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Edible river fish-derived extended-spectrum β-lactamase (ESBL)-producing Enterobacterales harboring transferable plasmids encoding $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$, and $bla_{CTX\text{-}M\text{-}55}$

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ABSTRACT

Transmission of extended-spectrum β-lactamase (ESBL) genes has increased the global prevalence of ESBL-producing bacteria, especially in developing countries. Human infection with these bacteria may be food-mediated but has not been fully elucidated. Therefore, we aimed to examine ESBL-producing bacteria in edible river fish and elucidate their potential for horizontal gene transfer. A total of 173 ESBL-producing Enterobacterales were isolated (*Escherichia coli* [$n = 87$], *Klebsiella pneumoniae* [$n = 52$], *Enterobacter cloacae* complex [$n = 18$], *Citrobacter freundii* complex [$n = 14$], *Atlantibacter hermannii* [n = 1] and *Serratia fonticola* [n = 1]) from 56 of 80 fish intestinal contents sampled. Among the bacterial $bla_{CTX\text{-}M}$ genotypes, $bla_{CTX\text{-}M\text{-}55}$ was the most predominant, followed by $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$, and $bla_{CTX\text{-}M\text{-}65}$. Furthermore, we found that ESBL-producing Enterobacterales were able to transfer their $bla_{CTX\text{-}M}$ genes to *E. coli*. In summary, our results suggest that ESBL-producing Enterobacterales transfer $bla_{CTX\text{-}M}$ to indigenous gut *E. coli* in humans, following the consumption of contaminated fish.

1. Introduction

Extended-spectrum β-lactamases (ESBL) are enzymes that degrade antibiotics with β-lactam rings, up to third-generation cephalosporins, and the genes are transmitted across species via plasmids [1]. ESBL-producing Enterobacterales (ESBL-EN) were first reported in Europe during the early 1980s and have since spread globally, becoming more prevalent in developing countries [2,3]. ESBL-producing bacteria are known to cause nosocomial infections, urinary tract infections, and bacteremia [2,4,5]. Hence, attention should be paid to ESBL-producing bacteria carried by humans.

In Vietnam from 2015 to 2022, AmpC/ESBL-producing bacteria were detected in 93% of chicken meat, 51% of residents, and 39% of edible fish, and ESBL-genes in the river [6–9]. Currently, there is insufficient scientific data available to establish a link between food and human carriers of ESBL-producing bacteria worldwide, including Vietnam. Therefore, in this study, we aimed to examine and detect ESBL-EN in edible river fish and elucidate their potential to transfer genes to *E. coli* in the human gut.

2. Materials and methods

2.1. Isolation and identification of Enterobacterales

A total of 80 edible river fish were purchased from local markets in Ho Chi Minh City, Vietnam, in March 2020 (Supplementary Table 1). Intestinal contents were aseptically removed (1 g and 5 g samples) and incubated in buffered peptone water (9 mL and 45 mL, respectively;

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Merck, Darmstadt, Germany). After incubation at 37 °C for 22 h, 10 μL of bacterial broth was spread on CHROMagar ECC (CHROMagar, Paris, France) containing 2 mg/L of cefotaxime (CTX). One to three blue or mauve colonies were picked and inoculated on Mueller-Hinton agar (Becton, Dickinson, NJ, USA) for further identification using matrix-assisted laser desorption/ionization time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).

Bacterial DNA was extracted using alkaline heat extraction. Briefly, bacterial cells were suspended in 100 μL of 25 mM NaOH and incubated at 95 °C for 10 min. After incubation, 8 μL of 1 M Tris-HCl was added. The solution was centrifuged at 12000 ×*g* for 10 min, and the supernatant was used as the template DNA. To identify *E. coli*, PCR was performed according to the method published in a previous study [10].

2.2. ESBL confirmation and antibiotic susceptibility tests

The presence of the ESBL phenotype was confirmed using CTX (30 μg) and ceftazidime (30 μg) with and without clavulanic acid (10 μg). In addition, antibiotic susceptibility testing for ampicillin (10 μg), cefoxitin (30 μg), meropenem (10 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), nalidixic acid (30 μg), trimethoprim-sulfamethoxazole (23.75/1.25 μg), chloramphenicol (30 μg), and fosfomycin (50 μg) was performed. Both the ESBL phenotype confirming test and the antibiotic susceptibility test were performed using the disk diffusion method recommended by the Clinical and Laboratory Standard Institute (M100-S23) [11].

