phenotypic methods included plating the stool samples on MacConkey agar supplemented with 2  $\mu$ g/ml cefotaxime (CTX-MacConkey). The results were confirmed using cefotaxime and ceftazidime with and without clavulanic acid, as recommended by the Clinical and Laboratory Standards Institute [6]. Isolates were identified using conventional biochemical tests. Tests for

U.-O. Luvsansharav · I. Hirai · M. Niki · A. Nakata · A. Yoshinaga · Y. Yamamoto (✉)  
Department of Bioinformatics, Osaka University Graduate School of Medicine, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan  
e-mail: yyamamoto@sahs.med.osaka-u.ac.jp

T. Moriyama  
Health Care Center, Osaka University, Osaka 560-0043, Japan

susceptibility to imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, and tetracycline were conducted by the disc diffusion method using an SN Disc (Nissui Pharmaceutical, Tokyo, Japan).

Bacterial DNA was extracted from the isolates by boiling the bacterial suspensions. DNA samples with a concentration of 0.1 ng/μl were used as the templates for polymerase chain reaction (PCR) analysis. The universal primers CTX-M-U1 (5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3') and CTX-M-U2 (5'-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG-3') were used to detect the *bla*<sub>CTX-M</sub> gene, and the primers bla-SHV.SE (5'-ATG CGT TAT ATT CGC CTG TG-3') and bla-SHV.AS (5'-TGC TTT GTT ATT CGG GCC AA-3') were used to detect the *bla*<sub>SHV</sub> gene, as described previously [7]. DNA from the reference *E. coli* *bla*<sub>CTX-M</sub>-positive strain was used as the positive control. The PCR products were visualized by 2% agarose gel electrophoresis, followed by staining with GelRed Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA). DNA sequencing was used for genotyping the *bla*<sub>CTX-M</sub> genes. The nucleotide sequences were analyzed with CLUSTALW and BLAST software (<http://clustalw.ddbj.nig.ac.jp/top-j.html>).

Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA). Variables were analyzed by univariate analysis using  $\chi^2$  or Fisher's exact test, as appropriate. Statistical significance was set at  $p < 0.05$ .

The characteristics of the study participants are shown in Table 1. The participants were aged between 20 and 70 years, with a median age of 41.9 years. Most had not used antibiotics within the 3 months prior to

sample collection and most did not have a history of hospitalization.

Of the 218 samples, 65 (29.8%) showed bacterial growth on CTX-MacConkey agar and 14 (6.4%) showed ESBL production (Table 2). All the 14 isolates were identified from different participants. On PCR analysis, 92.9% (13 of 14) of the ESBL-positive isolates were positive for the *bla*<sub>CTX-M</sub> gene, and 1 sample carried the *bla*<sub>SHV</sub> gene. By genotyping of *bla*<sub>CTX-M</sub> genes, CTX-M-14 (38.5%) and CTX-M-2 (30.8%) ESBL types were predominantly identified. The other samples harbored the CTX-M-8, CTX-M-3, and CTX-M-15 types (15.4, 7.7, and 7.7%, respectively).

Among the CTX-M ESBL-producing *Enterobacteriaceae*, 84.6% (11 of 13) were identified as *E. coli* and 15.4% (2 of 13) as *K. pneumoniae*. The SHV-type ESBL producer was identified as *E. coli*. We did not identify any participants who harbored both ESBL-producing *K. pneumoniae* and *E. coli* as well as different CTX-M genotypes. Antibiotic susceptibility tests showed that 92.9% (13 of 14) of the ESBL-positive bacteria were susceptible to all the tested antibiotics, except for tetracycline. Five of the 14 ESBL producers were resistant to only tetracycline, and 1 was resistant to gentamicin and quinolones in addition to tetracycline.

Statistical analysis did not reveal any significant association between ESBL production and antibiotic usage or hospitalization experience, although these findings may be attributed to the small number of ESBL-positive cases identified in this study.

