Consumption of edible ice contaminated with *Acinetobacter*, *Pseudomonas*, and *Stenotrophomonas* is a risk factor for fecal colonization with extended-spectrum β-lactamase-producing *Escherichia coli* in Vietnam

Tatsuya Nakayama, Nguyen Cong Ha, Phong Quoc Le, Ryuji Kawahara, Yuko Kumeda, Yoshinori Sumimura and Yoshimasa Yamamoto

ABSTRACT

Although Vietnamese residents frequently harbor extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-E), it is unclear which foods/beverages are risk factors for acquiring these bacteria. The aim of this study was to evaluate the frequency with which edible ice served in restaurants is contaminated with antibiotic-resistant bacteria, and thereby clarify whether this product poses a risk for ESBL-E carriage in humans. Ice from restaurants in Vietnam and Japan was screened for bacteria capable of growing on agar containing cefotaxime (BG-CTX). Of the 119 BG-CTX strains isolated in Vietnam, 40%, 39%, and 12% were identified as *Pseudomonas* spp., *Acinetobacter* spp., and *Stenotrophomonas maltophilia*, respectively. Meanwhile, of the six such strains isolated in Japan, five were identified as *Acinetobacter* spp. and one as *Pseudomonas* spp. More than 10% of the *Acinetobacter* isolates exhibited cefotaxime, ceftazidime, and sulfa/trimethoprim resistance, while 21% of *Pseudomonas* and 14% of *S. maltophilia* isolates exhibited meropenem and sulfa/trimethoprim resistance, respectively. Subsequent multiplex polymerase chain reaction (PCR) analyses detected ESBL-encoding genes in 10% of the BG-CTX. Notably, feces harvested from mice administered water contaminated with BG-CTX contained *E. coli* harboring the $bla_{CTX\text{-}M\text{-}9}$ gene. In conclusion, our findings indicate that consumption of contaminated edible ice is a risk factor for human ESBL-E carriage.

Key words | *Acinetobacter*, edible ice, ESBL-producing bacteria, *Pseudomonas*, *Stenotrophomonas*

Tatsuya Nakayama (corresponding author)
Division of Food Medical Science,
National Institute of Health Science,
1-18-1 Kamiyoga,
Setagaya, Tokyo, Japan
E-mail: *t.nakayama@nihs.go.jp*

Tatsuya Nakayama
Yoshinori Sumimura
Center for Global Initiatives,
Osaka University,
Suita, Osaka, Japan

Nguyen Cong Ha
Food Technology Department,
College of Agriculture and Applied Biology,
Can Tho University,
Can Tho, Vietnam

Phong Quoc Le
Center for Food Safety Analysis of Central Provinces,
Nha Trang Pasteur Institute,
Nha Trang, Vietnam

Ryuji Kawahara
Division of Microbiology,
Osaka Institute of Public Health,
Higashinari, Osaka, Japan

Yuko Kumeda
Research Center for Microbial Control
Osaka Prefecture University
Sakai, Osaka, Japan

Yoshimasa Yamamoto
Graduate School of Pharmaceutical Sciences,
Osaka University,
Suita, Osaka, Japan

INTRODUCTION

The continued emergence of antibiotic-resistant bacteria (ARB) is a serious threat to global health (Al-Sultan *et al.* 2015). In particular, there have been increasing reports of infections caused by extended-spectrum β-lactamase (ESBL)-producing bacteria (ESBL-B), including ESBL-producing *Escherichia coli* (ESBL-E) strains, in developing countries (Sasaki *et al.* 2010). A previous study found that >50% of Vietnamese residents harbored ESBL-E, suggesting a high prevalence of ARB among healthy individuals in Vietnam (Nakayama *et al.* 2015). To reduce the prevalence of ESBL-E colonization, it is necessary to characterize the route through which humans are exposed to these bacteria. Notably, previous reports detected markedly higher frequencies of ESBL-E contamination in foods sold at local Vietnamese markets, compared to foods sold at Japanese markets (Kawamura *et al.* 2014), particularly among chicken products (>80%

doi: 10.2166/wh.2017.054

contamination rate) (Le *et al.* 2015a, 2015b). Although many foods produced in Vietnam are contaminated with ESBL-E, it has been suggested that cooking of these foods should result in a lower prevalence of these strains. Indeed, in a preliminary study, we failed to isolate ESBL-E from certain cooked foods, even when other contaminants were present, suggesting that there might be a low frequency of ESBL-E contamination in the food served in Vietnamese restaurants.

Because daily temperatures in Vietnam typically exceed 25°C year-round, edible ice is commonly consumed. Given that this ice is used without heating or disinfection, it could be a source of ARB contamination. Indeed, previous research demonstrated that ice consumption is a potential vehicle for consumer infection. Gerokomou *et al.* (2011) reported that commercial ice and ice used for fish and seafood were widely contaminated with *E. coli*, *Pseudomonas aeruginosa*, *Yersinia* spp., and *Clostridium perfringens*. Furthermore, antibiotic-resistant *Vibrio* strains were previously isolated from edible ice in Indonesia (Waturangi *et al.* 2013), and ice-cream products were found to harbor *Bacillus cereus* group strains, including strains harboring genes encoding β-lactamases and other virulence determinants (Arsian *et al.* 2014). Thus, foods or beverages that come in to contact with untreated ice can easily become contaminated with bacteria, and can thereby constitute a risk factor for acquiring pathogenic organisms, including ARB.