2.3. ESBL genotyping and identification in Enterobacterales

The multiplex PCR assay described by Le et al. was used to detect *bla* genes including $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, and $bla_{CTX\text{-}M\text{-}25}$ groups, bla_{SHV}, and bla_{TEM} [12]. Multiplex PCR was performed using 1 μL of template DNA and the QIAGEN Multiplex PCR kit (QIAGEN, Hilden, Germany). In case the multiplex PCR shows positive bands of the $bla_{CTX\text{-}M}$ groups, PCR suitable for each $bla_{CTX\text{-}M}$ group and sequencing of the PCR products were performed using the previously reported method (Supplementary Table 2) [13–16]. Basic local alignment search was performed using the obtained sequence to determine the $bla_{CTX\text{-}M}$ genotype (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4. Multiplex PCR for replicon typing and phylogenetic grouping

The strains carrying the $bla_{CTX\text{-}M}$ gene underwent replicon typing, a method that identifies plasmid incompatibility based on replication regions. Replicon typing was performed using a PCR-based method from a previous report, which classified the types into FIA, FIB, FIC, HI1, HI2, I1-1γ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA [17].

E. coli phylogenetic grouping was also performed using multiplex PCR with primer pairs ChuA.1 and ChuA.2, YjaA.1 and YjaA.2, and TspE4C2.1 and TspE4C2.2. The protocol that was followed was reported previously [18]. Primer information is provided in Supplementary Table 2.

2.5. Conjugation assay

All ESBL-producing isolates in this study, except *E. coli,* were used as donors and *E. coli* ATCC 25922 and TB1 (an environmental strain from Vietnam) were used as recipients in intergeneric conjugation experiments. The cells were inoculated in Mueller-Hinton broth (Becton, Dickinson and Company, NJ, USA) and incubated overnight at 37 °C. The proliferated cells were pelleted by centrifugation and subsequently adjusted to McFarland 1.0 with Luria–Bertani broth, which includes 1 L of distilled water, 10 g of NaCl, 10 g of tryptone (Becton, Dickinson and Company), and 5 g of yeast extract (Becton, Dickinson and Company) [19]. Ten microliters of the broth containing the recipient cells were spotted and fully penetrated on Mueller-Hinton agar. Subsequently, the same amount of donor cells were spotted on the same position of the recipient cells, and incubated for 24 h at 37 °C. After incubation, the cells of the conjugation plate were spread on CHROMagar ECC containing 2 mg/L of CTX and incubated at 37 °C for 22 h to screen for $bla_{CTX\text{-}M}$-transmitted *E. coli*. Single blue colonies were picked and their DNA was extracted using alkaline heat extraction. To confirm the gene transfer, *bla* genes of acquired *E. coli* colonies were detected using multiplex PCR described above [12].

3. Results

3.1. ESBL-EN members in the intestinal contents of freshwater fish

A total of 173 ESBL-EN isolates were obtained from 56 (70.0%) of the 80 fish intestinal content samples. *Escherichia coli* was the most abundant (52.5%), followed by *K. pneumoniae* (35.0%,), *Enterobacter cloacae* complex (15.0%), *Citrobacter freundii* complex (11.3%), *Atlantibacter hermannii* (1.3%), and *Serratia fonticola* (1.3%) (Table 1). ESBL-EN were detected in 53.3–90.0% of the samples of edible freshwater fish species examined, excluding *Cynoglossidae* and *Mugil* spp. In all fish species positive for ESBL-EN, *E. coli* was most common (40.0–70.0%), followed by *K. pneumoniae* (6.7–50.0%) (Supplementary Table1).

