Antimicrobial Resistant *Escherichia coli* Distribution along the Lower Part of the Chao Phraya River, Thailand

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Abstract. Currently, antimicrobial drugs are not eliminated during the wastewater treatment process, especially in developing countries where antimicrobial drugs are used with less controls. An increase in contamination from effluent entering river systems is a major contributor to antimicrobial drug resistance and the spread of antimicrobial-resistant genes throughout rivers. This study aims to investigate the occurrence of antimicrobial-resistant *Escherichia coli* (*E. coli*) and evaluate the impact of urbanization on the distribution of antimicrobial-resistant *E. coli* along the lower part of the Chao Phraya River in Thailand. It was found that the geometric mean *E. coli* concentration was 624 CFU/100 mL. In addition, the geometric mean concentrations of *E. coli* resistant to cefotaxime and ciprofloxacin were 86 and 182 CFU/100 mL, respectively. A positive correlation coefficient was found between the concentration of antimicrobial-resistant *E. coli* and the number of population. The concentrations of *E. coli* were significantly positively correlated with antimicrobial-resistant *E. coli* (*R* = 0.950, *P* < 0.001). Therefore, this study indicated that antimicrobial-resistant *E. coli* were widely distributed in the Chao Phraya River with the highest concentrations in the Bangkok metropolitan area. These populations have been linked to an increase in antimicrobial-resistant *E. coli* contamination.

1. Introduction

The presence of antimicrobial-resistant bacteria (ARB) in aquatic environments has become a serious global problem in accordance with the World Health Organization (WHO) because of the misuse and overuse of antimicrobial drugs for infectious diseases in humans and animals [1,2]. The aquatic environment, including rivers, has been described as a gigantic reservoir that facilitates the spread of antimicrobial resistance (AMR) [3]. The accumulation of antimicrobial resistance in aquatic environments may arise from waterborne disease infection outbreaks thus acting as an essential dissemination mechanism of antimicrobial resistance genes (ARGs). Therefore, the presence of antimicrobial resistance and antimicrobial-resistant genes has become an increasing environmental risk in aquatic environments, which may lead to possible infections in humans [1]. In addition, the impact of AMR on public health and mortality worldwide was reported by Jim’ O NEILL. It was found that every year, 700,000 people die as a result of antimicrobial drug resistance [4].
This study focused on the drugs cefotaxime and ciprofloxacin, as both of these antimicrobial drugs are classified as the highest-priority critically-important antimicrobials in human medicine according to the WHO [5]. Cefotaxime is part of the third-generation cephalosporin group, and ciprofloxacin is a synthetic broad-spectrum fluoroquinolone. Third-generation cephalosporins and fluoroquinolones are a major threat to antimicrobial-resistant bacteria in humans, especially *E. coli* in Thailand [6].

The Chao Phraya River receives water from various sources, including treated wastewater from domestic use, industries, hospitals, and non-treated wastewater discharged directly from households into rivers [7]. In addition, the lower part of the Chao Phraya River is an area of high population growth. We hypothesize that the growing population of this area is likely to influence the quantity of fecal matter released, leading to antimicrobial resistance in the lower part of the Chao Phraya River. Since *E. coli* is a known microbiological indicator of fecal contamination [8], this investigation will help understand the emergence of fecal contamination and pollution density caused by antimicrobial-resistant bacteria. The present study aimed to investigate the occurrence of *E. coli*, which was resistant to cefotaxime and ciprofloxacin along the lower part of the Chao Phraya River, and to evaluate the impacts of urbanization, focusing on the role of the population density in the distribution of cefotaxime and ciprofloxacin resistant *E. coli*.

