

RESEARCH ARTICLE

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## Characteristics of Antibiotic-resistant *E. coli* Isolated from Perfume River in Hue City, Vietnam

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### Abstract

Antibiotic resistant bacteria (ARB) in aquatic environments is attracting increasing attention. However, the spread of ARB along Perfume River in Hue City is poorly understood. This study aimed to phenotypically and genotypically characterize  $\beta$ -lactam- and fluoroquinolone-resistant *Escherichia coli* isolates from this river. Water samples were collected from the urban, rural, agricultural, and less-affected areas in March 2020. *E. coli* susceptibility to seven commonly employed antibiotics was analyzed using the disk diffusion method, and the antibiotic resistance genes (ARGs), *qnrA*, *qnrB*, *qnrS*, *TEM*, *SHV*, and *CTX-M*, were identified using polymerase chain reaction (PCR) and DNA sequencing. The antibiotic susceptibility patterns of *E. coli* revealed that the rate of amoxicillin resistance was the highest (60%). PCR assays and sequencing of 12  $\beta$ -lactam-resistant *E. coli* isolates indicated the presence of *bla*<sub>TEM</sub> and *bla*<sub>CTX-M-15</sub> in 58.3% and 16.7% of the isolates, respectively. Only one of four fluoroquinolone-resistant *E. coli* isolates harbored the *qnrS*, while *qnrA* or *qnrB* genes were not detected. These findings suggest that this water may be an essential source of transmissible ARGs in Hue City, which may have a detrimental impact on the people living in this area.

**Keywords:** Aquatic Environments, *Escherichia coli*, Antibiotic Resistance, Perfume River

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## INTRODUCTION

Since their discovery, antibiotics have been extensively used to treat infectious diseases caused by various bacteria in humans and animals. However, one of the biggest global health concerns is the increase in antibiotic resistance (AR) in hospitals, communities, and the environment. Antibiotic-resistant illnesses cause more than 700,000 deaths annually, and this number is estimated to reach 10 million by 2050.<sup>1</sup> The misuse and overuse of antibiotics for treating infectious diseases in humans and animals are thought to be the main contributors to AR.<sup>2</sup> In addition, environmental antibiotic residues exert selective pressures on the evolution of antibiotic resistance genes (ARGs). It is known that these ARGs can spread between different microorganisms via mobile genetic components like plasmids, integrons, and transposons,<sup>3,4</sup> promoting the emergence of antibiotic-resistant bacteria (ARB). The environmental presence of ARB may endanger human health through direct or indirect contact with contaminated water (for drinking and recreational use).<sup>5</sup>

*Escherichia coli*, one of the most prevalent indicator microorganisms of fecal pollution in the environment, acts as the main reservoir of ARGs because of its ability to collect and preserve resistance genes from other bacteria.<sup>6</sup> *E. coli* strains that are resistant to antibiotics are becoming more frequently reported in various environments, including surface water,<sup>3,5,7,8</sup> and drinking water.<sup>9</sup> Strains of *E. coli* producing enzymes known as extended-spectrum lactamases (ESBLs) have threatened public health since they were first reported in 1979. They can hydrolyze lactam antibiotics, such as penicillins and cephalosporins, rendering them ineffective. This mechanism is particularly concerning because it can lead to resistance to multiple classes of antibiotics, making treatment difficult. Recently, there has been an increased occurrence of ESBL-producing *E. coli* in surface water, particularly in Southeast Asia.<sup>10</sup> Plasmid-mediated quinolone resistance (PMQR) refers to resistance to quinolone antibiotics, which are commonly used to treat *E. coli* infections. This mechanism is mediated by plasmids, small DNA molecules that can be transferred between bacteria. PMQR has also been found in *E. coli*

strains globally and is an increasing public health concern.<sup>11,12</sup>

Antibiotic use is high in Vietnam and Asia. Their consumption is associated with high rates of misuse and overuse.<sup>4</sup> In 2015, 3,838 tons of antibiotics were consumed in Vietnam, with 71.7% for animal use and the remainder for human use.<sup>13</sup> According to a study published in 2019, Vietnam uses the most antibiotics in the aquaculture industry, and antimicrobial use has increased over the past 10 years as a result of uncontrolled drug use in this country.<sup>14</sup> In Vietnam, the discovery of *E. coli* strains resistant to antibiotics and residual antimicrobials in river water are causes of concern.<sup>15,16</sup> The isolation of these antibiotic-resistant strains of *E. coli* from river water in Vietnam raises questions regarding the source of contamination. It is possible that these bacteria originated from untreated human or animal waste discharged into the river. The overuse of antibiotics in the agricultural sector may also have played a role, as antibiotic-resistant bacterial strains can develop in livestock treated with antibiotics.