In Japan, there are few reports of foodborne illness due to consumption of edible ice in foods or beverages, and the percentage of ESBL-E carriage in humans is approximately ten times lower than that in Vietnam (Nakayama *et al.* 2015). The aim of this study was therefore to evaluate the frequency at which edible ice in Vietnam and Japan is contaminated with ESBL-B, and to determine whether contaminated ice is responsible, at least in part, for the ESBL-E carriage rate among Vietnamese citizens.

METHODS

Sample collection

Edible ice samples were collected from 62 restaurants in Vietnam (15 in Hanoi, 13 in Can Tho, 13 in Nha Trang, 12 in Thai Binh, and nine in Ho Chi Min City; Figure 1) between March 2014 and March 2016. For comparison, edible ice was also collected from 26 restaurants in Japan. All restaurants were described as middle class restaurants (3 to 20 US dollars per person) in international tourist books. For collection, edible ice was ordered in a cup, which was wiped by a paper towel containing ethanol, and allowed to melt. The melted water was then sampled using a transport swab with Cary-Blair transport media (Eiken Chemical, Tokyo, Japan) and maintained at 4°C.

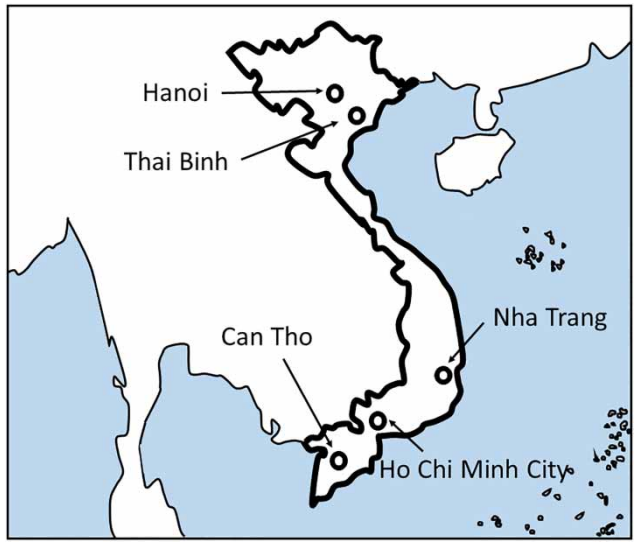

Figure 1 | Locations where edible ice samples were harvested in this study. Samples were obtained from restaurants in Hanoi, Thai Binh, Nha Trang, Ho Chi Minh City, and Can Tho, Vietnam.

Bacterial isolation and identification

Transport swabs were streaked on CHROMagar ECC chromogenic medium (CHROMagar, Paris, France) supplemented with 1 μg/mL of cefotaxime (chromo-CTX) (Kanto Chemical Co., Inc., Tokyo, Japan) and on LB agar plates (Sigma-Aldrich, St Louis, MO, USA). Colonies of distinct phenotypes were picked and subcultured in LB medium (Sigma-Aldrich) at 37°C for 24 h, and genomic DNA was extracted by the boiling method (Sasaki *et al.* 2010). An approximately 1,500 base pair region of the 16S rRNA gene was then amplified from each DNA sample (1 ng/μL) using 16S rRNA-specific primers (forward, 5′-AGA GTT TGA TCC TGG CTC AG-3′; reverse, 5′-TAC GGT TAC CTT GTT ACG ACT T-3′; Gene Design, Osaka, Japan) (Nakayama & Oishi 2013) and a polymerase chain reaction (PCR) thermal cycler device (Takara Bio, Shiga, Japan). The resulting products were purified using a

QIAquick PCR purification kit (Qiagen, Venlo, The Netherlands) and subjected to nucleotide sequencing at Macrogen Japan (Tokyo, Japan). Sequencing results were analyzed using leBIBI software (https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi).

Antibiotic susceptibility

Antibiotic susceptibility was determined by the disk diffusion method using antibiotic disks (Beckton Dickinson and Co., Franklin Lakes, NJ, USA), according to the standard procedure of the Clinical & Laboratory Standards Institute (CLSI) (Cockerill 2011). Strains were cultivated in the presence of antibiotics from the following classes: β-lactams (ampicillin, ceftazidime [CAZ], cefoxitin, and meropenem [MEM]); quinolones (nalidixic acid and ciprofloxacin); aminoglycosides (kanamycin, streptomycin, and gentamicin); tetracycline; phenicols (chloramphenicol); and folic acid inhibitors (trimethoprim-sulfamethoxazole [SXT]). Because *Stenotrophomonas maltophilia* exhibits multi-drug resistance, both levofloxacin (LEV) and minocycline (MIN) were also included. The results of the susceptibility testing were interpreted using CLSI document M100-S24.

Confirmation of ESBL production and genotyping of the bla_{CTX-M}

ESBL production was confirmed by the disk diffusion method using CTX and CAZ, with and without clavulanic acid, as recommended by the CLSI. The quality control strains *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were included in each assay. Positive isolates were identified using the API 20E system (Sysmex-bioMerieux, Marcy l'Etoile, France). The API 20NE system (Sysmex-bioMerieux) was also used for *S. maltophilia* identification.

