RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2024.069

ScienceAsia 50 (3): 2024: ID 2024069: **1–9**

Alteration of antimicrobial phenotypic resistance of *E. coli* in municipal wastewater treatment process and receiving canal water in Bangkok, Thailand

Wilai Chiemchaisri[a,*], Chart Chiemchaisri[a], Nopparat Patchanee[b], Toru Watanabe[c]

[a] Department of Environmental Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900 Thailand
[b] Fisheries Program, Faculty of Agriculture Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000 Thailand
[c] Department of Food, Life and Environmental Science, Faculty of Agriculture, Yamagata University, Yamagata 990-0021 Japan

[*]Corresponding author, e-mail: fengwlc@ku.ac.th

Received 12 Sep 2023, Accepted 9 May 2024
Available online 21 Jul 2024

ABSTRACT: The phenotypic prevalence of antibiotic-resistant *E. coli* (ARE) in the sewage of Bangkok City, Thailand, during the conventional activated sludge (CAS) process was evaluated. The relationship of ARE in the CAS effluent/receiving water was investigated. The CAS *E. coli* isolates were tested against 20/21 antibiotics in 6 classes: 4 aminoglycosides, A; 3 tetracyclines, T; 1 chloramphenicol, C; 11 quinolones, Q; 1 sulfonamide, S; and 1 beta-lactam, B. The results showed that the sewage contained 84% ARE, of which 28% was single drug-resistance and 56% was multidrug-resistant *E. coli* (MRE). Poor removals of ARE/MRE were found in the CAS. Inversely, a more susceptive *E. coli* population to all tested antibiotics was detected in the CAS effluent (24%). When MRE was classified into low (L)-, medium (M)-, and high (H)-levels based on number of resistant antibiotics, it showed that the effluent L-MRE (2 to 5 antibiotics) was highest (46%) compared to the others (29–38%), while H-MRE (11 to 16 antibiotics) was relatively dominant in the dewatered sludge (24%). Additionally, Q-S-T was the most phenotypic resistant pattern in sludge, while Q-S was highly frequent in effluent. ARE in receiving water showed a high correlation to MRE of CAS effluent along receiving water due to high contamination of coliforms and *E. coli*.

KEYWORDS: activated sludge, antibiotic resistance, *E. coli*, sewage, wastewater

INTRODUCTION

Over 2 decades, there have been many reports that domestic wastewater contains mixtures of various residual chemical pollutants from the daily use of pharmaceutical products including flora/pathogenic microorganisms from human excreta [1]. Thailand is one of the largest consumers of antibiotics in the SEA, in which top-five antibiotics released into the water environment via discharged effluent from hospital wastewater are determined including amoxicillin, tetracycline, ampicillin, ciprofloxacin, and imipenem, respectively, mainly from the suspended growth systems [2]. The National Antimicrobial Resistance Surveillance Center of Thailand [3] reported that antimicrobial-resistant *Enterococcus* spp. from urinary tract infection patients from 2011 to 2022 showed very high antimicrobial resistance (AMR), especially to tetracycline (81.8% to 91.7%), erythromycin (67.5% to 72.6%), penicillin G (40.9% to 52.5%), and gentamycin (32.9% to 50.0%). While those of healthy adults working on farms showed 75.5%–77.3% positive for extended-spectrum beta-lactamase (ESBL) producing *E. coli* in stool specimens [4].

Domestic wastewater has been proven to be one of the important sources of surface water contamination of antibiotic-resistant bacteria (ARB) [1, 5]. A wastewater treatment plant (WTTP) is the most important facility to remove these impurities and eliminate waterborne pathogens in sewage before being discharged into the environmental water. However, the migration of human enteric microorganisms into water bodies/environments has been ubiquitous especially from WWTPs where disinfection is omitted [6]. ARB contamination is neither necessarily local nor dependent on the environment; they can disseminate among different bacterial species and distinct habitats [7]. The wild types of *E. coli* show a high correlation to antibiotic microbial resistance characteristics in environment water [8–10]. To understand ARB development, addressing the study of antibiotics and AMR genes, not only in the clinic but in natural non-clinical environments, is suggested [10]. Advanced biological techniques of AMR genes in WWTPs have been applied in Europe, Australia, America, and South Middle Asia [1, 11, 12]; however, few studies have been performed on ARB dissemination from WWTPs in Southeast Asia (SEA) region [13]. Unfortunately, the detection of ARGs in WWTPs is currently reported in units that cannot be directly used for assessing health consequences and risks [13]. Because disinfection of the effluents from many WWTPs in Bangkok metropolitan is rarely done [6] with a direct discharge of large volumes of treated wastewater into the canals and then the

Chao Praya River, the main river in the central part of Thailand, a large amount of enteric ARB has been reported in the Chao Praya River [14].

Because migration of ARB is possible worldwide [1], information on ARB sources in various parts of the world is still required to understand ARB development and transport behavior. Worldwide transport and commercial activities are helping the dissemination of bacteria even between different oceans and continents with rare reports from Southeast Asian countries. The number of coliform bacteria including ARB in the domestic wastewater and in the treatment process relative to those in the receiving water had been investigated. This study reports the prevalence of viable ARB in municipal WWTP and its receiving canal water. In this study, *E. coli* was used as the indicator to evaluate antimicrobial resistance prevalence in WWTP and receiving canal water.