In central Vietnam, the Perfume River is the largest river flowing through Hue City, which has a population of more than 1 million residents as of 2021. This river is a crucial water source for drinking, domestic use, irrigation, and other purposes. However, the water quality of the Perfume River has deteriorated in some locations over the past few decades because of rapid population and economic growth in Hue City. Despite increasing contamination of the Perfume River, there is limited information about the antibiotic resistance of *E. coli* in the surface water of Hue City. This preliminary investigation aimed to examine the antibiotic susceptibility patterns and molecular characteristics of *E. coli* isolates resistant to seven antibiotics, including lactams and fluoroquinolones, obtained from the Perfume River.

## MATERIALS AND METHODS

### Sampling sites

Water samples were collected along the Perfume River (length of the river channel: 104 km; catchment area: 2,830 km<sup>2</sup>) from upstream (Bang Lang Junction, Thuy Bang Commune, Hue

**Table 1.** Description and coordinates for each sample site in the Perfume River

Human activities	Sample label	Description	Coordinates	
			Latitude	Longitude
Less affected areas (L)	L1	This site is under Tuan Bridge, upstream of the Perfume River, which is close to Bang Lang junction.	16°23'59.6"N	107°34'29.3"E
	L2	Suburban area with sparse residential apartments	16°24'36.4"N	107°34'01.2"E
	L3	Sparse residential apartment, close to Hon Chen Temple	16°25'17.0"N	107°33'49.0"E
	L4	Perfume River and Bach Yen River junction close to Long Ho Bridge	16°26'54.9"N	107°31'48.4"E
	L5	This site is close to Thien Mu Pagoda, which is about 5 km from urban areas in Hue City	16°27'06.1"N	107°33'02.2"E
Rural areas (R)	R1	Rural area; the junction between Perfume River and Pho Loi River	16°30'00.4"N	107°35'19.0"E
	R2	Rural area with residential apartments	16°29'45.6"N	107°35'01.0"E
	R3	Sampled at the junction of the canal, Dong Ba River and Perfume River; residential and business area	16°29'40.9"N	107°34'41.5"E
Urban areas (U)	R4	Rural area with residence	16°30'05.0"N	107°34'35.4"E
	R5	Rural and residential area	16°30'51.1"N	107°34'28.2"E
	U1	Residential and business area with shopping malls, Dong Ba Market and floating restaurant on Perfume River	16°28'14.5"N	107°35'23.3"E
	U2	Residential apartment area, behind a local market	16°28'58.1"N	107°35'30.1"E
	U3	This site is below the Con Bridge, which connects Con Island to the city	16°28'54.8"N	107°35'41.3"E
	U4	Sampled at the Stock Dam where Nhu Y River connects with the main river; tourist boat docking area	16°28'24.2"N	107°35'41.6"E
	U5	This site is close to Cho Dinh Bridge and an aquatic processing company (small scale)	16°29'23.6"N	107°35'34.1"E
Agricultural areas (A)	A1	Rural and agricultural area; sampled at the junction between Bo River and the main river	16°31'39.9"N	107°34'24.7"E
	A2	Rural area with agriculture and sparse residential apartments; junction of Pho Loi River and Perfume River	16°32'16.4"N	107°35'13.9"E
	A3	Rural, sparse residential and agricultural area	16°32'47.4"N	107°35'32.6"E
	A4	Rural and agricultural area	16°32'59.6"N	107°35'56.4"E
	A5	Rural and agricultural area; sampled near Thao Long Bridge, where there are several livestock farms	16°32'49.6"N	107°36'57.6"E

City) to downstream (Thao Long Bridge, Phu Thanh Commune, Hue City). Figure and Table 1 show detailed maps of the locations and descriptions of all sampling locations, respectively. Twenty sampling locations were chosen based on the predominant land use in Hue City. Four distinct activities were represented by the sites: five were less-affected areas, five were urban areas, five were rural areas, and five were agricultural areas.

