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Research article

Public Health Implications of Antimicrobial-Resistant Bacteria in the U-Tapao Canal, South of Thailand: A Study of *Escherichia coli* and Associated Gram-Negative Bacteria

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Abstract: Water-borne diseases are a major global public health concern, leading to significant morbidity and mortality worldwide. In this study, *E. coli* and the associated culturable bacteria were investigated from 7 water sampling locations along the 26 kilometers of U-Tapao Canal, an important aquatic source in southern Thailand. Five *E. coli* strains were obtained from 3 water samples (3/21, prevalence of 14%). Two of five were multidrug-resistant (MDR) *E. coli*. One *E. coli* strain was resistant to imipenem, suggesting that it was a carbapenem-resistant *E. coli*. All five *E. coli* strains exhibited γ-hemolysis on blood agar and produced catalase, suggesting their virulence to some extent. Fifteen diverse bacterial strains other than *E. coli* were also found and classified into 12 distinct bacterial species using MALDI-TOF MS. The finding of *E. coli* and other bacterial species in the U-Tapao Canal in this study highlights the microbial contamination inhabiting this canal and emphasizes the potential risk of water-borne diseases among inhabitants residing in the vicinity. This study strengthens the need for systematic microbiological monitoring of water quality, promoting public health and environmental safety.

Keywords: Bacterial diversity; *Escherichia coli*; fecal; multidrug resistance; water-borne

1. Introduction

Fecal contamination of water has been a major public health concern since it is a source of numerous pathogens [1]. Fecal indicator bacteria are a group of bacteria used to assess water fecal contaminations and their abundance should correspond to the presence of fecal pathogens [2]. One of the major pathogens that serves as an indicator of fecal water contamination supplies is *Escherichia coli* [3]. *E. coli* contamination at a level of zero per 100 mL is considered the threshold for safe potable water [4]. Numerous *E. coli* strains carry distinct virulent genes that are capable of causing a wide variety of diseases, including diarrhea, septicemia, and urinary tract infection [5, 6]. Water-borne outbreaks have been reported to implicate Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) [7]. STEC producing Shiga toxin 2 can result in hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), leading to high mortality rates [8]. In addition, EPEC, comprising *bfp* (coding for bundle-forming pili) and *eae* (coding for intimin), including locus of enterocyte

effacement (LEE), can lead to non-bloody diarrhea [5]. More importantly, they can acquire antimicrobial-resistant genes through horizontal gene transfer, contributing to their intractability.

The U-Tapao Canal originates from the Thai-Malaysian border and terminates at the lower area of Songkhla Lake, covering a distance of approximately 130 kilometers [9]. It serves as a catchment area and effectively aggregates microbial contaminants from diverse upstream sources traversing Hat-Yai city, which is an urban center with a population exceeding hundreds of thousands of people. This hydrological pathway is serving as a potential stream for the spread of water-borne pathogens to the urban populations. In Thailand, there is a report of a high level of multidrug-resistant (MDR) and extended-spectrum beta-lactamases (ESBL)-producing *E. coli* from river water. However, the data on *E. coli*, including other bacterial species, are scarce in southern Thailand. Therefore, this study discovers the information in terms of prevalence, virulence, antimicrobial resistance (AMR), and bacterial diversity in fresh water in the southern Thai area. This is beneficial to Thailand's public health intervention.

2. Materials and Methods

2.1 Sample collection and bacterial isolation

Water samples were collected from 7 locations, including 5 locations along 26 kilometers of U-Tapao Canal (UTP-1 to UTP-5), and the 2 water reservoirs near cattle farms (Rattana farm, RTN, and Ruengkitt farm, RKF) (Figure 1). Briefly, 100 mL of water was acquired at a depth of 30 cm below the water surface. Ten mL of water was mixed with 90 mL of tryptic soy broth (TSB) (Becton Dickinson, Sparks, USA) and incubated at 37℃ for 1 h. One loop-full of the bacterial culture was streaked on eosin methylene blue agar (EMB agar) (Becton Dickinson, Sparks, USA) and incubated at 37℃ for 18 h for *E. coli* isolation. Green metallic sheen colonies were selected and kept in stock at -80℃ using 10% glycerol (final concentration) as the cryoprotectant.