MATERIALS AND METHODS

Wastewater treatment process and sampling location

Dindaeng WWTP is the biggest central treatment plant in Thailand. It receives wastewater from 2.3% of the total Bangkok metropolitan area (37 km^2) serving 1,080,000 people in 8 districts: Phomprapsatruphai, Samphanthawong, Patumwan, Rajtawee, and some parts of Phranakhon, Dusit, Phayathai, and Dindaeng districts. Dindaeng WWTP is a conventional activated sludge (CAS), one type of suspended growth treatment systems, with a treatment capacity of 350,000 m^3/d with an inflow of 204,000–206,000 m^3/d. The operation condition of the WWTP is 4 h-HRT (hydraulic retention time) with mixed liquor suspended solids (MLSS) of 6,000 mg/l in aeration tank [15]. The excess sludge from the clarifier is concentrated to 20% biosolids by the belt filter press. Then, lime powder ($Ca(OH)_2$) is mixed into the dewatered sludge to control odor during transportation. The treated wastewater (effluent) is discharged without disinfection into the pond, namely Rama VI, joined to the "Samsane canal". In this study, 2 sampling rounds of *E. coli* collection were performed in the dry period of the year to avoid interference with rainwater effect. The 1st round (January to April) was to evaluate the phenotypic alteration of *E. coli* in WWTP (300 colonies in total), thus *E. coli* isolates from each location of WWTP (each $n = 100$): influent, effluent, and dewatered sludge, had been performed monthly with 20–30 *E. coli* colony collection. The 2nd round was carried out monthly from November to December by 20–30 *E. coli* colony collection to investigate the phenotypic alteration of *E. coli* in receiving water in correlation to those from WWTP. The *E. coli* was isolated from influent/effluent of WWTP including the water samples from the receiving canal at 1, 2, 3, and 4 km from the WWTP effluent discharged point (each $n = 60$, and 360 colonies in total). The samples for biological analysis were kept in 500 ml sterile bottles stored in the ice box and immediately transported to the laboratory. All samples were microbial analysis within 2 h. Concurrently, separate sample bottles (1 l) for the common chemical properties of wastewaters ($n = 6$) were determined according to standard methods of wastewater analysis [16] to evaluate the monthly general performance of WWTP.

Bacterial enumeration and examination of antibiotic resistance of *E. coli*

Total coliforms/fecal coliforms/*E. coli* and *Enterobacter* spp. were enumerated by the 5 tubes-MPN technique for the 1st sampling [16]. In addition, *E. coli* was isolated by spread plate method and membrane filtration technique using Chromocult coliform agar (ChromoCult® Coliform Agar, Merck KGaA, Darmstadt, Germany) [17]. It is noted that for the samples with high bacterial concentrations such as influent/dewatered sludge, the spread plate method was used, while for those of low bacterial concentrations such as effluent, the filtration technique was employed. For the 2nd sampling, only total coliforms and *E. coli* were enumerated using the spread plate method (influent) and membrane filtration technique (effluent/canal water). For susceptibility test, the *E. coli* isolates were cultured in Tryptic soy (TS) broth (Perlcore, EIKEN Chemical®, Tokyo, Japan) at 35 ± 2 °C until the turbidity was equivalent to the McFarland No. 0.5. Thereafter, each *E. coli* culture was smeared on Mueller Hinton agar plates (MH Agar, BBL™, New Jersey, USA) for disk diffusion test. Twenty antibiotics were placed on MH agar to assess *E. coli* antibiotic resistance. All test plates were incubated at 35 ± 2 °C for 18 to 22 h [18]. Evaluation of antibiotic resistance of each antibiotic was carried out by measuring a clear diameter around the disks. Three characteristics of antibiotic susceptibility for sensitive (S), intermediate (I), and resistant (R) were assessed according to the leaflet of the EIKEN Chemical®. The susceptible control in disk assay was *E. coli* TISTR780 (Microbiological Resources Centre, Thailand Institute of Scientific and Technological Research, Thailand). Those tested antibiotics (KB Disk®, EIKEN Chemical) are different bacterial resistant mechanisms categorized into 5 classes: (1) aminoglycosides, 4 types: kanamycin (KM); gentamycin (GM); tobramycin (TOB); amikacin (AMK), (2) tetracyclines, 3 types: tetracycline (TC); doxycycline (DO); and minomycin (MNO), (3) chloramphenicol (CP), (4) quinolones, 11 types: nalidixic acid (NA); cinoxacin (CIN); norfloxacin (NFX); ofloxacin (OFX); enoxacin (ENX); ciprofloxacin (CIP); lomefloxacin (LFX); fleroxacin (FLX); levofloxacin (LVX); gatifloxacin (GFLX); and sitafloxacin (STFX), and (5) Sulfonamide (ST). It is noted that only cephalothin, (beta-lactams, KF) was tested additionally for the 2nd round strains of *E. coli* because high prevalent AMR of beta-lactams in healthy worker adults in Thailand has been reported [4].

RESULTS AND DISCUSSION

Wastewater characterization and CAS performance

The chemical characteristics of sewage treated effluent of the CAS are shown in Table 1. Overall properties indicate that treatment efficiencies (22–88%) for the major chemical parameters had been achieved for the quality standard of domestic discharged wastewater of Thailand. In terms of bacterial elimination, it was found in the range of 92–94% equivalent to a reduction of 1.0–1.7, 0.9–1.3, 0.3–1.4, and 0.4–1.5 log MPN/100 ml for total coliforms, fecal coliforms, *E. coli*, and *Enterobacter* spp., respectively. These efficiencies were slightly higher relative to the general CAS performance of microbial removal (80–90%) as reported elsewhere [19]. This discharged bacterial concentrations of the CAS was above the guidelines of wastewater reuse for agriculture and the standard water quality of the surface water (Type 3) for agricultural applications in Thailand (Table 1).