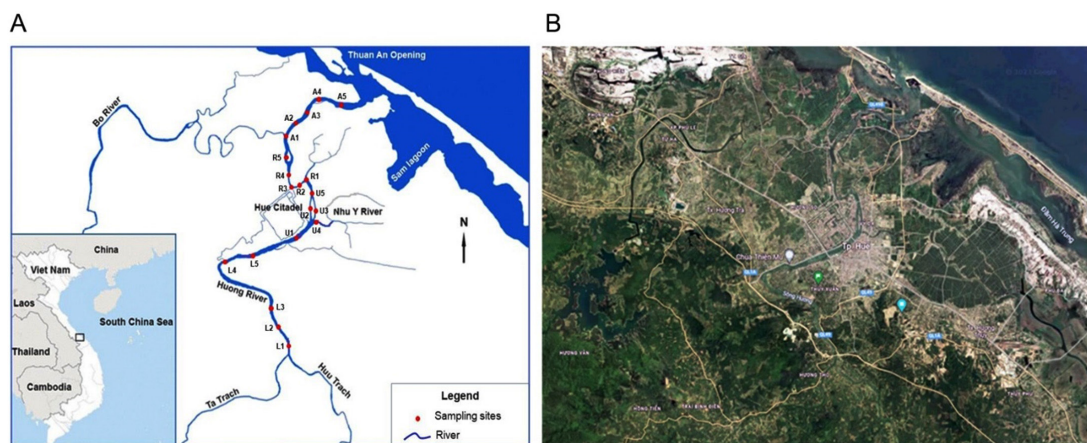
In March 2020, all water samples were collected between 08:00 h and 10:00 h in the morning at a depth of 50 cm below the water surface to avoid surface scum and debris. There was no rainfall for a couple of days. The samples were collected into 0.5-L glass containers previously sterilized by autoclaving at 121°C for 15 minutes. A total volume of 500 mL was collected from each location, and the samples were labeled, stored on ice for transportation to the laboratory, and processed for further analysis within 24 hours.

#### ***E. coli* isolation and identification**

Each water sample was filtered to collect microbiological cells using a sterile membrane filter with a 0.45- $\mu$ m pore (47 mm diameter, cellulose nitrate; Sartorius, Gottingen, Germany). Membranes were placed on m-FC agar (Criterion<sup>TM</sup>; Hardy Diagnostics, Santa Maria, CA, USA) plates and cultured at 44.5°C for 24 hours. Fecal coliform bacteria were detected as blue colonies. A maximum of three blue colonies were picked from each m-FC dish and grown on

CHROM agar plates (Difco, Franklin Lakes, NJ, USA) for *E. coli* isolation. Dark pink to reddish colonies, thought to be *E. coli*, were chosen and sub-cultured on nutrient agar plates (Merck, Darmstadt, Germany). BHI medium supplemented with sterile glycerol at a final concentration of 20% was stored at -80°C for further analysis. Approximately 4–5 colonies of putative *E. coli* isolates were grown overnight at 37°C on nutrient agar plates and suspended in tubes containing 500  $\mu$ L of sterile distilled water. DNA was extracted using the phenol-chloroform method, according to the manufacturer's instructions, using an iVApDNA Extraction Kit (Viet A Technology Corporation, Ho Chi Minh, Vietnam).

The *uidA* gene, which is specific to *E. coli* species, was examined in all putative *E. coli* isolates. The forward primer (52 -CCAAAAGCCAGACAGAGT-32 ) and reverse primer (52 -GCACAGCACATCAAAGAG-32 ) were used in the polymerase chain reaction (PCR) assay, and the amplicon was 623 bp. One microliter of each template DNA sample was added to 24  $\mu$ L of the 1 $\times$  Q5 High-Fidelity Master Mix (New England Biolabs, Ipswich, MA, USA) reaction mixture, containing each primer at a final concentration of 0.2  $\mu$ M. The PCR cycling conditions were an initial denaturation at 94°C for 3 minutes; followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 50 seconds; and a final extension at 72°C for 7 minutes. GelRed<sup>TM</sup> Nucleic Acid Stain



**Figure.** Location of sample collection. Water samples were collected from Perfume River (20 locations): A. A map from Department of Natural Resources and Environment, Thua Thien Hue province; B. A satellite image from Goolge Map