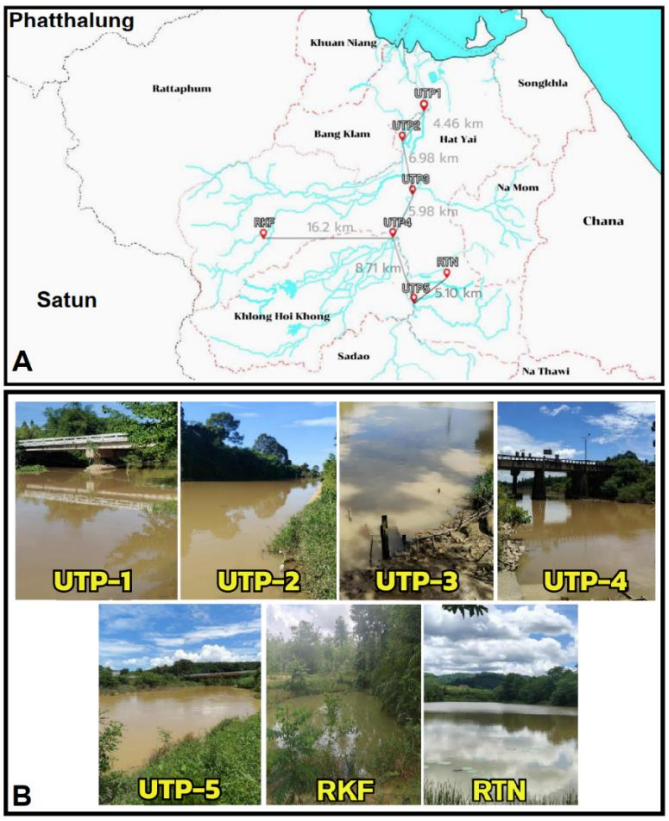

Figure 1. Water sampling locations. A. Water samples were collected from 7 locations, including 5 locations (UTP-1 to UTP-5) along the 26 kilometer-U-Tapao Canal, and 2 water reservoirs from nearby farms (Ruengkitt farm, RKF, and Rattana farm, RTN farm) to assess the bacterial contamination. B, actual field sites where water samples were collected.

2.2 Bacterial identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

A total of 27 suspected *E. coli* isolates were subjected to bacterial identification by MALDI-TOF MS [10]. In brief, a tiny amount of bacterial colony was spotted onto a steel target plate. The sample was overlaid with 1 µl of 100% formic acid. After drying, 1 µl of Bruker HCCA matrix (α-Cyano-4-hydroxycinnamic acid) was prepared followed the manufacturer's instructions, and was added. The sample was processed and analyzed in a Microflex Biotyper (Bruker Daltonik GmbH, Germany). All isolates were done in duplicates. The highest score value and the identification provided by MALDI-TOF MS were recorded. Score values ≤ 2.0 and ≥1.7 were established as the cut-off for species-level and genus-level identification, respectively [11]. Species-level sample identities were determined by the MTB software searching against the Bruker BDAL MSP library database.

2.3 Hemolysis and catalase production

Hemolysis on blood agar and catalase production were also examined to seek their additional virulence characteristics. For the hemolysis assay, an overnight bacterial colony was spotted on human blood agar and incubated at 37ºC for 18 hours. Alpha (α), beta (β), or gamma (γ) hemolysis was observed macroscopically. Catalase production assay was examined using 3% H_2O_2 solution as described by Sukhumungoon et al. [12].

2.4 Antimicrobial susceptibility assay

All *E. coli* strains were examined for antimicrobial susceptibility using the disk diffusion method [13]. Twelve antimicrobial agents used in this assay were amikacin, AK (30 µg), ampicillin, AMP (10 µg), ceftazidime, CAZ (10 µg), ceftriaxone, CRO (30 µg), cephalothin, KF (30 µg), chloramphenicol, C (30 µg), ciprofloxacin, CIP (5 µg), gentamicin, CN (10 µg), kanamycin, K (30 µg), imipenem, IPM (10 µg), streptomycin, S (10 µg), and tetracycline, TE (30 µg) (Oxoid Hampshire, UK). The clear zone was measured by a vernier caliper. *E. coli* ATCC 25922 was used as a control. MDR was determined by the resistance to 2 or more antibiotic classes.

2.5 Statistical analyses

Fisher's Exact Test was employed to determine the significant difference in the relationship between the detection of *E. coli* and the sampling locations along the U-Tapao Canal. *P*-value was set at 0.05.