The following primer sets were used for genotyping of bla_{CTX-M} genes, as described elsewhere (Hon *et al.* 2016): $bla_{CTX-M-1}$ group forward, 5′-GAA TTA GAG CGG CAG TCG GG-3′ and reverse, 5′-CAC AAC CCA GGA AGC AGG C-3′; $bla_{CTX-M-2}$ group forward, 5′-GAT GGC GAC GCT ACC CC-3′ and reverse, 5′-CAA GCC GAC CTC CCG AAC-3′; $bla_{CTX-M-9}$ forward, 5′-GTG CAA CGG ATG ATG TTC GC-3′ and reverse, 5′-GAA GCG TCT CAT CGC CGA TC-3′; and $bla_{CTX-M-8/25}$ group forward, 5′-GCG ACC CGC GCG ATA C-3′ and reverse, 5′-TGC CGG TTT TAT CCC CG-3′. All primers were obtained from Gene Design. The resulting PCR products were visualized by 2% agarose gel electrophoresis and staining with GelRed Nucleic Acid Stain (Biotium, Hayward, CA, USA).

Animal experiments

Specific pathogen-free 12-week-old C57BL/6 mice (Japan Clea, Tokyo, Japan) were acclimatized to standard laboratory conditions and provided free access to rodent chow and water for 1 week. To avoid fighting and follow the guidelines, three female mice were used. Prior to the experiment, transporter swabs used for sampling of Hanoi restaurants were suspended in 5 mL of LB medium and incubated at 37°C for 18 h. Approximately 50 μL of the resulting suspension containing *Acinetobacter* spp., *Pseudomonas* spp. harboring $bla_{CTX-M-9}$, and *S. maltophilia* was subcultured in 5 mL LB medium at 37°C for an additional 18 h, after which the 5 mL of bacterial broth was added to 250 mL of sterile water. Mice were then provided with this contaminated water, and fecal samples were harvested immediately after evacuation. Samples (100 mg each) were homogenized in 500 μL sterile PBS, serially diluted, and spread on chromo-CTX medium. After incubation at 37°C for 18 h, bacterial colony forming units (CFU) were calculated. Colonies were subsequently picked and subjected to bacterial identification and bla_{CTX-M} gene analyses, as described above. The experimental use of the animals in this study was in accordance with the guidelines of the Animal Care Committee of Osaka University.

Statistical analysis

Data were analyzed using the Pearson's chi-square test using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, USA). *P*-values <0.05 were considered statistically significant.

RESULTS

Isolation of bacteria from edible ice

Edible ice samples were harvested from 62 restaurants in Vietnam and 26 restaurants in Japan, and screened for bacteria

capable of growing on chromo-CTX (BG-CTX). In total, 119 and six BG-CTX strains were isolated from 59 (95%) and four (15%) of the restaurants tested in Vietnam and Japan, respectively. Contamination of edible ice with total bacteria and with BG-CTX was significantly more prevalent among restaurants in Vietnam than those in Japan (Table 1).

Characterization of BG-CTX strains

The BG-CTX strains isolated in Vietnam comprised six bacterial genera: *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Enterobacter*, *Aeromonas*, and *Klebsiella* (Table 2). The predominant strains were identified as *Pseudomonas* spp. (48/119; 40%), *Acinetobacter* spp. (47/119; 39%), and *Stenotrophomonas* (14/119; 12%). Specifically, *Acinetobacter* was represented primarily by *A. baumannii* (15/47; 32%) and *A. calcoaceticus* (6/47; 12.8%), while *Pseudomonas* was primarily represented by *P. putida* (9/48; 19%), *Pseudomonas* spp. (8/48; 17%), and *P. aeruginosa* (4/48; 8%) (Figure 3). All 14 strains of *Stenotrophomonas* were identified as *S. maltophilia*. Meanwhile, only two genera, *Acinetobacter* (5/6) and *Pseudomonas* (1/6), were identified in the ice samples harvested in Japan. Of the *Acinetobacter* strains isolated, two were identified as *A. baumannii* and one as *A. junii* (Figure 2).

Table 1 | Frequency of edible ice contamination among restaurants in Vietnam ($n = 62$) and Japan ($n = 26$)

Sites		Total number of samples	Samples contaminated with bacteria	Samples contaminated with BG-CTX
Vietnam	Hanoi	15	15	13
	Thai Binh	12	11	11
	Nha Trang	13	13	13
	Ho Chi Minh City	9	9	9
	Can Tho	13	13	13
	Total	62	61 (98%)[a]	59 (95%)[a]
Japan		26	6 (23%)	4 (15%)

BG-CTX, bacteria growing on media containing cefotaxime.
[a]$P < 0.0001$ compared to restaurants in Japan.