**Table 2.** Oligonucleotide primers for PCR detection of  $\beta$ -lactamase resistance and PMQR genes in *E. coli*

Target gene	Primer sequence (5' 3')	Product size (bp)	Ref.
<i>bla</i> <sub>TEM</sub>	F: AGTGCTGCCATAACCATGAGTG R: CTGACTCCCCGTCGTGTAGATA	431	[18]
<i>bla</i> <sub>SHV</sub>	F: GATGAACGCTTTCCCATGATG R: CGCTGTTATCGCTCATGGTAA	214	
<i>bla</i> <sub>CTX-M</sub>	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	593	
<i>qnrA</i>	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	[19]
<i>qnrB</i>	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	
<i>qnrS</i>	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCACCTGTAGGC	417	

10,000× (Biotium, Fremont, CA, USA) was used to stain the PCR products on a 1.5% agarose gel. Electrophoresis was performed using a 100V direct current for 30 minutes, and the bands were observed under ultraviolet light and compared using a 100 bp ladder (Invitrogen, Carlsbad, CA, USA).

#### Antibiotic susceptibility tests

Following the recommendations of the Clinical Laboratory Standards Institute (CLSI) recommendations, antibiotic susceptibility was evaluated using an agar diffusion assay with antibiotic-impregnated paper disks (disk diffusion method).<sup>17</sup> The following antibiotics were used: amoxicillin (10  $\mu$ g), cefotaxime (30  $\mu$ g), ceftazidime (30  $\mu$ g), ceftriaxone (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), nalidixic acid (30  $\mu$ g), and meropenem (10  $\mu$ g).

Confirmed *E. coli* strains were suspended in nutritional broth and cultured at 35  $\pm$  2°C for 5 hours. The broth was diluted in a normal saline solution to achieve the density of a 0.5 McFarland turbidity standard. The diluted broth was applied to cotton swabs on Mueller-Hinton agar plates (Merck). Antibiotic platelets were placed 30 mm apart and 10 mm from the edge of the disk after air drying. The plates were incubated under aerobic conditions at 35  $\pm$  2°C for 16–18 h. CLSI breakpoints were used to analyze the data after measuring and recording the zones of inhibition and resistance.<sup>17</sup> Strains that were resistant to three or more antibiotics were classified as multidrug-resistant (MDR) *E. coli* strains.

**Table 3.** Antibiotic susceptibility profiles of *E. coli* strains (N=20)

Name of antibiotics	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Amoxicillin	12 (60)	0	8 (40)
Nalidixic acid	4 (20)	2 (10)	14 (70)
Ciprofloxacin	3 (15)	10 (50)	7 (35)
Cefotaxime	3 (15)	1 (5)	16 (80)
Ceftriaxone	3 (15)	0	17 (85)
Ceftazidime	2 (10)	1 (5)	17 (85)
Meropenem	0	1 (5)	19 (95)

The presence of ESBL was identified using a combination disk test (CDT) in isolates that exhibited resistance to cephalosporins. Pairs of cephalosporin-containing disks (30  $\mu$ g of cefotaxime or ceftazidime), with and without 10  $\mu$ g of clavulanic acid, were put on opposite sides of a Mueller-Hinton plate (Merck) inoculated with a 0.5 McFarland suspension of the test isolate. After overnight incubation in the air at 37°C, zones of inhibition were observed. If the zone of inhibition around the combination disk was at least 5 mm, the test isolate was considered an ESBL producer.

#### Detection of antibiotic resistance genes

##### Detecting $\beta$ -lactamase genes

The *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> genes were detected by PCR amplification of isolates resistant to penicillins or cephalosporins. The primers were chosen based on previous studies. Table 2 lists the target genes, primer sequences, product sizes, and references. PCR was performed

**Table 4.** The number of environmental isolates resistant to six antibiotics based on their sample locations (N=20)

Location	<i>E. coli</i> isolates	The number of antibiotic-resistant <i>E. coli</i> isolates					
		AX	CTX	CAZ	CRO	CIP	NA
Less affected area	5	2	0	0	0	0	0
Urban area	5	4	3	2	3	2	2
Rural area	5	3	0	0	0	0	1
Agricultural area	5	3	0	0	0	1	1
Total	20	12	3	2	3	3	4

AX: amoxicillin, CTX: cefotaxime, CAZ: ceftazidime, CRO: ceftriaxone, CIP: ciprofloxacin, NA: nalidixic acid

in a 25 µL final reaction volume containing 1× Q5 High-Fidelity Master Mix (New England Biolabs), 0.2 µM primer pairs for *bla*<sub>TEM</sub> or 0.4 µM primer pairs for *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub>, nuclease-free water, and 1 µL of DNA. The amplification protocol was as follows: 94°C for 5 minutes; 30 cycles of 95°C for 20 seconds, 61°C for 30 seconds, and 72°C for 1 min; and finally, 72°C for 5 minutes. Agarose gels (1.5%) were used to separate and visualize all PCR products, as described previously.