3.2. Antibiotic susceptibility testing

Among the ESBL-EN isolates, those resistant to ampicillin were the most common (100.0%), followed by those resistant to CTX (97.7%), tetracycline (85.5%), trimethoprim-sulfamethoxazole (76.9%), chloramphenicol (72.3%), nalidixic acid (59.0%), streptomycin (57.8%), and ciprofloxacin (50.3%). Meropenem-resistant isolates were not detected (Table 1). Of the 173 ESBL-EN isolates, 161 (93.1%) were resistant to three or more antibiotic classes, making them multidrug-resistant bacteria. The highest proportion of multidrug-resistant bacteria was found in *K. pneumoniae* (51/51; 100%) and *C. freundii* complex (14/14; 100%), followed by *E. coli* (80/87; 92.0%) and *E. cloacae* complex (16/19; 84.2%). Both *A. hermannii* and *S. fonticola* were resistant to two antibiotic classes, and thus, were not multidrug-resistant strains (Supplementary Fig. 1).

3.3. ESBL genotypes and replicon typing of Enterobacterales

ESBL-related genes were identified. The results showed that $bla_{CTX\text{-}M\text{-}55}$ was the most predominant in ESBL-producing *E. coli* (71.1%), *Klebsiella* (26.9%), *Enterobacter* (38.9%), and *Citrobacter* (50%). In most cases, ESBL-EN contained $bla_{CTX\text{-}M\text{-}55}$ (47.4%), followed by $bla_{CTX\text{-}M\text{-}15}$ (17.3%) and $bla_{CTX\text{-}M\text{-}27}$ (9.2%) (Table 1).

3.4. Multiple ESBL-EN isolation in same fish

Multiple ESBL-EN were isolated from 14 fish (17.5%) (Table 2). In fish no. 16, ESBL-producing *E. coli* strain 175 and *K. pneumoniae* strain 173 co-harbored both bla_{TEM} and $bla_{CTX\text{-}M\text{-}55}$, and plasmid replicon type F. In fish no. 37B, ESBL-producing *E. coli* strain 256 and 257 also harbored $bla_{CTX\text{-}M\text{-}55}$ and plasmid replicon type FIB and F. The phylogenetic group of *E. coli* strain 256 was type D, which differed from type B1 of *E. coli* strain 257 (Table 2).

3.5. Transfer of $bla_{CTX\text{-}M}$ to *E. coli*

Conjugation assays showed $bla_{CTX\text{-}M}$ transfer from 1 (1.2%) and 6 (7.0%) of 86 ESBL-EN strains, excluding *E. coli*, to *E. coli* ATCC25955 and TB1, respectively. Out of 80 samples of edible fish, 5 samples (6.3%) were detected with these Enterobacterales that carried transferable $bla_{CTX\text{-}M}$ to *E. coli* (Table 1).

Table 1
Breakdown of bla_{CTX-M} type, antibiotic resistance, and transmission rate to *E. coli* in ESBL-producing Enterobacterales in this study.