Table 2 | Isolation of six bacteria genera from edible ice samples

		Bacteria isolated from edible ice using CHROMagar containing cefotaxime						
Sites		*Acinetobacter*	*Pseudomonas*	*Stenotrophomonas maltophilia*	*Enterobacter*	*Aeromonas*	*Klebsiella pneumoniae*	Total bacteria
Vietnam	Hanoi	13	6	3	0	0	0	22
	Thai Binh	3	9	2	1	2	0	17
	Nha Trang	8	12	4	1	2	1	28
	Ho Chi Minh City	14	7	1	0	1	1	23
	Can Tho	9	14	4	2	0	0	29
	Total	47 (40%)	48 (40%)	14 (12%)	4 (3%)	5 (4%)	2 (2%)	119
Japan		5 (83%)	1 (17%)	0	0	0	0	6

Evaluation of the antimicrobial susceptibility of strains isolated from edible ice

Among the Vietnamese strains, 15%, 11%, and 21% of the *Acinetobacter* isolates exhibited resistance to CTX, CAZ, and SXT, while 21% of the *Pseudomonas* isolates and 14% of the *S. maltophilia* strains exhibited resistance to MEM and SXT, respectively. Conversely, only one of the five Japanese *Acinetobacter* strains exhibited CTX, CAZ, MEM, and SXT resistance (Table 3).

Detection of $bla_{\text{CTX-M}}$ gene

ESBL-positive strains were subsequently screened for the $bla_{\text{CTX-M}}$ gene. While none of the *Acinetobacter* isolates possessed $bla_{\text{CTX-M}}$ genes, several *Pseudomonas*, *S. maltophilia*, and *Enterobacter* strains were found to possess both the $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ genes. None of the strains harbored the $bla_{\text{CTX-M-2}}$ or $bla_{\text{CTX-M-8}}$ genes (Table 4).

Acquisition of antibiotic resistance by *E. coli* after consumption of contaminated water in mice

To evaluate whether the consumption of contaminated ice/water could lead to the acquisition of antibiotic resistance by