Alteration of antibiotic-resistant *E. coli* population in CAS

Table 2 shows changes in the antibiotic resistance of *E. coli* strains in various samples of CAS (the 1st sampling). It is noted that MRE was classified into 3 categories by amount of resistant antibiotics: a low resistant degree (2 to 5 antibiotics); a medium resistant degree (6 to 10 antibiotics); and a high resistant degree (>10 antibiotics). In the case of *E. coli* resistance to only one antibiotic in 20 tested antibiotics, it was classified as ARE. It shows that ARE was eliminated by the WWTP by about 37%. Inversely, a more susceptive *E. coli* population to all tested antibiotics was detected in the CAS effluent (24%). It seems that the antibiotic resistance character of *E. coli* disappeared after the CAS process. Likewise, in the case of MRE, only the medium resistance (6 to 10 antibiotics) decreased from 12% to 9% of effluent, while the low resistance (2 to 5 antibiotics) increased from 38% to 46%. This evidence suggests that the number of antibiotic resistance expressions of the MRE in the effluent could reverse after the CAS process. Although most activated sludge processes treating urban wastewater generated high ARE concentrations with 5 to 7 antibiotic resistances in various antibiotic groups [20], the mechanisms by which biological processes/conditions influence the development/selection of ARB and ARG transfer are still poorly understood up to date [5, 21]. Unfortunately, there has not been the same classification of MRE in the previous study as this study presented. Therefore, information on antibiotic resistance alteration of *E. coli* in a WWTP has been limited. Nevertheless, there have been few reports on the removal of ARB in domestic WWTPs. Some studies suggest that multidrug-resistant and susceptible microbial populations are not equally affected. It is proposed that the removal efficacy of ARB by a WWTP is influenced by many factors such as the bacterial species, influent properties, bacterial loads [1, 2] as well as the design/operation of the WWTP [13, 22]. Pathogenic ARB seems to be removed highly in WWTP relative to the coliform ARB. The percentage of coliforms and fecal coliforms carrying transmissible resistance genes was higher in the treated sewage compared to the raw sewage, while the number of selected resistant pathogens was reduced up to 99% [20]. It is mentioned that the mechanism of antibiotic resistance development is via horizontal transferring of the mobile genetic elements such as plasmids, transposons, and integrons between bacterial species in the activated sludge process [13]. Inversely, the reverse of antibiotic resistance expression could be found in bacteria that live in a non-stress condition. It is proposed that the main biological parameter that influences the rate of development of resistance, the stability of the resistance, and the rate at which the resistance might decrease if antibiotic is used was reduced [23].

In the case of the dewatered sludge, the dewatered sludge of this CAS with lime addition showed a higher proportion of susceptibility to the resistance of *E. coli* relative to that of the influent and effluent. However, disinfection of sludge by chemicals such as $Ca(OH)_2$ can generally reduce the significant number of microorganisms [24]. However, given the selection pressure, bacterial strains with appropriate mechanisms of resistance may have a better chance of survival [24]. Remarkably, the MRE of 11–16 antibiotics increased about 4 times (24%) compared to those of the influent/effluent (6%). Oppositely, only 29% of medium MRE (2–5 antibiotic resistances) was a lower proportion than that detected in the influent/effluent. Because dewatered sludge contains high condenses of biomass (20%, ∼200,000 mg solids/l), which are very high compared to those of influent and effluent (11–26 mg/l). It is stated that horizontal gene transfer is possible in technical equipment wherever bacteria concentration is high [13, 25]. It might be presumable that medium MRE altered to high MRE during sedimentation or dewatered process.

Table 3 shows the total 26 phenotyping classifications of 300 resistant *E. coli* strains in the CAS samples into 5 resistant antibiotic groups. Considering the ARE of each antibiotic group, the highest percentage (10.3%) of ARE belonged to Q. The Q resistance percentage tended to reduce from the influent (19%) to the effluent (7.9%) and the sludge (13.2%). For other antibiotics, the percentage of ARE in all samples was lower (0% to 7.9%). In the case of 2 groups of antibiotic resistances, there were 9 phenotyping patterns of

Table 1 Performance of the studied CAS of Bangkok during studied period.

Parameter	Range (min–max)			Thailand WWTP effluent standard	Thailand surface water quality standard (Type 3)
	INF	EFF	Removal, %		
BOD, mg/l	32–40	4–6	85–88	20	< 2
COD, mg/l	160–320	32–60	79–84	–	–
SS, mg/l	18–26	11–14	22–54	50	–
TKN, mg/l	16–19	3–4	74–77	–	–
TN, mg/l	33–34	8–9	73–77	20	–
pH	6.6–6.8	7.3–7.5	–	5.5–9.0	–
Total coliform, log MPN/100 ml	7.2–7.5	5.6–6.3	1.0–1.7 log	–	4.3
Fecal coliform, log MPN/100 ml	7.1–7.5	6.7–7.4	0.9–1.3 log	–	3.6
E. coli, log MPN/100 ml	6.1–7.0	5.6–6.0	0.3–1.4 log	–	–
Enterobacter spp. log MPN/100 ml	6.0–7.3	5.6–6.2	0.4–1.5 log	–	–

INF, influent; EFF, effluent; and WWTP, wastewater treatment plant (chemical parameters, $n = 6$; biological parameters, $n = 4$).

Table 2 Resistant percentages of *E. coli* strains of the CAS samples to 20 antibiotics (the 1st sampling).