	Percentage of the edible fish isolating ESBL-producing bacteria isolates (%)	Percentage of the edible fish containing ESBL-producing bacteria transmissison to *E. coli*	Number of bacteria isolates	Percentage of indentification bla_{CTX-M} (%)					Percentage of antibiotic resistance (%)														Percentage of bla_{CTX-M} transmission to *E. coli* (%)	
				$bla_{CTX-M-1}$ group		$bla_{CTX-M-9}$ group		Others	β-lactams					Quinolones		Aminoglycosides			Folic acid inhibitors	Tetracycline	Phenicols	Fosfomycins		
				$bla_{CTX-M-15}$	$bla_{CTX-M-55}$	$bla_{CTX-M-27}$	$bla_{CTX-M-65}$		AMP	CTX	CAZ	CFX	MEM	NAL	CIP	STR	KAN	GEN	SXT	TET	CHL	FOS	ATCC 25955	TB1
ESBL-producing *E. coli*	52.5 (42/80)	-	87	9.2 (8/87)	62.1 (54/87)	3.4 (3/87)	10.3 (9/87)	14.9 (13/87)	100 (87/87)	97.7 (85/87)	24.1 (21/87)	13.8 (12/87)	0	69 (60/87)	64.4 (56/87)	60.9 (53/87)	40.2 (35/87)	49.4 (43/87)	73.6 (64/87)	86.2 (75/87)	79.3 (69/87)	27.6 (24/87)	-	-
ESBL-producing *Klebsiella*	35 (28/80)	2.5 (2/80)	52	32.7 (17/52)	26.9 (14/52)	11.5 (6/52)	3.8 (2/52)	25 (13/52)	100 (52/52)	100 (52/52)	26.9 (14/52)	17.3 (9/52)	0	51.9 (27/52)	48.1 (25/52)	57.7 (30/52)	51.9 (27/52)	53.8 (28/52)	96.2 (50/52)	96.2 (50/52)	69.2 (35/52)	75 (39/52)	1.9 (1/52)	1.9 (1/52)
ESBL-producing *Enterobacter*	15 (12/80)	2.5 (2/80)	18	22.2 (4/18)	38.9 (7/18)	16.7 (3/18)	0	22.2 (4/18)	100 (18/18)	100 (18/18)	33.3 (6/18)	100 (18/18)	0	33.3 (6/18)	27.8 (5/18)	27.8 (5/18)	5.6 (1/18)	27.8 (5/18)	61.1 (11/18)	50 (9/18)	38.9 (7/18)	61.1 (11/18)	0	16.7 (3/18)
ESBL-producing *Citrobacter*	11.3 (9/80)	1.3 (1/80)	14	7.1 (1/14)	50 (7/14)	21.4 (3/14)	0	21.4 (3/14)	100 (14/14)	85.7 (12/14)	35.7 (5/14)	100 (14/14)	0	64.3 (9/14)	7.1 (1/14)	85.7 (12/14)	28.6 (4/14)	71.4 (10/14)	92.9 (13/14)	100 (14/14)	92.9 (13/14)	0	0	14.3 (2/14)
ESBL-producing *A. hermannii*	1.3 (1/80)	0	1	0	0	100 (1/1)	0	0	100 (1/1)	100 (1/1)	0	0	0	0	0	0	0	0	100 (1/1)	0	0	0	0	0
ESBL-producing *S. fonticola*	1.3 (1/80)	0	1	0	0	0	0	100 (1/1)	100 (1/1)	100 (1/1)	0	100 (1/1)	0	0	0	0	0	0	100 (1/1)	0	0	0	0	0
ESBL-producing bacteria	70 (56/80)	6.3 (5/80)	173	17.3 (30/173)	47.4 (82/173)	9.2 (16/173)	6.4 (11/173)	19.7 (34/173)	100 (173/173)	97.7 (169/173)	26.6 (46/173)	31.2 (54/173)	0	59 (102/173)	50.3 (87/173)	57.8 (100/173)	38.7 (67/173)	49.7 (86/173)	76.9 (133/173)	85.5 (148/173)	72.3 (125/173)	42.8 (74/173)	1.2 (1/86)	7 (6/86)

AMP:ampicillin, CTX: cefotaxime, CAZ: ceftazidime, CFX: cefoxitin, MEM: meropenem, NAL: nalidixic acid, CIP: ciprofloxacin, STR: streptomycin, KAN: kanamycin, GEN: gentamicin, SXT: trimethoprim/sulfamethoxazole, TET: tetracycline, CHL: chloramphenicol, FOS: fosfomycin

Table 2
Multiple ESBL-producing Enterobacterales isolated from same edible fish.