2. Methodology

2.1 Description of study area
The Chao Phraya River or Chao Phraya Basin is a major river in Thailand, whose source is at the conjunction of the Nan and Ping rivers in the Nakhon Sawan Province. The Chao Phraya River flows through Bangkok City and outwards to the Gulf of Thailand (Samut Prakan province). It covers an area of 158,000 km², accounting for approximately 35% of Thailand’s surface area and its banks are home to 40% of the country’s population [9]. The 12 sampling stations for this study were chosen from those listed in the Thailand Pollution Control Department (PCD) which are monitored regularly. The sampling points covered four provinces (Pathum Thani: CH17 and CH16.1, Nonthaburi: CH15 and CH13, Bangkok: CH12 - CH05, Samut Prakan: CH04 and CH02). The sampling point at CH17 is the upstream point, and the sampling point at CH02 is the downstream point.

2.2 River water sample collection
The river water samples were collected from the lower part of the Chao Phraya River in March 2021, which is the dry season in Thailand, from 12 sampling points using the grab sampling technique. All river samples were collected in sterilized bottles (2,000 mL) at each sampling point. Subsequently, the samples were placed in a cooler box. They were then transported to the Water Quality Engineering Laboratory and thereafter stored at 4 °C in a refrigerator.

2.3 Water quality measurement
Water quality parameters, including temperature (°C), dissolved oxygen (DO), electrical conductivity (EC), and pH were measured on-site at each river sampling point using portable water quality meters. The water quality analysis in the laboratory included total suspended solids (TSS) and biochemical oxygen demand (BODs) measurements.

2.4 Enumeration and isolation of antimicrobial-resistant *E. coli*
All samples were filtered using the membrane filtration method with filtration equipment (STU-3G ADVANTEC, Microbial Detection Combination) and sterilized filter papers (Sterile MCE Filters, 0.45 µm, 47 mm, ADVANTEC, Japan) on the same day that the samples were collected. The volume of each sample used was pre-tested and estimated to contain a concentration of *E. coli* of approximately 30–300 or 8-80 CFU/filter depending on the sampling point [10]. The filter was placed on a specific enzyme-substrate medium, selected for the simultaneous detection and enumeration of *E. coli* and other coliforms isolated from water and environmental samples in this study (CHROMagar™ ECC, CHROMagar,
France). *E. coli* was cultured under three different conditions: (a) CHROMagar (without antimicrobial drugs), (b) CHROMagar with cefotaxime (1 mg/L), and (c) CHROMagar with ciprofloxacin (1 mg/L). Then, the colonies of antimicrobial-resistant *E. coli* were denoted by a blue color on agar plates after incubation for 24 h at 37±2°C. The concentrations of antimicrobial-resistant *E. coli* were determined by culturing the blue colonies on a specific enzyme-substrate medium and then calculating the antimicrobial-resistant *E. coli* concentration at each sampling point.

### 3. Results and Discussion

#### 3.1 Water quality status of the lower reaches of the Chao Phraya River

The characteristics of water quality along the lower reaches of the Chao Phraya River at each sampling point are shown in Figure 1. The pH values showed a small range of 7.2 – 7.7, with the values falling within the recommended limit of 6-9 set by the surface water quality standard in Thailand [11]. The highest electrical conductivity (EC) was greater than or equal to 20 mS/cm at CH02. CH04 and CH06, which are downstream of the Chao Phraya River, and were high in EC value due to their proximity to the Chao Phraya River mouth, which is affected by tidal forces and seawater intrusion.