3. Results and Discussion

3.1. Bacterial isolation and bacterial identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

The existence of *E. coli* in natural aquatic sources constitutes a critical public health concern, as these sources supply water to municipal systems for domestic consumption. In this study, a total of five *E. coli* strains were isolated. These 5 strains were recovered from 3 positive water samples (out of 21 total samples, prevalence of 14%). The 3 positive samples were obtained from three distinct locations: one from UTP-1 (yielding 1 isolate), one from UTP-2 (yielding 1 isolate), and the last one from the RKF cattle farm (yielding 3 isolates). (Figure 1 and Table 1). Despite the fact that the presence of *E. coli* was not significantly associated with the water sampling sites (*P*-value > 0.05), its detection is still a public health problem (Table 1). In the course of *E. coli* investigation from the northeastern region of Thailand, a total of 34 surface water samples were collected and yielded 89 *E. coli* isolates, suggesting a high level of *E. coli* contamination [14]. Likewise, the detection of *E. coli* in water has also been documented in several countries worldwide. Bong et al [15] investigated the prevalence and diversity of antimicrobial-resistant *E. coli* under anthropogenic pressure in the Larut River, Malaysia. *E. coli* was detected at all sampling sites, and its estimated abundance was up to 4.1×10^5 CFU/100 mL.

Table 1. Prevalence of *E. coli* from U-Tapao Canal, May, 2025

Sample	GPS location	No. of positive sample / No. of total sample (%)	No. of positive isolate / No. of total isolate (%)
*UTP-1	7°04'30.3"N 100°28'32.6"E	1/3 (33)	1/6 (17)
UTP-2	7°02'31.3"N 100°27'06.8"E	1/3 (33)	1/4 (25)
UTP-3	6°58'48.5"N 100°27'48.1"E	0/3 (0)	0/2 (0)
UTP-4	6°55'53.0"N 100°26'24.2"E	0/3 (0)	0/5 (0)
UTP-5	6°51'22.7"N 100°27'52.3"E	0/3 (0)	0/4 (0)
RKF	6°55'48.5"N 100°17'30.6"E	1/3 (33)	3/3 (100)
RTN	6°53'06.4"N 100°30'09.0"E	0/3 (0)	0/3 (0)
Total		3/21 (14%)	5/27 (19)

*UTP, U-Tapao, RKF, Ruengkitt farm, RTN, Rattana farm.

In addition to the detection of *E. coli*, we demonstrated a high degree of bacterial diversity within the fluvial environment. A total of 15 associated culturable Gram-negative isolates recovered from 6 of 7 sampling locations were classified into 12 distinct bacterial species using MALDI-TOF MS, including *Aeromonas caviae*, *Aeromonas hydrophila*, *Enterobacter kobei*, *Klebsiella pneumoniae*, *Kluyvera geogiana*, *Kosakonia radicincitans*, *Enterobacter cloacae*, *Phytobacter ursingii*, *Pantoea dispersa*, *Aeromonas veronii*, *Raoultella ornithinolytica*, and *Leclercia adecarboxylata,* all of which were capable of growing on the selective EMB agar. (Table 3). These bacteria are classified in the families *Enterobacteriaceae* and *Aeromonadaceae*. In addition, some of them are opportunistic pathogens such as *Aeromonas caviae*, *Aeromonas hydrophila*, *and Klebsiella pneumoniae* [16, 17]. Hence, our result underscores the complexity of bacterial communities inhabiting the U-Tapao Canal and highlights the possibility of infection caused by bacteria other than *E. coli* in the future. Based on the results of our investigation, the presence of multiple bacterial species detected in the U-Tapao Canal strongly suggests that these microorganisms are likely introduced through fecal discharges from both humans and animals. Nopprapun et al. [18] examined the origins of fecal bacterial contamination in the Mae Klong River by employing the H8 biomarker, a human-associated genetic marker specific to *Escherichia coli*. Using real-time PCR, 500 *E. coli* isolates were analyzed from ten sampling sites distributed longitudinally from the upstream to the downstream reaches of the river. Their findings revealed that between 10% and 46% of the isolates carried the human-associated H8 marker, indicating a substantial contribution of untreated or inadequately treated domestic wastewater to the river. This serves as compelling evidence that household effluents constitute a major source of microbial pollution in the aquatic environment. Moreover, Díaz-Gavidia et al. [19] reported that humans and cattle constitute the primary sources of fecal microorganism contamination in the Maipo and Maule Rivers of central Chile.