Number of resistant antibiotic(s)	Resistant percentage of *E. coli* isolate (%)		
	Influent ($n = 100$)	Effluent ($n = 100$)	Dewatered sludge ($n = 100$)
Sensitive to 20 antibiotics	16	24	32
Resistance (ARE+MRE)	84	76	68
ARE	28	15	11
MRE Category			
a) Low: 2–5 antibiotics	38	46	29
b) Medium: 6–10 antibiotics	12	9	4
c) High: 11–16 antibiotics	6	6	24

ARE: antibiotic-resistant *E. coli* and MRE: multidrug-resistant *E. coli*.

antibiotic resistances. The Q-S MRE was mostly found especially in the influent/effluent (10.7% to 13.2%) relative to the other group of antibiotics (0% to 4.8%). The similarity of the bacterial resistance mechanism of quinolone and sulfonamide is an alteration of the target site in DNA synthesis, while that of quinolone and tetracycline is an active efflux pump [26]. Different from the S-T MRE, it was found in the range of 7.1% to 10.3% of all samples. Nevertheless, the highest percentage of resistance phenotypes belongs to the Q-S-T MRE of the sludge sample (17.6%). The Q-S-T MRE had increased markedly relative to that of the influent. Likewise, the Q-S-T-C-A MRE showed the same trend as the Q-S-T ARE. Nevertheless, the Q-S-T-C MRE was slightly decreased because of a decrease in the C-ARE percentage. Overall results suggest that ARE of the CAS tended to decrease after the treatment process, while more MRE was detected in the sludge. The Q-S-T MRE was mostly found in the sludge while the Q-S MRE was more frequently detected in the effluent.

Antibiotic-resistant phenotypes of *E. coli*

Antibiotic-resistant phenotypes of *E. coli* strains in the CAS sample against 20 antibiotics are shown in Fig. S1. The top 3 highest percentages of resistance to antibiotics were NA (nalidixic acid, 54%), ST (sulfamethoxazole, 50%), and TC (tetracycline, 42%), respectively. After the treatment process, these high resistance percentages to 3 antibiotics remained in effluent/sludge. The ST-ARE was remarkably high percentages relative to that from previous reports in the activated sludge processes (up to 20%, Table 4) in many countries in Europe [24, 27–29]. Nevertheless, the WWTP in Portugal [19] addressed a constant ARE percentage in influent/effluent (22%), and only one plant shows a similar trend of ARE as of this study. Inversely, TC ARE slightly decreased in the effluent but increased in the sludge. TC is one of the common antibiotics that most ARE in sewage tend to develop resistance to during the treatment process (Table 4). It was reported that the resistance to TC and ST is influenced by their residuals in WWTP and temperature [11]. Different from NA, ARE tended to decrease after treatment of both the effluent and sludge. As compared to the available data of NA-ARE in 10 years (4% to 10%) [24], the NA-ARE of this CAS was about 4 times significantly higher. Besides, it is seen that ARE in the sludge had higher resistance to the latest generations of quinolones (LFX, ENX, FLX, CIN, OFLX, LVX, NFX, and STFX). Neverthe-

Table 3 Antibiotic resistance phenotypes of *E. coli* isolates in WWTP categorized by class of antibiotics.

Phenotype pattern No.	Phenotype	Amount of resistant strain (n=100 each)			Total (n=300)	Frequency over total resistance (%)			Total (%)
		INF	EFF	SLD		INF	EFF	SLD	
1.1	Q	16	6	9	31	19.0	7.9	13.2	10.3
1.2	T	5	6	4	15	6.0	7.9	5.9	5.0
1.3	S	5	2	4	11	6.0	2.6	5.9	3.4
1.4	C	4	2	0	6	4.8	2.6	0	2.0
1.5	A	0	1	1	2	0	1.3	1.5	0.7
2.1	Q-S	9	10	1	20	10.7	13.2	1.5	6.7
2.2	Q-T	4	1	2	7	4.8	1.3	2.9	2.3
2.3	Q-C	0	3	0	3	0	3.9	0	1.0
2.4	Q-A	0	0	1	1	0	0	1.5	0.3
2.5	S-T	6	7	7	20	7.1	9.2	10.3	6.7
2.6	S-C	0	0	1	1	0	0	1.5	0.3
2.7	S-A	1	0	0	1	1.2	0	0	0.3
2.8	T-A	0	2	1	3	0	2.6	1.5	1.0
2.9	T-C	2	2	0	4	2.4	2.6	0	1.3
3.1	Q-S-T	3	7	12	22	3.6	9.2	17.6	7.3
3.2	Q-S-C	5	7	2	14	6.0	9.2	2.9	4.7
3.3	Q-T-C	2	2	0	4	2.4	2.6	0	1.3
3.4	Q-S-A	0	2	0	2	0	2.6	0	0.7
3.5	S-T-C	2	1	2	5	2.4	1.3	2.9	1.7
3.6	S-T-A	2	2	1	5	2.4	2.6	1.5	1.7
4.1	Q-S-T-C	8	6	6	20	9.5	7.9	8.8	6.7
4.2	Q-S-T-A	3	2	2	7	3.6	2.6	2.9	2.3
4.3	Q-S-C-A	1	1	3	5	1.2	1.3	4.4	1.7
4.4	Q-T-A-C	1	1	0	2	1.2	1.3	0	0.7
4.5	S-T-A-C	0	1	2	3	–	1.3	2.9	1.0
5.1	Q-S-T-C-A	5	2	7	14	6.0	2.6	10.3	4.7
	Overall	84	76	68	228	100.0	100.0	100.0	100.0

Number of tested antibiotics; A: 4 aminoglycosides; T: 3 tetracyclines; C: 1 chloramphenicol; Q: 9 quinolones; S: 1 sulfonamide; INF, influent; EFF, effluent; SLD, sludge; and WWTP, wastewater treatment plant.