Fish No.	Strain No.	Bacterial species	Antibiotic susceptibility														Sub-group of ESBL-related gene	Identification of bla_{CTX-M}	Replicon typing	*E. coli* phylogenetic group
			β-lactam					Quinolone		Aminoglycoside										
			AMP	CTX	CAZ	CFX	MEM	NAL	CIP	STR	KAN	GEN	SXT	TET	CHL	FOS				
5B	123	*Klebsiella pneumoniae*	R	R	S	S	S	S	S	R	S	S	R	R	S	I	SHV, TEM, CTX-M-1g	$bla_{CTX-M-15}$	N.D.	-
5B	128re	*Escherichia coli*	R	R	I	S	S	S	S	S	S	S	S	S	S	S	CTX-M-1g	$bla_{CTX-M-15}$	N.D.	B1
16B	173	*Klebsiella pneumoniae*	R	R	I	S	S	I	S	R	S	R	R	R	R	R	SHV, TEM, CTX-M-1g	$bla_{CTX-M-55}$	FIB,F	-
16B	174	*Klebsiella pneumoniae*	R	R	R	R	S	R	R	R	R	S	R	R	R	R	SHV, TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
16B	175	*Escherichia coli*	R	R	I	I	S	R	S	R	S	R	R	R	R	R	TEM, CTX-M-1g	$bla_{CTX-M-55}$	F	A
23B	204	*Klebsiella pneumoniae*	R	R	I	S	S	R	R	R	R	R	R	R	R	R	SHV, CTX-M-1g	$bla_{CTX-M-15}$	N.D.	-
23B	205	*Escherichia coli*	R	R	S	S	S	S	S	S	S	S	S	S	S	S	CTX-M-1g	$bla_{CTX-M-15}$	N.D.	B1
23B	206	*Escherichia coli*	R	R	I	S	S	S	S	S	S	S	S	S	S	S	CTX-M-1g	$bla_{CTX-M-15}$	N.D.	B1
27B	217①	*Citrobacter freundii* complex	R	R	I	R	S	R	I	R	R	R	R	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
27B	218	*Citrobacter freundii* complex	R	R	I	R	S	I	S	R	R	R	R	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
27B	219	*Citrobacter freundii* complex	R	R	R	R	S	R	I	R	R	R	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
27B	220	*Escherichia coli*	R	R	I	I	S	R	R	R	I	S	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	FIB, Y, F	A
27B	221	*Escherichia coli*	R	R	I	R	S	R	R	R	S	S	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	FIB, Y, F	A
27B	222	*Escherichia coli*	R	R	I	S	S	R	R	R	I	S	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	FIB, Y, F	A
29B	229	*Citrobacter freundii* complex	R	R	I	R	S	I	S	S	S	R	S	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
29B	230	*Citrobacter freundii* complex	R	R	I	R	S	I	S	S	S	R	S	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
29B	231	*Citrobacter freundii* complex	R	R	R	R	S	I	S	S	S	R	S	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
29B	232	*Escherichia coli*	R	R	S	S	S	R	R	R	I	S	R	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	FIB, Y, F	A
29B	233	*Escherichia coli*	R	R	S	S	S	R	R	R	I	S	R	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	FIB, Y, F	A
32B	236	*Klebsiella pneumoniae*	R	R	R	R	S	R	R	R	R	R	R	R	R	R	SHV, TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
32B	238	*Escherichia coli*	R	R	I	S	S	I	S	S	S	S	S	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	HI1	A
32B	239	*Escherichia coli*	R	R	I	S	S	S	S	S	S	S	S	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	HI1, A/C	A
32B	240	*Escherichia coli*	R	R	S	S	S	I	S	S	S	S	S	R	R	I	TEM, CTX-M-1g	$bla_{CTX-M-55}$	HI1	A