In accordance with the surface water quality standards in Thailand, the CH16.1 – CH17 areas were in Class 3, which constitutes a moderately clean fresh surface water resource that is used for both agriculture and drinking. In addition, the CH02 – CH15 areas were in Class 4, which is a relatively clean fresh surface water resource that is used for industry and drinking but requires a special water treatment process before use. The water quality standards for surface water in Thailand recommended limits of dissolved oxygen (DO), biochemical oxygen demand (BOD5), and fecal coliform bacteria for Class 3 and Class 4, which are different criteria according to the Pollution Control Department reporting (Class 3: DO > 4 mg/L, BOD < 2 mg/L, fecal coliform bacteria < 4000 MPN/100 mL; Class 4: DO > 2 mg/L, BOD < 4 mg/L) [11]. The result of DO in 6 sampling points was lower than the criteria in Class 4 (CH04: 1.5 mg/L, CH05: 0.8 mg/L, CH10: 1.4 mg/L, CH11: 1.1 mg/L, CH12: 1.8 mg/L, and CH13: 1.8 mg/L). In addition, CH17 fell into the same criteria as Class 3 (4.1 mg/L) while CH16.1 was lower than the criteria of Class 3 (3.5 mg/L). BOD5 concentrations of CH04, CH13, and CH15 passed the surface water quality standards in Thailand, while the other sampling points exceeded the standard level. The highest BOD5 was observed at CH05 (6.4 mg/L) and CH06 (6.8 mg/L), which are sampling points in the Bangkok metropolitan area, indicating the impact of urbanization on river water quality.

![Figure 1](image-url)

*Figure 1. The transition of water quality from upstream to downstream along the lower reaches of the Chao Phraya River.*
3.2 Abundance of E. coli concentration

The concentration of E. coli in the lower part of the Chao Phraya River was in the range of 100 – 2,433 CFU/100 mL. The overall geometric mean of E. coli concentration was 624 CFU/100 mL, which exceeded 4.9 times the maximum permissible E. coli concentration in the fresh recreational water standard of the USEPA (126 CFU/100 mL) [12]. In addition, the geometric mean of E. coli concentration in the Tama River, Japan (44 CFU/100 mL) was lower than the geometric mean of E. coli concentration in this study [13]. This is due to the inefficiency of wastewater management facilities in Thailand, such as sewerage systems, which are unable to gather all the wastewater [7]. However, the E. coli concentration in the Saigon River, Vietnam (724 CFU/100 mL) was found to be higher than that in this study [14]. High concentrations of E. coli were detected at CH05 (2,433 CFU/100 mL), CH06 (1,700 CFU/100 mL), and CH11 (1,480 CFU/100 mL), which are sampling points in Bangkok city. The lowest concentration of E. coli was observed upstream of this sampling study at CH17 (100 CFU/100 mL) and CH16.1 (230 CFU/100 mL), as shown in Figure 2.

The large population density in Bangkok could affect the level of E. coli concentration, as shown in the sampling point at CH12, where it is upstream of Bangkok and E. coli concentration gradually increased [15]. The downstream area of the Chao Phraya River had low E. coli concentrations at sites CH04 and CH02, where a high concentration of salinity suppresses the growth of E. coli [16]. This result indicated that the E. coli concentration was found to be higher along the river flows, especially in Bangkok (urbanization area) due to a high population density.

![Graph showing the concentration of E. coli and antimicrobial-resistant E. coli along the lower part of the Chao Phraya River.](image)

Figure 2. The concentration of E. coli and antimicrobial-resistant E. coli along the lower part of the Chao Phraya River.

3.3 Prevalence of antimicrobial-resistant E. coli associated with the sampling points

The concentration of E. coli resistant to cefotaxime and ciprofloxacin along the lower part of the Chao Phraya River for the duration of this study ranged from 5 CFU/100 mL to 533 CFU/100 mL, and 20 CFU/100 mL to 1,540 CFU/100 mL, respectively, as shown in Figure 2. The overall geometric mean of the antimicrobial-resistant E. coli concentration was 125 CFU/100mL. The highest concentration of antimicrobial-resistant E. coli was found at CH05 (CTX: 533 CFU/100 mL, CIP: 1,540 CFU/100 mL) because the sample point is in the Phra Khanong canal, which not only receives wastewater from Phra Khanong District but also receives wastewater from Saen Saep canal, Khlong Toey canal, Lad Phrao canal, and Pravet canal. In addition, the sampling point at CH11 was found to have the third-highest concentration (CTX: 193 CFU/100 mL, CIP: 580 CFU/100 mL), where the sampling area was at the Thewet Canal connecting to the Phadung Krong Kasem canal. The sampling point at CH06 (CTX: 280 CFU/100 mL, CIP: 585 CFU/100 mL) was the second-highest, where the sampling location was close to the Chong Nonsi canal and the Chong Nonsi wastewater treatment plant that directly discharged treated wastewater to the Chao Phraya River without chlorination. It has been reported that the Chong
Nonsi wastewater treatment plant discharges effluent of antimicrobial-resistant *E. coli* at a concentration of $3.23 \times 10^3$ CFU/100mL [17].