3.2. Hemolysis and catalase production

Hemolysis of blood indicates the bacterial virulence potential to humans. In this study, five *E. coli* strains from U-Tapao Canal and the nearby cattle farm demonstrated γ-hemolysis. The absence of hemolytic activity has been previously described in certain *E. coli* strains. The study from Hai Phong, Vietnam, that investigated the *E. coli* strains isolated from chicken and duck feces reported that 47% (7 of 15) exhibited γ-hemolysis [20]. Moreover, Ibrahim et al. [21] investigated the hemolysis caused by *E. coli* isolated from water and clinical samples in Baghdad, Iraq, in 2013. They showed that 60% (12 of 20 strains) displayed γ-hemolysis. Even though *E. coli* strains in this study lacked the hemolysis capability; it cannot be conclusively inferred that these isolates possess low virulence, as numerous other virulence factors may remain uncharacterized within them. Catalase is shown to be one of the virulence factors of *E. coli*. In this study, all *E. coli* strains were found to produce it (Table 2), supporting that they were pathogenic. Macrophages are essential constituents of the human innate immune system and may be a first line of defense to combat pathogens in the intestine [22]. Macrophages can produce and release reactive oxygen species (ROS) in response to phagocytosis, leading to a bactericidal event [23]. To survive this antimicrobial process, *E. coli* needs to surmount the oxidative stress produced by macrophages. Generally, they overcome oxidative stress by producing enzymes such as

peroxidases, superoxide dismutases, and catalases [24]. The latter enzymes catalyze the decomposition of H_2O_2 into the harmless byproducts, water and oxygen, thereby protecting cells from oxidative destruction [25].

Table 3 Diversity of bacteria in U-Tapao Canal analyzed by MALDI-TOF MS, May, 2025.

Sample	Bacterial strain	Identification result	[a]MALDI-TOF MS score
UTP-1	UTP-1.3.1	*Aeromonas caviae*	2.16
	UTP-1.2.1	*Aeromonas hydrophila*	2.06
	UTP-1.2.2	*Enterobacter kobei*	2.20
	UTP-1.1.3	*Klebsiella pneumoniae*	2.21
	UTP-1.1.2	*Kluyvera geogiana*	2.24
UTP-2	UTP-2.2.2	*NOIP	0
	UTP-2.3.1	NOIP	1.42
	UTP-2.3.2	NOIP	1.50
UTP-3	UTP-3.1.1	NOIP	0
	UTP-3.1.3	*Aeromonas caviae*	2.14
UTP-4	UTP-4.1.2	*Kosakonia radicincitans*	1.75
	UTP-4.1.3	*Enterobacter cloacae*	2.31
	UTP-4.2.1	*Phytobacter ursingii*	1.78
	UTP-4.2.2	NOIP	0
	UTP-4.2.3	*Phytobacter ursingii*	2.12
UTP-5	UTP-5.1.1	*Pantoea dispersa*	2.15
	UTP-5.1.2	*Aeromonas veronii*	2.24
	UTP-5.3.1	*Raoultella ornithinolytica*	2.05
	UTP-5.3.2	*Raoultella ornithinolytica*	2.30
RTN-1	RTN-1.2	NOIP	1.46
	RTN-1.3	*Leclercia adecarboxylata*	1.95
	RTN-3.1	NOIP	1.54

*NOIP, no organism identification possible. [a]MALDI-TOF MS score, Score values 2.00-3.00 indicate high-confidence identification; 1.70-1.99 indicates low-confidence identification; 0.00-1.69 indicates no organism identification possible.

3.3. Antimicrobial susceptibility assay

Bacterial resistance to antimicrobial agents is an escalating public health concern, particularly the emergence and spread of MDR bacteria, posing a significant global health threat. In this study, among the five *E. coli* strains obtained, 2 exhibited the MDR traits. *E. coli* strain UTP-1.1.1 revealed the resistant pattern to ampicillin, ciprofloxacin, kanamycin, streptomycin, and tetracycline, while strain UTP-2.2.1 was resistant to ampicillin, cephalothin, imipenem, and streptomycin (Table 2). The detection of *E. coli* strain UTP-2.2.1 exhibiting resistance to imipenem indicates the presence of a presumptive carbapenem-resistant *Escherichia coli* (CREC). This critical finding warrants urgent confirmatory testing. The other three *E. coli* strains from the RKF cattle farm were not MDR *E. coli* but displayed similar antimicrobial resistance patterns (Table 2). The results in this study are in concordance with the work from Tabut et al. [14] that investigated the AMR of *E. coli* from surface water, wastewater, and discharged water in the Namsuay watershed, northeastern Thailand. The results exhibited that *E. coli* was resistant to fluoroquinolone, third-generation cephalosporin, polymyxin, and carbapenem. In addition, Bong et al. [15] examined the prevalence and diversity of AMR in *E. coli* from the Larut River, Malaysia, using 20 antimicrobial agents that represented 11 different antimicrobial classes. They found that the highest resistance frequency was detected for the tetracycline class, followed by quinolones, penicillins, sulfonamides, amphenicols, fluoroquinolones, and aminoglycosides. The bacterial resistance to tetracycline is not surprising. Owing to the cost-effectiveness of tetracycline, it has been widely employed for prophylaxis and treatment of infectious diseases in humans and animals. In addition, at sub-therapeutic concentrations, it is used as an animal zootechnical additive [26]. The research from Vietnam revealed that resistance to tetracycline was the major antimicrobial resistance observed in raw meat samples