#### Detecting fluoroquinolone resistance genes

PCR amplification was used to detect PMQR genes, including *qnrA*, *qnrB*, and *qnrS*, in isolates resistant to ciprofloxacin and nalidixic acid. The primers were selected from those used in previous studies. Table 2 lists the target genes, primer sequences, and sizes of the products and references. PCR was performed in a 25 µL final reaction volume containing 1× Q5 High-Fidelity Master Mix (New England Biolabs), 0.4 µM each primer, nuclease-free water, and 1 µL of DNA. The following amplification protocol was used: 94°C for 5 minutes; 32 cycles of 94°C for 45 seconds, 58°C for 45 seconds, and 72°C for 1 minute; and finally, 72°C for 5 minutes. All PCR products were separated on 2% agarose gels and electrophoresis was performed under 100V of direct current for 60 minutes.

#### Sequencing analysis of ESBL genes

To identify the ESBL gene types detected by the multiplex PCR assay, the DNA sequences of the amplicons were analyzed. A Clean & ConcentratorTM-5 (Zymo Research, Irvine, CA, USA) was used to purify the amplified PCR products before bidirectional sequencing was

**Table 5.** Distribution of the resistance genotypes in β-lactam- and fluoroquinolone-resistant isolates

Site	Resistance pattern	Presence of ARGs
L2	AX	-
L5	AX	<i>bla</i> <sub>TEM</sub>
U1	CIP/NA	-
U2	AX	<i>bla</i> <sub>TEM</sub>
U3	AX/CTX/CAZ/CRO	<i>bla</i> <sub>CTX-M15</sub>
U4	AX/CTX/CAZ/CRO	<i>bla</i> <sub>CTX-M15</sub>
U5	AX/CTX/CRO/CIP/NA	<i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>CTX-M65</sub>
R1	AX	-
R2	AX/NA	<i>bla</i> <sub>TEM</sub>
R3	AX	<i>bla</i> <sub>TEM</sub>
A1	AX	<i>bla</i> <sub>TEM</sub>
A2	CIP/NA	<i>qnrS</i>
A4	AX	<i>bla</i> <sub>TEM</sub>
A5	AX	<i>bla</i> <sub>TEM</sub>

AX: amoxicillin, CTX: cefotaxime, CAZ: ceftazidime, CRO: ceftriaxone, CIP: ciprofloxacin, NA: nalidixic acid.

performed. The resulting sequences were analyzed using Geneious R11 software and compared with known ESBL gene sequences by multiple sequence alignment using the BLAST program.

#### Data analysis

The statistical analyses were performed using Excel software.

## RESULTS

### *E. coli* antibiotic resistance pattern

Twenty different bacterial strains of *E. coli* were detected in the surface water samples and evaluated for their resistance to seven antibiotics. All isolates demonstrated susceptibility to meropenem but showed different levels of

resistance to the six other antibiotics. The AR rate of *E. coli* was highest for amoxicillin (Table 3).

Table 4 displays the number of *E. coli* isolates that exhibit antibiotic resistance at various sampling locations along the Perfume River, which are impacted by diverse human activities in Hue City. In general, urban areas showed the largest concentration of antibiotic-resistant *E. coli*.

Three of 20 *E. coli* isolates tested positive for cephalosporin resistance and underwent confirmatory testing (CDT). They were discovered to be ESBL producers, and all were MDR.

### Identification of genes in *E. coli* causing $\beta$ -lactam and fluoroquinolone resistance

#### $\beta$ -lactam

All 12 penicillin- or cephalosporin-resistant isolates were selected and screened for the presence of  $\beta$ -lactamase genes, including *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub>. *bla*<sub>TEM</sub> and *bla*<sub>CTX-M-15</sub> were present in 58.3% and 16.7% of the *E. coli* isolates, respectively, whereas *bla*<sub>TEM</sub> and *bla*<sub>CTX-M-65</sub> were present in 8.3% of the isolates. No isolates contained *bla*<sub>SHV</sub> genes (Table 5).