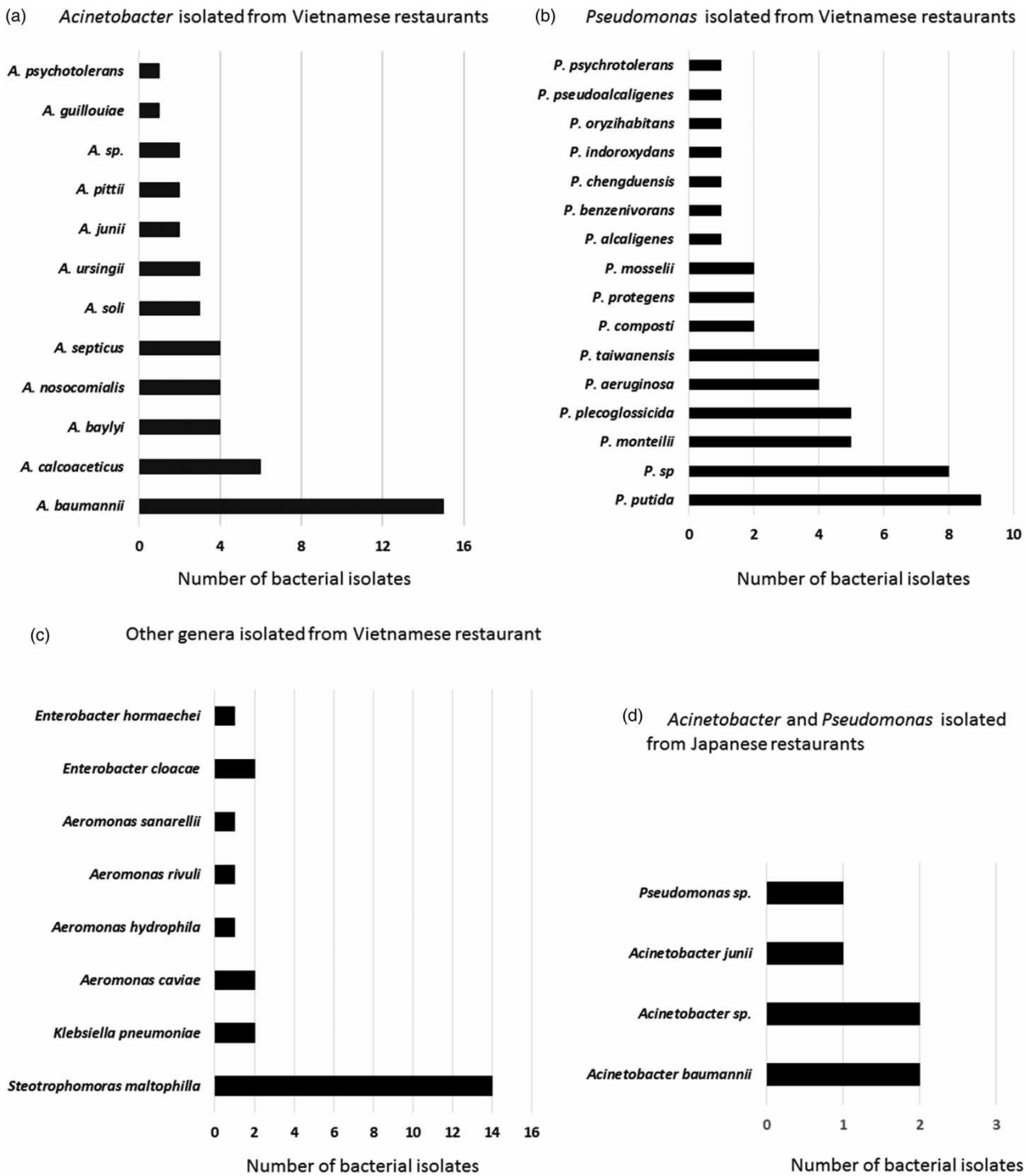

Figure 2 | Prevalence of bacterial species among edible ice samples obtained from Vietnamese restaurants. Ice samples acquired from restaurants in Vietnam and Japan were melted and screened for the presence of bacterial strains capable of growing on agar containing cefotaxime (BG-CTX strains). These strains were then identified by 16S rRNA gene sequencing analysis. Graphic depictions of the prevalence of (a) *Acinetobacter*, (b) *Pseudomonas*, and (c) other genera in ice samples obtained from restaurants in Vietnam. (d) Graphic depiction of the prevalence of *Acinetobacter* and *Pseudomonas* strains in ice samples obtained from restaurants in Japan.

Table 3 | Antibiotic susceptibility of strains (percentage and numbers) isolated from edible ice on agar containing cefotaxime (CTX)

Vietnam

Total number of *Acinetobacter*	Antibiotic susceptibility (%)																	
	CTX			CAZ			MEM			CIP			SXT			GEN		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
47	10.6 (5/47)	74.5 (35/47)	14.9 (7/47)	76.6 (36/47)	12.8 (6/47)	10.6 (5/47)	9 (45/47)	0	2 (2/47)	83 (39/47)	14.9 (7/47)	2.1 (1/47)	63.8 (30/47)	14.9 (7/47)	21.3 (10/47)	97.9 (46/47)	0	2.1 (1/48)

Total number of *Pseudomonas*	Antibiotic susceptibility (%)											
	CAZ			MEM			GEN			CIP		
	S	I	R	S	I	R	S	I	R	S	I	R
48	87.5 (42/48)	8.3 (4/48)	4.2 (2/48)	56.3 (27/48)	22.9 (11/48)	20.8 (10/48)	97.9 (47/48)	2.1 (1/48)	0	91.7 (44/48)	8.3 (4/48)	0

Total number of *S. maltophila*	Antibiotic susceptibility (%)								
	LEV			MIN			SXT		
	S	I	R	S	I	R	S	I	R
14	100 (14/14)	0	0	85.7 (12/14)	14.3 (2/14)	0	10 (10/14)	2 (2/14)	2 (2/14)

Japan

Total number of *Acinetobacter*	Antibiotic susceptibility (%)																	
	CTX			CAZ			MEM			CIP			SXT			GEN		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
5	20 (1/5)	60 (3/5)	20 (1/5)	60 (3/5)	20 (1/5)	20 (1/5)	80 (4/5)	0	20 (1/5)	80 (4/5)	20 (1/5)	0	60 (3/5)	20 (1/5)	20 (1/5)	100 (5/5)	0	0

CAZ, ceftazidime; MEM, meropenem; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; S, susceptible; I, intermediate; R, resistant.

Table 4 | Prevalence of the bla_{CTX-M} gene among strains isolated from edible ice samples

		Acinetobacter	*Pseudomonas*	*Stenotrophomonas maltophilia*	*Enterobacter*	*Aeromonas*	*Klebsiella pneumoniae*	Total bacteria
BG-CTX		47	48	14	4	5	2	119
ESBL-phenotype		10 (21%)	8 (17%)	4 (29%)	2 (50%)	0	0	24 (20%)
CTX-M genotype group	CTX-M-1	0	4	2	1	0	0	7
	CTX-M-2	0	0	0	0	0	0	0
	CTX-M-8	0	0	0	0	0	0	0
	CTX-M-9	0	2	2	1	0	0	5

BG-CTX, bacteria growing on media containing cefotaxime; ESBL, extended-spectrum beta-lactam.

intestinal microbes, mice were provided with water inoculated with BG-CTX strains for 2 weeks. Notably, while none of the mice harbored ESBL-E prior to the experiment (time 0), approximately 8.6×10^3 CFU/mL and 9.0×10^4 CFU/mL BG-CTX were detected at 4 and 7 days post-administration, respectively (Figure 3(a)). BG-CTX strains of *E. coli*, *Enterobacter cloacae*, and *S. maltophilia* were then isolated from mice feces and characterized. Of these, only *E. coli* strains were found to harbor the $bla_{CTX-M-9}$ gene *via* PCR analysis (Figure 3(b)).