37B	253	*Citrobacter freundii* complex	R	R	R	R	S	R	I	R	R	R	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
37B	256	*Escherichia coli*	R	R	R	R	S	R	R	R	R	R	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	FIB, F	D
37B	257	*Escherichia coli*	R	R	I	S	S	R	R	S	S	S	S	R	R	R	CTX-M-1g	$bla_{CTX-M-55}$	FIB, I1-γ, F	B1
44B	273①	*Enterobacter cloacae* complex	R	R	R	R	S	S	S	S	S	S	S	S	S	I	CTX-M-1g	$bla_{CTX-M-15}$	N.D.	-
44B	274	*Enterobacter cloacae* complex	R	R	R	R	S	I	S	S	S	S	S	S	S	R	CTX-M-1g	$bla_{CTX-M-15}$	N.D.	-
44B	275	*Enterobacter cloacae* complex	R	R	R	R	S	I	S	R	S	S	R	S	S	R	CTX-M-1g	$bla_{CTX-M-15}$	N.D.	-
55B	309	*Enterobacter cloacae* complex	R	R	I	R	S	R	R	R	R	R	R	R	R	R	CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
55B	310	*Klebsiella pneumoniae*	R	R	R	S	S	R	R	R	R	R	R	R	R	I	SHV, TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
55B	312	*Escherichia coli*	R	R	R	R	S	R	R	R	R	R	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	A
55B	313	*Escherichia coli*	R	R	R	R	S	R	R	R	R	R	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	A
56B	314	*Enterobacter cloacae* complex	R	R	R	R	S	R	R	R	I	R	R	R	R	I	TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
56B	316	*Escherichia coli*	R	R	I	S	S	R	R	R	R	R	R	R	R	R	TEM, CTX-M-1g	$bla_{CTX-M-55}$	F	A
56B	317	*Escherichia coli*	R	R	S	S	S	R	R	R	I	R	R	R	R	R	CTX-M-1g	$bla_{CTX-M-55}$	F	A
57B	319	*Klebsiella pneumoniae*	R	R	R	R	S	R	R	R	R	R	R	R	R	R	SHV, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
57B	320	*Citrobacter freundii* complex	R	R	S	R	S	S	S	S	S	S	S	R	S	S	CTX-M-1g	$bla_{CTX-M-15}$	N.D.	-
57B	321	*Escherichia coli*	R	R	I	S	S	I	S	S	S	S	S	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	X	B1
57B	322	*Escherichia coli*	R	R	S	R	S	S	S	S	S	S	S	R	S	S	TEM, CTX-M-1g	$bla_{CTX-M-15}$	N, Y	A
57B	323②	*Escherichia coli*	R	R	S	R	S	S	S	S	S	S	S	R	S	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	A
69B	364	*Enterobacter cloacae* complex	R	R	I	R	S	R	R	R	S	R	R	R	R	R	TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
69B	365	*Klebsiella pneumoniae*	R	R	R	S	S	R	R	R	R	R	R	R	I	R	SHV, TEM, CTX-M-1g	$bla_{CTX-M-55}$	FIIAs	-
69B	366	*Escherichia coli*	R	R	I	S	S	R	I	S	S	S	S	R	R	S	CTX-M-1g	$bla_{CTX-M-55}$	N.D.	B1
69B	368	*Escherichia coli*	R	R	S	S	S	R	R	R	R	R	R	R	R	S	TEM, CTX-M-1g	$bla_{CTX-M-55}$	F	A
72B	383	*Klebsiella pneumoniae*	R	R	R	S	S	R	I	R	S	S	R	R	S	R	SHV, TEM, CTX-M-1g	$bla_{CTX-M-55}$	N.D.	-
72B	384	*Escherichia coli*	R	R	R	S	S	R	R	R	R	R	R	R	R	R	TEM, CTX-M-1g	$bla_{CTX-M-55}$	F	A
72B	385	*Escherichia coli*	R	R	I	S	S	R	R	R	R	R	R	R	R	I	CTX-M-1g	$bla_{CTX-M-55}$	FIB,F	A
80B	411	*Citrobacter freundii* complex	R	R	S	R	S	R	S	R	I	S	R	R	R	S	CTX-M-9g	$bla_{CTX-M-27}$	N.D.	-
80B	413	*Klebsiella pneumoniae*	R	R	S	S	S	S	S	R	S	S	R	R	R	R	SHV, CTX-M-9g	$bla_{CTX-M-27}$	N.D.	-