Table 4 Antibiotic resistance percentages (disk diffusion assay) of *E. coli* isolates from wastewater treatment plants in comparison to other countries.

Country	Treatment type	Sample	% of *E. coli* resistant strain									
			KM	GM	TC	CP	NA	NFX	OFX	LVX	CIP	ST
Thailand (This study)	AS	INF	8	7	42	30	54	10	9	6	12	50
		EFF	9	5	37	25	44	5	5	7	6	51
		SLD	9	15	48	20	39	20	23	22	23	51
Finland [27]	na	INF	na	na	10–17	2–7	na	na	na	na	na	16–20
		EFF	na	na	5–14	na	na	na	na	na	na	0–7
Austria [24]	AS	INF	na	na	6–29	0	0–10	0	0	na	0	2–4
		EFF	na	na	16–35	0–8	0–4	0	0	na	0	0–10
		SLD	na	na	0–57	0–2	0–15	0–2	0–2	na	0–2	0–13
Germany [38]	AS	INF	0	0–15	0–5	0	na	na	na	na	na	0–15
		EFF	0	0	0–26	0	na	0	0	na	0	0
Portugal [19]	AS	INF	na	3.8	32.1	na	na	na	na	na	2.5	22.2
		EFF	na	5.6	36.8	na	na	na	na	na	9.7	22.5
USA [39]	na	EFF	0	na	14	na	1	na	na	na	na	na
Poland [29]	AS	EFF	na	2	na	na	na	na	na	9.5	10.5	11.1

“na” = data not available; INF, influent; EFF, effluent; and SLD, sludge.

less, the ARE percentage tended to decrease with a new generation, or in other words, the more generation, the more decrease in resistant character (Table S1). Compared to other sources of ARE in Thailand, the NFX-ARE isolated from in-patients tended to increase from 78% to 87%, while a decreasing trend was reported for the OFX-ARE from 72% to 62% from 1998 to 2010 [3]. There are reports of the correlation between antibiotic usage and releases into domestic sewage. CIP, the most prescribed fluoroquinolone, has been detected at levels of hundreds of ng/l in surface water and up to 5 µg/l in the effluent of wastewater treatment plants [2, 30, 31]. Most of the quinolones entering the wastewater treatment system were mainly adsorbed on the sludge as a major elimination mechanism in the range of 60% to 100% [2, 32, 33]. These findings explain that sludge is the main reservoir of quinolones/derivatives. For this reason, microorganisms including *E. coli* in the sludge in the WWTP have high potency to quinolone exposure subsequently in resistance development.

Relationship of antibiotic-resistant *E. coli* in the CAS effluent and the receiving water

The total coliforms and *E. coli* in water samples of the CAS and the receiving water at 1 km to 4 km of the discharge point were enumerated and examined for antibiotic resistance (Table 5). In 2nd sampling, the total coliforms and *E. coli* population were eliminated by the CAS about 2 log orders from 4–5 log orders to 2–3 log orders which was a similar trend as in the 1st sampling, indicating consistency of the treatment performance. Total coliforms and *E. coli* population were about 1–2 log orders higher in the receiving water along 1 km to 4 km. The known factors, i.e. available organic substances and water temperature, are most affected by the dynamic population of microorganisms in wastewater [1, 11]. Data from the Department of Drainage, Bangkok Metropolitan, show that the BOD concentration of the receiving water (Samsane canal) was in the range of 10–20 mg/l [15], which was significantly higher than that in the CAS effluent (4–6 mg/l). Most of the canal water in Bangkok generally had high BOD concentrations while the coliforms were up to 7 log MPN orders [34]. This is because some canals in Bangkok function as large open channels directly receiving sewage from households. The high population of coliforms and *E. coli* in the receiving water provides more opportunity for resistance gene exchanges between the same species/family of ARE of the CAS effluent and the natural water bacteria [10].

Medium to high percentages of ARE in the CAS samples in the 1st sampling were similar groups as of the 2nd sampling data; NA, TC, ST, and CP showed consistency of CAS bacterial loads/efficiency. The ARE prevalence in the effluent and the receiving water was plotted to find the least square correlation (Fig. 1). The results show the high correlation of ARE prevalence of 6 antibiotics in the CAS effluent and that in the receiving water ($R^2 = 0.81$–0.96) along 1 to 4 km downstream. The percentage of ARE of NA, ST, and TC was high in the CAS effluent and tended to increase with the canal distance. The ARE percentages of those antibiotics increased to 30.0–66.7% at 3 to 4 km from the receiving point. When more antibiotics, beta-lactam, KF and AMK were tested, the highest percentage of ARE was KF resistance at 90% and 98% ARE for the influent and effluent, respectively. KF is the first generation of beta-lactams to which ARE shows commonly high resistance in various sources such as in patient specimens (71–88%) and leachate from a solid waste carried truck (57–61%) [17, 35]. Additionally, ARE of AMK in the samples from the 1st sampling shows high susceptibility (100%) in all wastewater samples, but that from the 2nd sampling shows intermediate susceptibility (23.3–36.7%) and resistance (10.0–15.0%) of the *E. coli* strains (Table 5). Increasing trends of antibiotic resistance prevalence of many AMRs including AMK have been reported [3]. Resistance prevalence could be a result of the use of antibiotics and the incidence of disease which varied by season and year [36]. According to the reports of NARST, increasing trends of AMK resistance of opportunistic AMR increase from 2000 to 2022 around 44.3% to 54.2% or ∼0.5% per year [3]. Moreover, it is seen that the AMK-ARE population increased 2 times in the canal water (30%) relative to that in the effluent (15%). Likewise, it has been reported that the proportion of ARE was generally high in a WWTP effluent and higher at 640 m downstream for trimethoprim and sulfamethoxazole whereas tetracycline was fairly constant across sites. It is mentioned that the resistance changed quickly within a short distance from 640 m to 2000 m although chemical water properties in effluent and the stream resembled [9]. Similarly, the proportion of ampicillin- and tetracycline-resistant coliform increased downstream up to 16 to 20 km of WWTP effluent inputs [37]. In conclusion, ARE generated from the CAS of Bangkok was the important source of ARE dissemination in the receiving water. As a result, ARE prevalence was highly enhanced and more populous in the receiving water environment with high coliforms and *E. coli* contamination.