To the best of our knowledge, this is the first published data regarding the prevalence of ESBL-producing

**Table 1** Characteristics of the study participants

Variable	Extended-spectrum $\beta$ -lactamase (ESBL) (–)	ESBL (+)	Total number
No. of participants			218
Age group (years)			
20–39	62 (91.2%)	6 (8.8%)	68
40–59	119 (95.2%)	6 (4.8%)	125
Over 60	23 (92.0%)	2 (8.0%)	25
Gender			
Male	103 (92.8%)	8 (7.2%)	111
Female	101 (94.4%)	6 (5.6%)	107
Antibiotic usage within 3 months			
Yes	35 (89.7%)	4 (10.3%)	39
No	160 (94.7%)	9 (5.3%)	169
Don't remember	9 (90.0%)	1 (10.0%)	10
Lifetime history of hospitalization			
Yes	72 (94.7%)	4 (5.3%)	76
No	130 (92.9%)	10 (7.1%)	140
Don't remember	2 (100.0%)	0 (0.0%)	2

**Table 2** Detection of CTX-M-type ESBL-producing *Enterobacteriaceae*

Participants tested	ESBL phenotype <sup>a</sup>	CTX-M gene <sup>b</sup>	Organisms	Genotype of CTX-M genes <sup>c</sup>				
				CTX-M-1 group <sup>d</sup>	CTX-M-2 group <sup>e</sup>	CTX-M-8 group <sup>f</sup>	CTX-M-9 group <sup>g</sup>	
218	14 (6.4%)	13 (92.9%)	<i>Escherichia coli</i>	11 (84.6%)	1 (CTX-M-15)	4 (CTX-M-2)	2 (CTX-M-8)	4 (CTX-M-14)
			<i>Klebsiella pneumoniae</i>	2 (15.4%)	1 (CTX-M-3)	0	0	1 (CTX-M-14)
			Total	13 (100%)	2 (15.4%)	4 (30.8%)	2 (15.4%)	5 (38.5%)

<sup>a</sup> ESBL phenotype was determined according to the Clinical and Laboratory Standards Institute recommendations

<sup>b</sup> The CTX-M gene was determined by polymerase chain reaction (PCR)

<sup>c</sup> The genotype of CTX-M genes was determined by DNA sequencing

<sup>d</sup> The CTX-M-1 group includes CTX-M-1 and several other variants, such as CTX-M-3 and CTX-M-15

<sup>e</sup> The CTX-M-2 group includes CTX-M-2 and several other variants

<sup>f</sup> The CTX-M-8 group includes CTX-M-8

<sup>g</sup> The CTX-M-9 group includes CTX-M-9 and several other variants, such as CTX-M-14

*Enterobacteriaceae* among healthy Japanese adults. Other countries have reported a 2.4–13.1% prevalence of fecal carriage of ESBL-producing *Enterobacteriaceae* in healthy people [8–12]. This value reached 58.2% in developing countries, such as Thailand, where antibiotics are used without prescription [12]. However, in Japan, where antibiotic usage is strictly regulated, the 6.4% prevalence of ESBL-producing *Enterobacteriaceae* among healthy individuals is relatively high.

The high prevalence (92.9%) of CTX-M-type ESBLs identified in the present study is consistent with the global trend of CTX-M dominance. The prevalence of the CTX-M-14 enzyme belonging to the CTX-M-9 group and the CTX-M-2 enzyme belonging to the CTX-M-2 group was compatible with the findings of previous studies carried out in hospital settings, which have reported that the CTX-M-9, CTX-M-2, and CTX-M-1 groups are the predominant CTX-M-type ESBLs in *E. coli* in Japan [4, 5, 13, 14]. This similarity between the CTX-M-type ESBL groups identified in community and hospital settings may indicate that people who are already carriers of ESBL producers are visiting hospitals. In previous studies of clinical isolates in Japan, ESBL-producing bacteria showed coresistance to fluoroquinolones [5, 15]. However, our data showed that most of the ESBL producers (92.9%) identified from healthy individuals were susceptible to all antibiotics, except tetracycline. Furthermore, our study did not show any significant association between the fecal carriage of ESBL-positive *Enterobacteriaceae* and recent antibiotic use or hospitalization, which indicates the existence of other risk factors. Because the study enrolled only a limited number of participants, possible factors that contribute to

the prevalence of ESBL-producing bacteria in healthy people in Japan should be investigated in a larger number of participants.

In summary, our results suggest that the prevalence of the fecal carriage of CTX-M-type ESBL producers, mostly *E. coli*, by healthy Japanese people is rapidly increasing. This may be one of the causes of the dissemination of ESBL-producing bacteria in hospitals. A broader and more detailed study of the prevalence of ESBL producers in the community is needed to prevent potential treatment failures associated with serious infections caused by these bacteria.

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**Conflict of interest** There is no conflict to declare.

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