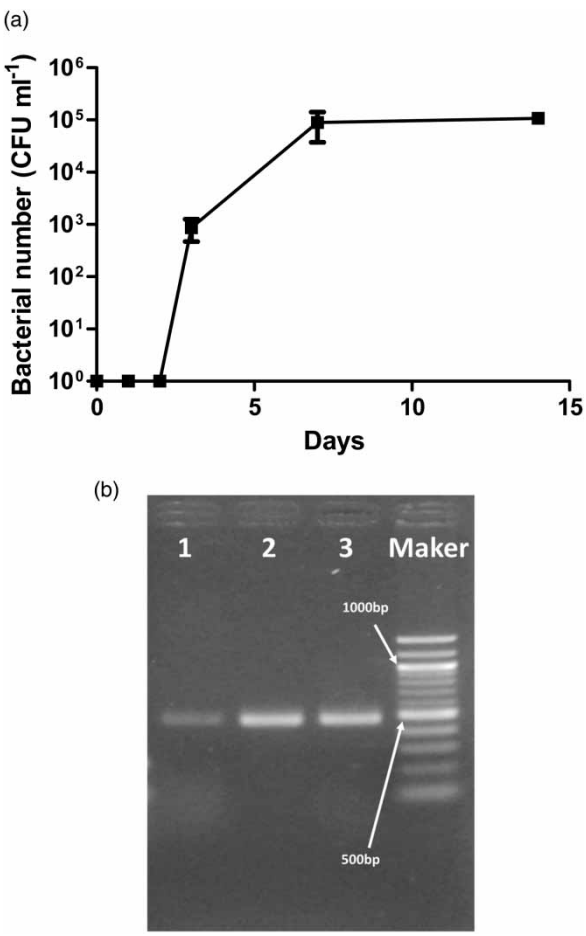

Figure 3 | Acquisition of the $bla_{CTX-M-9}$ gene by intestinal *Escherichia coli* after consumption of edible ice contaminated with antibiotic resistant bacteria (ARB). (a) Total number of BG-CTX strains in mouse feces. (b) Confirmation of the presence of the $bla_{CTX-M-9}$ gene in *E. coli* harvested from mouse feces by PCR, followed by agarose gel electrophoresis, analysis. Lane 1, edible ice; lane 2, *Pseudomonas* spp. isolates from edible ice; lane 3, *E. coli* isolated from feces.

DISCUSSION

There are multiple reports of travelers visiting developing countries and becoming colonized with ESBL-E strains (Paltansing *et al.* 2013; Yaita *et al.* 2014). It is suspected that this colonization occurs via consumption of foods contaminated with ESBL-E; however, the foods served in developing countries are typically subjected to heat treatment (i.e., boiling, grilling, or frying), which should be sufficient to prevent the spread of these organisms. Indeed, in preliminary experiments, we failed to detect abundant contamination of cooked foods with ESBL-E in Vietnam. Likewise, ESBL-E strains were not detected among edible ice samples. We, therefore, expanded our initial investigation to characterize the bacterial contaminants of edible ice, and to determine whether these contaminating bacteria harbor ESBL-related genes that could be transmitted to *E. coli* within the human gut.

Because Vietnam is located in the tropics, the use of ice is a common part of daily life; however, the handling of these ice products is typically rough, and hygiene is often neglected or insufficient. According to information from local people, many wholesalers in Vietnam will transport edible ice blocks on cargo truck beds, covered with straw.

Subsequently, upon delivery, restaurants will often store the ice in buckets on the floor. The restaurant workers then serve the ice in beverages such as water, tea, and beer. For comparison with the ice served in Vietnam, we used ice samples taken from restaurants in Japan. Nearly all of these restaurants used ice machines for ice production, with many being capable of automatically dispensing ice into the cup, thereby eliminating human handling. However, several samples were obtained from restaurants in which ice-machines were used, but the ice was subsequently stored in an uncovered bucket. Notably, these were the samples from which ARB strains were isolated. Based on these findings, it is evident that hygienic conditions are essential for preventing contamination of ice by ARB.

In this study, to investigate whether consumption of contaminated ice can result in the acquisition of antibiotic resistance by intestinal bacteria, mice were given water spiked with BG-CTX isolated from Vietnamese ice samples. Remarkably, after the mice had consumed the contaminated water, we isolated intestinal *E. coli* strains that had acquired the $bla_{\text{CTX-M-9}}$ gene, indicating that exposure to ice/water containing ARB harboring this gene might result in ESBL-E carriage in humans.

Developing countries often have problems with bacterial contamination of ice or water. For example, multidrug-resistant *Vibrio cholerae* was isolated from edible ice in Indonesia (Waturangi *et al.* 2013), while drinking water systems in Iran were found to be polluted by enterococci (Enayati *et al.* 2015). In addition, multidrug-resistant *Pseudomonas* spp. were detected in drinking water distribution systems located in southwest Nigeria (Adesoji *et al.* 2015). In the current study, we also isolated enterococci from ice samples in Vietnam, which was likely the result of fecal contamination. Notably, there have been numerous reports in recent years of the emergence of carbapenem-resistant *Pseudomonas* and *Acinetobacter* strains (Dahdouh *et al.* 2014; Kateete *et al.* 2016). As such, special attention should be paid to identifying and eliminating contamination by these genera. Interestingly, in this study, *Acinetobacter* strains were predominant in samples harvested from megacities (Hanoi and Ho Chi Minh City), while *Pseudomonas* was predominant in samples harvested from rural core cities (Thai Binh, Nha Trang, and Can Tho). Moreover, while only six contaminants were isolated from Japanese ice samples, five of these strains were *Acinetobacter*. *Pseudomonas* is highly prevalent in river water and soils, and *P. putida*, the predominant species isolated from edible ice in Vietnam, is a typical soil-dwelling organism (Iyer & Damania 2016; Morimoto *et al.* 2016). In a previous study (Nakayama *et al.* 2017), *Pseudomonas* was significantly more prevalent than *Acinetobacter* in the Vietnamese water system (Supplement Figure 1, available with the online version of this paper), indicating that *Pseudomonas*-specific hygiene is less effective than that for *Acinetobacter*.