CONCLUSION

The Bangkok sewage contained high percentages of MRE (56%). The top 3 highest percentages of resistance to antibiotics were NA (54%), ST (50%), and TC (42%), respectively. The treatment plant, activated sludge process, showed poor elimination of *antibiotic-resistant E. coli* (ARE/MRE, <37%). Alteration of antibiotic resistance of *E. coli* in the activated sludge process was found. MRE population of 11–16 antibiotic resistances remarkably increased in the dewatered sludge. The Q-S-T (quinolones-sulfonamides-

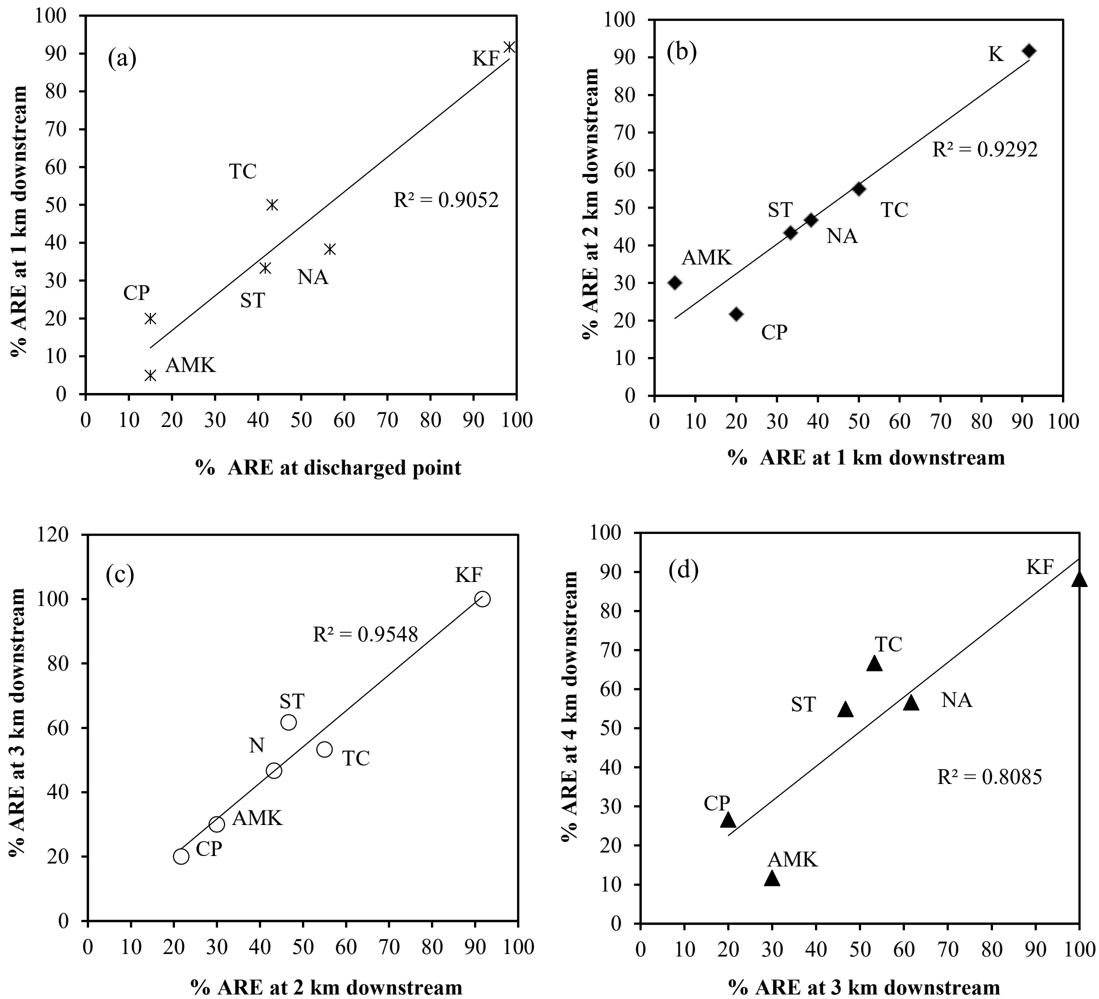

Fig. 1 Correlation of prevalence of ARE between the CAS effluent and the receiving water: (a) discharged point vs. 1 km downstream; (b) 1 km vs. 2 km downstream; (c) 2 km vs. 3 km downstream; and (d) 3 km vs. 4 km downstream.

Table 5 Amount of enteric bacteria in sewage and treated sewage and their resistances to 6 groups of antibiotics.