AMP:ampicillin, CTX: cefotaxime, CAZ: ceftazidime, CFX: cefoxitin, MEM: meropenem, NAL: nalidixic acid, CIP: ciprofloxacin, STR: streptomycin, KAN: kanamycin, GEN: gentamicin, SXT: trimethoprim/sulfamethoxazole, TET: tetracycline, CHL: chloramphenicol, FOS: fosfomycin
CTX-M-1g: CTX-M-1 group, CTX-M-9g: CTX-M-9 group N.D.: non-detection
Resistance
Intermediate
Suceptibility

4. Discussion

Antibiotic-resistant bacteria are found in humans, animals, and the environment, and these can be a source of infection to humans, with food considered one of the most important sources. It is need to understand the extent to which antibiotic-resistant bacteria contaminate foodstuffs. River fish are an important food source in Vietnam, not only for domestic consumption, but also as an export product.

Edible freshwater fish showed a 70% detection rate for ESBL-EN, with *E. coli* being the most common species in this study. *K. pneumoniae*, *C. freundii* complex, and *E. cloacae* complex were the next three most common species respectively. These three species had $bla_{CTX-M-55}$ and $bla_{CTX-M-15}$ genes, similar to *E. coli*, indicating no difference in antibiotic resistance genotypes. In some of the fish, the same type of plasmid was detected between *E. coli* and *Klebsiella* or non-clonal *E. coli*, suggesting that they share antibiotic-resistance plasmids. Based on these findings, it is hypothesized that antibiotic-resistance plasmids are horizontally transferred among bacteria within the same river fish. Furthermore, in our previous study in Vietnam, ESBL-producing *E. coli* was found in 28–50% of freshwater fish, depending on the region [8]. Although some studies have reported the presence of ESBL-EN in freshwater fish, such as 0.9% and 0.5% of snakehead fish (*Channa* spp.) and black carp (*Mylopharyngodon* spp.), respectively, in Hong Kong, and 13.3% of Nile tilapia fish (*Oreochromis niloticus*) in Tanzania [20,21], contamination rates in Vietnam were higher in comparison to these regions. Additionally, $bla_{CTX-M-1}$ and $_{CTX-M-9}$ groups were found in Vietnamese aquaculture sites and rivers [9]. These results suggest that freshwater fish can be contaminated by bla_{CTX-M} present in the river water and mud in Vietnam.

In our preliminary study, CTX-resistant bacteria were isolated from dishes served at 18 Vietnamese restaurants, and ESBL-producing bacteria, excluding ESBL-producing *E. coli*, were isolated (The data not published) (Supplementary Table 3). Evers et al. suggested that the processing method, such as heating, reduces the presence of ESBL-producing *E. coli* in a comparison study of beef and poultry up to ingestion [22]. Therefore, *E. coli* contamination during food consumption may be relatively low without raw meat. In this study, resistance gene transfer tests with ESBL-producing bacteria, excluding *E. coli*, showed that approximately 7% of the bacteria transferred bla_{CTX-M} to *E. coli* TB1 isolates from Vietnam. Notably, the transfer of resistance genes was more common in *E. coli* from Vietnam than in the ATCC standard strains. This indicates that ESBL-producing bacteria, excluding *E. coli*, have the potential to transfer bla_{CTX-M} to *E. coli* in the human gut.

In Vietnam, both $bla_{CTX-M-55}$ and $bla_{CTX-M-15}$ have been detected in

healthy people [6,23] and food [7]. Hoang et al. reported the detection of a 120 kbp plasmid encoding $bla_{CTX-M-55}$ in food, humans, and urinary tract infections, indicating the horizontal spread of the bla_{CTX-M} gene [23]. Similarly, in the present study, $bla_{CTX-M-55}$ and $bla_{CTX-M-15}$ were also frequently detected in edible river fish.

In this study, ESBL-ENs were detected in edible freshwater fish, and bla_{CTX-M} was found, which can be transferred to *E. coli*. These results suggest that Vietnam may be prone to a variety of ESBL-producing bacteria. Our previous study also showed that, in Japan, the type of ESBL-producing *E. coli* does not change once it is harbored in humans [24]. However, in Vietnam, it has been found that ESBL-producing *E. coli* carried in humans are violently replaced by other ESBL-producing *E. coli* [25]. This phenomenon may be due to the spread of ESBL-producing bacteria through food and the transfer of antibiotic resistance to *E. coli*.

5. Conclusion

In this study, ESBL-EN were isolated from edible river fish, including *E. coli*, *Klebsiella*, *Enterobacter*, and *Citrobacter*. These isolates harbored the $bla_{CTX-M-55}$, $bla_{CTX-M-27}$, and $bla_{CTX-M-15}$ genes and were found to transfer these genes to *E. coli*. These results indicate that ESBL-producing bacteria contaminating edible river fish may contribute to the presence of the bla_{CTX-M} gene in humans.

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CRediT authorship contribution statement

Michio Jinnai: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Takahiro Yamaguchi:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Doan Tran Nguyen Minh:** Investigation, Formal analysis. **Oanh Nguyen Hoang:** Investigation, Formal analysis. **Hien Le Thi:** Investigation, Formal analysis. **Phong Ngo Thanh:** Investigation, Formal analysis. **Phuong Hoang Hoai:** Investigation, Formal analysis. **Phuc Nguyen Do:** Supervision, Investigation, Formal analysis. **Chinh Dang Van:** Supervision. **Yuko Kumeda:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Atsushi Hase:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Tatsuya Nakayama:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

All authors declare that there are no conflicts of interest.

Data availability

No data was used for the research described in the article.

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