In addition to its importance for local citizens, clean drinking water is also a concern for travelers and tourists. In previous reports, bacteria, pesticides, and arsenic were detected in tap water and/or water stored for drinking in Vietnam (Seino *et al.* 2008; Agusa *et al.* 2014; Chau *et al.* 2015; Grady *et al.* 2015). Thus, travelers hesitate to use these sources of water because of concern for their health; however, while these individuals typically use bottled water for drinking, little is known regarding the microbial quality of this water. To address this point, we performed a preliminary evaluation of the levels of bacterial contamination of bottled drinking water and tap water in Vietnam. Notably, no bacterial contaminants were isolated from the 14 bottles of water purchased from Hanoi, Thai Binh, Ho Chi Minh City, and Can Tho. Furthermore, only one of the nine Vietnamese hotels evaluated (Hanoi, Thai Binh, Nha Trang, Ho Chi Minh City, and Can Tho) exhibited contamination of tap water with BG-CTX (*A. junii* and *Pseudomonas alcaligenes*). However, while no BG-CTX strains were isolated from the Hanoi and Thai Binh hotels, *Sphingomonas mucosissima* and *Staphylococcus sciuri* were detected in the water sampled from these hotels, respectively. Based on these findings, there is not widespread contamination of bottled water and tap water in hotels in Vietnam. Thus, the contamination of ice with bacteria in Vietnamese restaurants is likely due, at least in part, to improper handling by staff members. As such, national regulatory guidelines are needed to prevent such contamination with ARB.

In 2009, the Japanese Official Development Assistance program was initiated to support the improvement of infrastructure for ensuring water quality in Ho Chi Min City. The goals of this project include enhancing the clean water supply capacity, and improving issues with water leakage, low water pressure, and hygiene. Thus, improvements in water quality are currently underway in this country.

CONCLUSIONS

Approximately 95% of the Vietnamese restaurants evaluated in this study were found to have ice contaminated with BG-CTX strains, particularly *Acinetobacter* spp., *Pseudomonas* spp., and *S. maltophilia*. Of these strains, 10% exhibited the ESBL phenotype. Remarkably, administration of ESBL-B derived from contaminated ice to mice resulted in the emergence of intestinal ESBL-E strains. Thus, consumption of contaminated edible ice might comprise a significant risk factor for the emergence and colonization of humans with ESBL-B.

ACKNOWLEDGEMENTS

This work was supported by the Japan Agency for Medical Research and Development (AMED)/Japan International Cooperation Agency (JICA), as part of the Science and Technology Research Partnership for Sustainable Development (SATREPS).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Adesoji, A. T., Ogunjobi, A. A. & Olatoye, I. O. 2015 Molecular characterization of selected multidrug resistant *Pseudomonas* from water distribution systems in southwestern Nigeria. *Ann. Clin. Microbiol. Antimicrob.* **14**, 39.

Agusa, T., Trang, P. T., Lan, V. M., Anh, D. H., Tanabe, S., Viet, P. H. & Berg, M. 2014 Human exposure to arsenic from drinking water in Vietnam. *Sci. Total Environ.* **488–489**, 562–569.

Al-Sultan, A. A., Evans, B. A., Aboulmagd, E., Al-Qahtanti, A. A., Bohol, M. F., Al-Ahdal, M. N., Opazo, A. F. & Amyes, S. G. 2015 Dissemination of multiple carbapenem-resistant clones of *Acinetobacter baumannii* in the eastern district of Saudi Arabia. *Front Microbiol.* **2**, 634.

Arsian, S., Eyi, A. & Kucuksari, R. 2014 Toxigenic genes, spoilage potential, and antimicrobial resistance of *Bacillus cereus* group strains from ice cream. *Anaerobe* **25**, 42–46.

Chau, N. D., Sebesvari, Z., Amelung, W. & Renaud, F. G. 2015 Pesticide pollution of multiple drinking water sources in the Mekong delta, Vietnam: evidence from two provinces. *Environ Sci. Pollut. Res. Int.* **22**, 9042–9058.

Cockerill, F. R. 2011 Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S21. CLSI, PA, USA.

Dahdouh, E., Shoucair, S. H., Salem, S. E. & Daoud, Z. 2014 Mutant prevention concentrations of imipenem and meropenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Scientific World Journal* **2014**, 979648.

Enayati, M., Sadeghi, J., Nahaei, M. R., Aghazadeh, M., Porshafie, M. R. & Talebi, M. 2015 Virulence and antimicrobial resistance of *Enterococcus faecium* isolated from water samples. *Lett. Appl. Microbiol.* **61**, 339–345.

Gerokomou, V., Voidarou, C., Vatopoulos, A., Velonakis, E., Rozos, G., Alexopoulos, A., Plessas, S., Stavropoulou, E., Bezirtzoglou, E., Demertzis, P. G. & Akrida-Demertzi, K. 2011 Physical, chemical and microbiological quality of ice used to cool drinks and foods in Greece and its public health implications. *Anaerobe* **17**, 351–353.

Grady, C. A., Kipkorir, E. C., Nguyen, K. & Blatchley, E. R. 3rd 2015 Microbial quality of improved drinking water sources: evidence from western Kenya and southern Vietnam. *J. Water Health* **13**, 607–612.