#### Fluoroquinolone

PMQR genes *qnrA*, *qnrB*, and *qnrS* were examined in four fluoroquinolone-resistant strains. The *qnrS* gene was present in only one sample, but *qnrA* and *qnrB* genes were absent in all isolates (Table 5).

## DISCUSSION

### Antibiotic resistance pattern of environmental *E. coli*

Aquatic environments, such as rivers, are ideal reservoirs for the spread of AR. Many studies have demonstrated that ARB or genes confer resistance in aquatic settings.<sup>8,12,20,21</sup> Interestingly, this has also been observed in countries where antibiotic use is strictly regulated. For example, ARGs have been discovered in Swedish waters.<sup>20</sup> This study assessed the antibiotic susceptibilities of 20 different *E. coli* strains recovered from the Perfume River. The highest prevalence of AR in *E. coli* was observed for amoxicillin (60%), followed by nalidixic acid (20%). The antibiotic resistance patterns of *E. coli* isolates observed in our study are common in other aquatic environments.<sup>8,12,21</sup>

This may be because these antibiotics are the most widely manufactured and prescribed in human and veterinary medicine in Vietnam. In addition,  $\beta$ -lactams and fluoroquinolones were prescribed for humans at eight defined daily doses per 1,000 individuals in 2015.<sup>13</sup> Therefore, the discovery of *E. coli* resistant to these antibiotics in environments is not surprising. As the final resort among  $\beta$ -lactams, meropenem is exclusively used for patients with particularly challenging infections that are unresponsive to other  $\beta$ -lactam antibiotics. Fortunately, no meropenem-resistant *E. coli* isolates were detected.

The number of *E. coli* isolates at the sampling sites along the Perfume River that were affected by anthropogenic activities in Hue City is shown in Table 4. In general, the highest number of antibiotic-resistant *E. coli* strains was observed in metropolitan areas, followed by agricultural locations. It is important to note that, although the majority of the municipal wastewater in Hue City is appropriately collected and treated at a wastewater treatment plant, untreated wastewater is discharged into the canals before entering the Perfume River. As a result, water samples collected from urban areas are at risk of being polluted by untreated wastewater from a variety of sources, including households, urban runoff, and commercial activities. Agricultural areas were also affected by contamination with antibiotic-resistant *E. coli*. This can be attributed to the overuse of chemical insecticides and fertilizers for growth-promotion purposes in farming practices. In addition, agricultural waste and untreated wastewater may be released into the environment without surveillance. Our results were similar to those of previous studies.<sup>4,12</sup> The results reflect how human activities along the river contributed to an increase in contamination with antibiotic-resistant *E. coli* from upstream to downstream, suggesting a potential risk of AR spreading in the aquatic environment.

ESBL-producing *E. coli* strains are becoming more common worldwide. They are not only present in clinical settings, but also found in environmental niches, such as livestock; wildlife; and most notably, water.<sup>22</sup> The spread of these isolates, especially MDR isolates, is a source of concern in both developing and developed countries, as they constitute a potential

public health risk.<sup>21</sup> In this study, 15% (n = 3) of isolates were ESBL-producing *E. coli* isolates, all of which were isolated in metropolitan settings. It is of particular concern that all of the ESBL producers identified in this study displayed an MDR phenotype. It is highly likely that the Perfume River has been contaminated by sewage discharge, such as municipal sewage and wastewater from animal farms, which are located in or close to the sampling sites. Hon et al. reported that most ESBL-producing *E. coli* isolated from wild and farmed fish in the Mekong Delta of Vietnam were MDR.<sup>23</sup> This is not surprising, given that ESBL producers are routinely resistant to several antibiotics, particularly fluoroquinolones. This is because *bla*<sub>ESBL</sub> genes are frequently found in conjugative plasmids, which usually contain genes conferring resistance to other antibiotics.<sup>24</sup>