Water sample	Bacteria population		Intermediate/resistance of *E. coli* isolate (%), $n = 60$–100 per sample											
	Total Coliform	*E. coli*	KF		AMK		TC		CP		NA		ST	
			I	R	I	R	I	R	I	R	I	R	I	R
Inf (1st sampling)[a]	7.2–7.5	6.1–7.0	nd	nd	0.0	0.0	11.0	42.0	20.0	30.0	10.0	54.0	2.0	50.0
Eff (1st sampling)[a]	5.6–6.3	5.6–6.0	nd	nd	0.0	0.0	3.0	37.0	12.0	25.0	7.0	44.0	3.0	51.0
Inf (2nd sampling)[b]	6.9×10^5	7.0×10^4	10.0	90.0	23.3	10.0	13.3	55.0	11.7	31.7	28.3	56.7	10.0	50.0
Eff (2nd sampling)[b]	6.8×10^3	5.0×10^2	1.7	98.3	36.7	15.0	16.7	43.3	28.3	15.0	33.3	56.7	26.7	41.7
1 km[c]	1.4×10^4	8.0×10^2	8.3	91.7	31.7	5.0	6.7	50.0	8.3	20.0	53.3	38.3	10.0	33.3
2 km[c]	1.5×10^5	1.8×10^4	8.3	91.7	21.7	30.0	1.7	55.0	16.7	21.7	45.0	46.7	10.0	43.3
3 km[c]	4.1×10^5	5.0×10^4	0.0	100.0	20.0	30.0	3.3	53.3	13.3	20.0	36.7	61.7	28.3	46.7
4 km[c]	2.5×10^5	4.0×10^4	11.7	88.3	51.7	11.7	1.7	66.7	20.0	26.7	31.7	56.7	8.3	55.0

[a] unit is log MPN/100 ml; [b] unit is CFU/100 ml; [c] canal water at the distance downstream from the effluent discharged point; TC, total coliform; I, intermediate; R, resistance; KF, cephalothin; AMK, amikacin, TC, tetracycline; CP, Chloramphenicol; NA, nalidixic acid; ST, Sulfamethoxazole; and nd, not determined.

tetracyclines) was the most phenotype resistance pattern of MRE in the dewatered sludge while the Q-S MRE was highly frequent in the effluent. There were high correlations of antibiotic resistance prevalence to the 6 selected antibiotics of *E. coli* strains in the discharged effluent and those in receiving water along 1 to 4 km downstream. ARE generated from the CAS of Bangkok was considered the cause of ARE dissemination in the receiving water. The ARE population was abundantly found in the receiving water environment with high coliforms and *E. coli* contamination.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.069.

Acknowledgements: This study was financially supported by JSPS-KAKENHI, Japan.

REFERENCES

1. Tiwari A, Kurittu P, Al-Mustapha AI, Heljanko V, Johansson V, Thakali O, Mishra SK, Lehto K-M, et al (2022) Wastewater surveillance of antibiotic-resistant bacterial pathogens: A systematic review. *Front Microbiol* **13**, 977106.
2. Chiemchaisri W, Chiemchaisri C, Hamjinda NS, Jeensalute C, Buranapakdee P, Thamlikitkul V (2022) Field investigation of antibiotic removal efficacies in different hospital wastewater treatment processes in Thailand. *Emerging Contam* **8**, 329–339.
3. NARST (2023) Antimicrobial Resistant 2000–2022 (12M). The National Antimicrobial Resistance Surveillance Center of Thailand. Available at: http://narst.dmsc.moph.go.th.
4. Boonyasiri A, Tangkoskul T, Seenama C, Saiyarin J, Tiengrim S, Thamlikitkul V (2014) Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. *Pathog Glob Health* **108**, 235–245.
5. Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Sci Total Environ* **447**, 345–360.
6. Chiemchaisri W, Chiemchaisri C, Witthayaphirom C, Mahavee K, Watanabe T (2023) Surveillance of antibiotic persistence adaptation of emerging antibiotic-resistant bacteria in wastewater treatment processes: Comparison between domestic and hospital wastewaters. *Environ Technol Innovation* **31**, 103161.
7. Pruksakorn C, Pimarn C, Boonsoongnern A, Narongsak W (2016) Detection and phenotypic characterization of vancomycin-resistant enterococci in pigs in Thailand. *Kasetsart J Nat Sci* **50**, 199–203.
8. Laroche E, Petit F, Fournier M, Pawlak B (2010) Transport of antibiotic-resistant *Escherichia coli* in a public rural karst water supply. *J Hydrol* **392**, 12–21.
9. Akiyama T, Savin MC (2010) Population of antibiotic-resistant coliform bacteria change rapidly in a wastewater effluent dominated stream. *Sci Total Environ* **408**, 6192–6201.
10. Nappier SP, Liguori K, Ichida AM, Stewart JR, Jones KR (2020) Antibiotic resistance in recreational waters: state of the science. *Int J Environ Res Public Health* **31**, 33142796.
11. Novo A, Arė S, Viana P, Nunes OC, Manaia CM (2013) Antibiotic resistance, antimicrobial residues and bacterial community composition in urban wastewater. *Water Res* **47**, 1875–1887.
12. Gao P, Munir M, Xagoraraki I (2012) Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. *Sci Total Environ* **421–422**, 173–183.
13. Nguyen AQ, Vu HP, Nguyen LN, Wang Q, Djordjevic SP, Donner E, Huabing YH, Nghiem LD (2021) Monitoring antibiotic resistance genes in wastewater treatment: Current strategies and future challenges. *Sci Total Environ* **783**, 146964.
14. Honda R, Watanabe T, Sawaittayotin V, Masago Y, Chulasak R, Tanong K, Chaminda GT, Wongsila K, et al (2016) Impacts of urbanization on the prevalence of antibiotic resistant *Escherichia coli* in the Chaophraya River and its tributaries. *Water Sci Technol* **73**, 362–374.
15. BMA (2022) Bangkok Metropolitan Administration. Available at: https://dds.bangkok.go.th/News_dds/information.
16. APHA (2012) *Standard Methods for the Examination of Water and Wastewater*, 21st edn, American Public Health Association, Washington, DC, USA.
17. Threedeach S, Chiemchaisri W, Watanabe T, Chiemchaisri C, Honda R, Yamamoto K (2012) Antibiotic resistance of *Escherichia coli* in leachates from municipal solid waste landfills: comparison between semi-aerobic and anaerobic operations. *Biores Technol* **113**, 253–258.
18. CLSI (2017) *Performance Standards for Antimicrobial Susceptibility Testing*, 27th edn, CLSI Supplement M100, Clinical Lab Standards Institute, Wayne, Pennsylvania, USA.
19. Ferreira da Silva M, Vaz-Moreira I, Gonzalez-Pajuelo M, Nunes OC, Manaia CM (2007) Antimicrobial resistance patterns in *Enterobacteriaceae* isolated from an urban wastewater treatment plant. *FEMS Microbiol Ecol* **60**, 166–176.
20. Bouki C, Venieri D, Diamadopoulos E (2013) Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: a review. *Ecotoxicol Environ Saf* **91**, 1–9.
21. Zhao Q, Guo W, Luo H, Xing C, Wang H, Liu B, Si Q, Ren N (2021) Deciphering the transfers of antibiotic resistance genes under antibiotic exposure conditions: driven by functional modules and bacterial community. *Water Res* **205**, 117672.
22. Chiemchaisri W, Chiemchaisri C, Witthayaphirom C, Saengam C, Mahavee K (2022) Reduction of antibiotic-resistant-*E. coli*, -*K. pneumoniae*, -*A. baumannii* in aged-sludge of membrane bioreactor treating hospital wastewater. *Sci Total Environ* **812**, 152470.
23. Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat Rev Microbiol* **4**, 260–271.
24. Reinthaler FF, Posch J, Feierl G, Wüst G, Haas D, Ruckenbauer G, Mascher F, Marth E (2003) Antibiotic resistance of *E. coli* in sewage and sludge. *Water Res* **37**,