Hon, N. T. N., Hoa, T. T. T., Thinh, N. Q., Hinenoya, A., Nakayama, T., Harada, K., Asayama, M., Warisaya, M., Hirata, K., Phuong, N. T. & Yamamoto, Y. 2016 Spread of antibiotic and antimicrobial susceptibility of ESBL-producing Escherichia coli isolated from wild and cultured fish in the Mekong Delta, Vietnam. *Fish Pathol.* **51**, 575–582.

Iyer, R. & Damania, A. 2016 Draft genome sequence of *Pseudomonas putida* CBF 10-2, a soil isolate with bioremediation potential in agricultural and industrial environmental settings. *Genome Announc.* **4**, e00670–16.

Kateete, D. P., Nakanjako, R., Namugenyi, J., Erume, J., Joloba, M. L. & Najjuka, C. F. 2016 Carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* at Mulago Hospital in Kampala, Uganda (2007–2009). *Springerplus* **5**, 1308.

Kawamura, K., Goto, K., Nakane, K. & Arakawa, Y. 2014 Molecular epidemiology of extended-spectrum beta-lactamase and *Escherichia coli* isolated from retail foods including chicken meat in Japan. *Foodborne Pathog. Dis.* **11**, 104–110.

Le, Q. P., Ueda, S., Nguyen, T. N. H., Dao, T. V. K., Khanh, D. T. V., Van, H. T. A., Tran, T. T. N., Hirai, I., Nakayama, T., Kawahara, R., Do, T. H., Vien, Q. M. & Yamamoto, Y. 2015a Characteristic of extended spectrum beta-lactamase-producing *Escherichia coli* in retail meats and shrimp at a local market in Vietnam. *Foodborn Pathog. Dis.* **12**, 719–725.

Le, H. V., Kawahara, R., Khong, D. T., Tran, H. T., Nguyen, T. N., Phan, K. N., Jinnai, M., Kumeda, Y., Nakayama, T., Ueda, S. & Yamamoto, Y. 2015b Widespread dissemination of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in livestock and fishery products in Vietnam. *Int. J. Food Contaminat.* **2**, 17.

Morimoto, H., Kadoya, R., Takahashi, K. & Kasahara, Y. 2016 Proteome analysis of *Pseudomonas putida* F1 genes induced in soil environments. *Environ. Microbiol. Rep.* **8**, 825–832.

Nakayama, T. & Oishi, K. 2013 Influence of coffee (*Coffea arabica*) and galacto-oligosaccharide consumption on intestinal microbiota and the host responses. *FEMS Microbiol. Lett.* **343**, 161–168.

Nakayama, T., Ueda, S., Huong, B. T. M., Tuyen, L. D., Komalamisra, C., Kusolsuk, T., Hirai, I. & Yamamoto, Y. 2015 Wide dissemination of extended spectrum beta-lactamase-producing *Escherichia coli* in community residents in the Indochinese peninsula. *Infect. Drug Resist.* **23**, 1–5.

Nakayama, T., Hoa, T. T. T., Harada, K., Warisaya, M., Asayama, M., Hinenoya, A., Lee, J. W., Phu, T. M., Ueda, S., Sumimura, Y., Hirata, K., Phuong, N. T. & Yamamoto, Y. 2017 Water metagenomic analysis reveals low bacterial diversity and the presence of antimicrobial residues and resistance genes in a river containing wastewater from backyard aquacultures in the Mekong Delta, Vietnam. *Environ. Pollut.* **222**, 294–306.

Paltansing, S., Vlot, J. A., Krakman, M. E. M., Mesman, R., Bruijning, M. L., Bernards, A. T., Visser, L. G. & Veldakamp, K. E. 2013 Extended-spectrum β-lactamase-producing Enterobacteriaceae among travelers from the Netherlands. *Emerg. Infect. Dis.* **19**, 1206–1213.

Sasaki, T., Hirai, I., Niki, M., Nakamura, T., Komalamisra, C., Maipanich, W., Kusolsuk, T., Sanguankiat, S., Pubampen, S. & Yamamoto, Y. 2010 High prevalence of CTX-M beta-lactamase-producing Enterobacteriaceae in stool specimens obtained from healthy individuals in Thailand. *J. Antimicrob. Chemother.* **65**, 666–668.

Seino, K., Takano, T., Quang, N. K., Watanabe, M., Inose, T. & Nakamura, K. 2008 Bacterial quality of drinking water stored in containers by boat households in Hue city, Vietnam. *Environ. Health Prev. Med.* **13**, 198–206.

Waturangi, D. E., Subartono, M. X. & Wijaya, Y. 2013 Edible ice in Jakarta, Indonesia, is contaminated with multidrug-resistant *Vibrio cholera* with virulence potential. *J. Med. Microbiol.* **62**, 352–359.

Yaita, K., Suzuki, T., Nakaharai, K., Yoshimura, Y., Harada, S., Ishii, Y. & Tachikawa, N. 2014 Epidemiology of extended-spectrum beta-lactamase producing *Escherichia coli* in the stools of returning Japanese travelers, and the risk factors for colonization. *PLoS One* **9**, e98000.

First received 7 March 2017; accepted in revised form 30 June 2017. Available online 28 August 2017