The wastewater collection system in Thailand is a combined sewerage system with the possibility of sewer overflow and discharge directly to the Chao Phraya River. In addition, the capacity of centralized wastewater treatment plants is unable to process all the wastewater due to wastewater management limitations, which is a major reason for untreated wastewater from households being discharged directly into the lower part of the Chao Phraya River [9,18]. As a result, a high concentration of antimicrobial-resistant *E. coli* was found downstream along the lower reaches of the Chao Phraya River, and especially the sampling points located in Bangkok city. Since Bangkok has a large population, the sewer system covers only 40% of the city [18]. Therefore, untreated domestic wastewater and treated wastewater could become a major source of fecal and antimicrobial-resistant *E. coli* contamination along the lower part of the Chao Phraya River [13].

A substantial positive correlation coefficient ($R = 0.950$, $p < 0.001$) was detected between *E. coli* and antimicrobial-resistant *E. coli* concentrations, as displayed in Figure 3. Furthermore, the concentration of *E. coli* resistant to ciprofloxacin was higher than that of *E. coli* resistant to cefotaxime because the amount of cefotaxime consumption in Thailand was 4,068,398 defined daily doses (DDDs) which is much lower than that for ciprofloxacin (34,340,130 DDDS). This result was consistent with the Thai working group on Health Policy and Systems Research on Antimicrobial Resistance (HPSR-AMR, 2018), which reported antimicrobial consumption in Thailand in 2017 [19]. This study indicated that the abundance of *E. coli* and antimicrobial drug consumption might promote the antimicrobial-resistant *E. coli* concentration due to selection pressure for mutations along the lower part of the Chao Phraya River [20].

![Figure 3](image1.png)

**Figure 3.** Correlation between antimicrobial-resistant *E. coli* and *E. coli*.

![Figure 4](image2.png)

**Figure 4.** Relation of the population and geometric mean of antimicrobial-resistant *E. coli*.

### 3.4 Association between antimicrobial-resistant *E. coli* and the population

The geometric means of antimicrobial-resistant *E. coli* in Pathum Thani, Nonthaburi, Bangkok, and Samut Prakan were 37 CFU/100 mL, 46 CFU/100 mL, 399 CFU/100 mL, and 121 CFU/100 mL, respectively. The geometric mean of antimicrobial-resistant *E. coli* in Bangkok was 3.2 times higher than the USEPA standard [14]. The highest concentration of antimicrobial-resistant *E. coli* was detected in the river around Bangkok region because the population numbers along the Chao Phraya River in Bangkok was higher than in Pathum Thani, Nonthaburi, and Samut Prakan provinces as referred to at the National Statistical Office, who reported the population numbers in 2009 [21]. In addition, a positive correlation coefficient between the population numbers and antimicrobial-resistant *E. coli* concentration was found ($R = 0.673$, $p < 0.05$). The result was indicated that the population density along the lower part of the Chao Phraya River might be related to the prevalence of *E. coli* resistance to cefotaxime and ciprofloxacin. However, other factors also induce antimicrobial resistance in *E. coli* in aquatic environments, such as antimicrobial drugs, biocides, heavy metals, and bacterial density [20]. Therefore, to estimate antimicrobial-resistant *E. coli* concentrations produced from human excreta more accurately, further studies should be carried out on the antimicrobial-resistant bacteria along the lower part of the...
Chao Phraya River and their genetic structure by studying the genetic markers that detect microbial contamination sources based on host-associated genes found in bacteria from humans.