#### Distribution of the genes for $\beta$ -lactamase and PMQR

The most commonly administered antibacterial medicines are  $\beta$ -lactams, and the most prevalent and major mechanism by which bacteria resist these drugs is by the production of  $\beta$ -lactamase.<sup>25</sup> In our study,  $\beta$ -lactamase gene screening was performed on *E. coli* isolates. The percentages of *E. coli* harboring *bla*<sub>TEM</sub> and *bla*<sub>CTX-M-15</sub> were 58.3% and 16.7%, respectively. *bla*<sub>TEM</sub> appeared at the highest frequency, similar to the results reported in China.<sup>8,25</sup> In addition, one isolate carried *bla*<sub>TEM</sub> and *bla*<sub>CTX-M-65</sub>. Several drug-resistance genes have been identified in bacterial strains isolated from aquatic environments in previous reports.<sup>12,25</sup> This may be attributed to the coexistence of *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes in the plasmids.<sup>12</sup> However, the isolated *E. coli* strains did not possess *bla*<sub>SHV</sub> genes, as *bla*<sub>SHV</sub> is typically found in *Klebsiella* spp.<sup>12</sup>

Among the strains with ESBL phenotypes, *bla*<sub>CTX-M-15</sub> was the most common ESBL gene, which is in agreement with data from India and Turkey.<sup>3,12</sup> Since the beginning of the 21st century, *bla*<sub>CTX-M</sub>-producing *E. coli* isolates have spread and they are now major contributors to urinary tract and bloodstream infections in humans, occurring in both nosocomial settings and in the general population. This was not surprising because these enzymes constitute the majority of ESBLs in *E. coli* isolates recovered from surface water.

Fluoroquinolone resistance was assumed to be caused by mutations in gyrase and topoisomerase IV genes. However, it is now clear that it is also related to plasmid-associated resistance factors that may transmit antibiotic resistance in the environment. In our study, *qnrA*, *qnrB*, and *qnrS* were chosen to investigate PMQR. Although four *E. coli* strains were ciprofloxacin- or nalidixic-acid-resistant, only one strain possessed the *qnrS* gene. It has been reported that *E. coli* strains isolated from rivers contain the *qnrS* gene,<sup>11,12</sup> but not fluoroquinolone resistance determinants, such as *qnrA* and *qnrB*. The lack of *qnrA* and *qnrB* may be explained by the fact that these genes are frequently included in complex *sul1*-type integrons.<sup>26</sup> Resistance to fluoroquinolones in one *E. coli* strain was attributed to a plasmid-derived *qnrS* gene. The remaining isolates may be a result of point mutations in the gyrase and topoisomerase subunit genes, *gyrA*, *gyrB*, *parC*, or *parE* on the chromosome,<sup>27</sup> but this was not been investigated in this study.

However, our study has some limitations. The study was conducted for only 1 month; therefore, the impact of seasonal changes on the antibiotic-resistant rate of *E. coli* remains unclear. Furthermore, there may have been a correlation between water quality parameters and the prevalence of antibiotic-resistant *E. coli* in this study. Further research is required to investigate these possibilities.

#### CONCLUSION

The discovery of antibiotic-resistant *E. coli* in water samples from the Perfume River, Vietnam, suggests that this body of water was contaminated with feces. Our findings demonstrated that environmental isolates of *E. coli* have a significant prevalence of AR, particularly amoxicillin and nalidixic acid resistance. The proportion of antibiotic-resistant *E. coli* strains was higher in metropolitan regions, where large amounts of waste from human activities enter the river without treatment. ESBL-producing *E. coli* strains were also observed, and these showed an MDR phenotype for all antibiotic classes. In addition, the results of this study provide a clear genetic description of *E. coli* isolates resistant to  $\beta$ -lactams and fluoroquinolones from the Perfume River.



The rising prevalence of antibiotic resistance in aquatic environments should create concern for public health, as the Perfume River is utilized for irrigation, livestock, and domestic uses. Therefore, wastewater treatment systems should be improved to ensure the effective removal of bacteria and other contaminants from water sources. Antibiotics should only be used when they are truly necessary, and efforts should be made to reduce their overuse in agriculture. Furthermore, the development of monitoring programs to track the occurrence of ARB and ARGs in the Perfume River and other major water bodies around the country is recommended. By taking these steps, it may be possible to reduce the prevalence of ARB in water sources and prevent the spread of infections that are difficult to treat using standard antibiotics.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTION

NVQT, NHB, PTTT contributed in conceptualization, study design, literature review, data collection, writing original draft and editing the manuscript. NHB wrote, reviewed and edited the manuscript. NVQT, NHB, PTTT, NTDK, NTT, PTHC, NTDH, TTCT, BP contributed in data analysis, interpretation, read and approved the final manuscript for publication.

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None.

#### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

#### ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Hue University of Medicine and Pharmacy, Hue University, Hue City, Vietnam, with reference number H2020/019.

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