[27]. More importantly, genes mediating tetracycline resistance are effectively transferred between different bacterial species. Therefore, these anthropogenic pressures might, to some extent, play a role in accelerating the AMR. The detection of imipenem-resistant *E. coli* in this study is predictable due to the consistent increase in reported cases of its incidence. The study on antimicrobial resistance of *E. coli* in natural aquatic environment in Khon Kaen Province, Thailand, using the disk diffusion approach, demonstrated an imipenem-resistant rate of 3.54%. Even though the proportion is relatively low, it reflects an escalating risk to public health.

It is estimated that antimicrobial-resistant bacteria have a great impact on around 2.8 million individuals and are responsible for at least 35,000 fatalities annually in the United States [28]. The U-Tapao River Basin covers an area of 2,840 square kilometers, spanning 7 districts of Songkhla province, including Sadao, Na-Mom, Hat-Yai, Khlong-Hoi-Khong, Bang-Klam, Rattaphum, and Khuan-Niang Districts. Therefore, the presence of diverse bacterial contaminants in U-Tapao Canal displaying high levels of antimicrobial resistance is considered a significant threat to public health. This canal serves as a water supply for domestic use, potable purposes, and recreational water activities to hundreds of thousands of people within this area. Pathogenic contaminants in significant quantities, combined with inadequate water management, are able to transmit harmful microorganisms to the residents. Consistent microbiological surveillance of river water plays a crucial role in mitigating and preventing the occurrence of water-borne disease outbreaks. In summary, Thai river systems commonly harbor substantial bacterial loads, many of which possess pathogenic potential to some extent and exhibit varying degrees of antimicrobial resistance. Consequently, practical recommendations for the public include maintaining strict personal hygiene after any contact with untreated river water, refraining from using canal or river water for bathing or other recreational activities, and avoiding the consumption of raw or undercooked aquatic organisms harvested from these waterways. Moreover, municipal authorities should rigorously inspect households and food establishments to ensure that wastewater is adequately treated before discharge into natural water bodies, and should implement routine monitoring of key water-quality indicators to safeguard public health and environmental integrity.

Table 2. Virulence and antimicrobial resistance pattern of *E. coli* strains from U-Tapao Canal, May, 2025.

Sample No.	*Strain name	Antimicrobial susceptibility												Hemolysis (human blood)	Catalase production
		**AK	AMP	C	CAZ	CRO	CIP	CN	K	KF	IPM	S	TE		
1	UTP-1.1.1	S	R	S	S	S	R	S	R	S	S	R	R	γ	+
2	UTP-2.2.1	S	R	S	S	S	S	S	S	R	R	R	S	γ	+
3	RKF-1.1	S	S	S	S	S	S	S	S	R	S	S	S	γ	+
	RKF-1.2	S	R	S	S	S	S	S	S	R	S	S	S	γ	+
	RKF-1.3	S	R	S	S	S	S	S	S	R	S	S	S	γ	+

*UTP, U-Tapao, RKF, Ruengkitt farm, RTN, Rattana farm; **AK, amikacin, AMP, ampicillin, C, chloramphenicol, CAZ, ceftazidime, CRO, ceftriaxone, CIP, ciprofloxacin, CN, gentamicin, K, kanamycin, KF, cephalothin, IPM, imipenem, S, streptomycin, TE, tetracycline; γ, gamma hemolysis; R, resistance, S, sensitive.

4. Conclusions

In the course of investigation, fecal indicator bacteria such as *E. coli* and other opportunistic pathogens (*e.g.*, *Aeromonas* spp.) were found in the U-Tapao Canal, indicating that the water is not safe for direct consumption. Even though the water undergoes water treatment in the municipal water system, water-borne illness risks remain, especially through recreational water activities. Our study raises a public health concern for the population in this area since some *E. coli* strains are MDR, with one strain identified as a presumptive carbapenem-resistant *E. coli* (CREC) and may carry crucial virulence factors. Regular water quality monitoring and stringent microbiological control measures are imperative in decreasing water-borne diseases. This study provides crucial baseline data for safeguarding public health.

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