4. Conclusions
This study confirmed that antimicrobial-resistant E. coli was observed in the lower part of the Chao Phraya River in Thailand, especially in Bangkok city (urbanization area), due to the inefficiency of wastewater management and the population density, which are directly related to antimicrobial resistance in E. coli. In addition, the concentration of ciprofloxacin-resistant E. coli was higher than that of cefotaxime, indicating that the amount of antimicrobial agent consumed is related to the antimicrobial resistance of E. coli. Hence, the E. coli concentration and antimicrobial drugs affected the concentration of antimicrobial-resistant E. coli in the river. The analysis by genetic markers should be studied further to identify the source of antimicrobial-resistant bacteria and genetic constituents contaminating the river.

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References
[1] Sharma V K, Johnson N, Cizmas L, Mcdonald T J and Kim H 2016 Chemosphere. 150 702–14
[2] Sinthuchai D, Boontanon S K, Piavyiriyakul P, Boontanon N, Jindal R, and Polprasert C 2021 Journal of Water Sanitation and Hygiene for Development.
[3] Amarasiri M, Sano D and Suzuki S 2020 Critical Reviews in Environmental Science and Technology. 50 2016–59
[4] O’Neill J 2016 Tackling drug-resistant infections globally: final report and recommendations (United Kingdom)
[5] World Health Organization and WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) 2017 Critically important antimicrobials for human medicine (World Health Organization)
[6] Ministry of Public Health and Ministry of Agriculture and Cooperatives 2020 National Strategic Plan on Antimicrobial Resistance 2017-2021 Thailand (Thailand)
[7] Pollution Control Department 2019 Booklet on Thailand State of Pollution 2018 (Thailand)
[8] Yamashita N, Katakawa Y, and Tanaka H 2017 Ecotoxicology and Environmental Safety. 143 38–45
[9] Padiyedath Gopalan S, Hanasaki N, Champathong A, and Tebakari T 2021 Hydrological Processes. 35 (1) 1–19
[10] Osińska A, Korzeniewska E, Harnisz M, and Niestępski S 2017 The Science of the Total Environment. 577 367–75
[11] Pollution Control Department 2002 Research on Water Quality Monitoring and Modeling Application in Thailand (Thailand)
[12] US-EPA 2012 Handbook on Recreational water quality criteria (EPA: United States)
[13] Ham Y, Kobori H, Kang J, Matsuzaki T, and Iino M 2012 Environmental Pollution. 162 98–103
[14] Widmer K, Van Ha N T, Vinitnantharat S, Sthiannopkao S, Wangsaatmaja S, Prasetiati M A N and Hur H G 2013 World Journal of Microbiology and Biotechnology. 29 (1) 2115–24
[15] Huang G, Xue H, Liu H, Ekawatpanit C, and Sukhapunnapha T 2019 Water. 11 (4)
[16] Abdulkarim S M, Fatimah A B, and Anderson J G 2009 Journal of Food, Agriculture and Environment. 7 (3–4) 51–4
[17] R. Sweattatut 2021 Occurrence of antimicrobial-resistant Escherichia coli in wastewater treatment plants and a fecal sludge treatment plant in Bangkok, Thailand (Master’s thesis, University of Kyoto University)
[18] Boontanon SK and Buathong T 2013 *On-site Management for Domestic Wastewater in Thailand* (Thailand)

[19] Thai working group on Health Policy and Systems Research on antimicrobial resistance 2017 *Consumption of antimicrobial agents in Thailand in 2017 First report* (Thailand)

[20] Karkman A, Do T T, Walsh F, and Virta M P J 2018 *Trends in Microbiology.* 26 (3) 220–8

[21] Hydro and Agro Informatics Institute 2012 *Data collection and data analysis for the development of databases of 25 river basins and flood and drought modelling: Chao Phraya river basin* (Thailand)