1685–1690.
25. Threedeach S, Chiemchaisri W, Chiemchaisri C (2016) Fate of antibiotic resistant *E. coli* in anoxic/aerobic membrane bioreactor treating municipal solid waste leachate. *Int Biodet Biodeg* **113**, 57–65.
26. Dale MM, Haylett DG (2009) *Pharmacology Condensed*, 2nd edn, Churchill Livingstone Inc., London, England.
27. Niemi M, Sibakov M, Niemela S (1983) Antibiotic resistance among different species of fecal coliforms isolated from water samples. *Appl Environ Microbiol* **45**, 79–83.
28. Galvin S, Boyle F, Hicke P, Velinga A, Morris D, Cormican M (2010) Enumeration and characterization of antimicrobial-resistant *Escherichia coli* bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *Appl Environ Microbiol* **76**, 4772–4779.
29. Łuczkiewicz A, Jankowska K, Fudala-Książek S, Olańczuk-Neyman K (2010) Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. *Water Res* **44**, 5089–5097.
30. Rosal R, Rodriguez A, Perdigon-Melon JA, Petre A, Garcia-Calvo E, Gomez MJ, Aguera A, Fernandez-Alba AR (2010) Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. *Water Res* **44**, 578–588.
31. Tello A, Austin B, Telfer TC (2012) Selective pressure of antibiotic pollution on bacteria of importance to public health. *Environ Health Perspect* **120**, 1100–1106.
32. Dorival-García N, Zafra-Gómez A, Camino-Sánchez FJ, Navalón A, Vílchez JL (2013) Analysis of quinolone antibiotic derivatives in sewage sludge samples by liquid chromatography-tandem mass spectrometry: Comparison of the efficiency of three extraction techniques. *Talanta* **106**, 104–118.
33. Hamjinda NS, Chiemchaisri W, Chiemchaisri C (2017) Upgrading two-stage membrane bioreactor by bioaugmentation of *Pseudomonas putida* entrapment in PVA/SA gel beads in treatment of ciprofloxacin. *Int Biodeterio Biodegr* **119**, 595–604.
34. Thongsri T, Petchkasem S, Muangkaew K (2014) An assessment of coliform and fecal coliform bacteria levels in surface water resources of Bangkok and vicinity area. *Bull Appl Sci* **3**, 59–67. [in Thai]
35. Kummerer K (2009) Antibiotics in the aquatic environment – a review – part I. *Chemosphere* **75**, 417–434.
36. Pulingam T, Parumasivam T, Gazzali AM, Sulaiman AM, Chee JY, Lakshmanan M, Chin CF, Sudesh K (2022) Antimicrobial resistance: prevalence, economic burden, mechanisms of resistance and strategies to overcome. *Eur J Pharm Sci* **170**, 34936936.
37. Goñi-Urriza M, Pineau L, Capdepuy M, Roques C, Caumette P, Quentin C (2000) Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. *J Antimicrob Chemother* **46**, 297–301.
38. Gallert C, Fund K, Winter J (2005) Antibiotic resistance of bacteria in raw and biologically treated sewage and in groundwater below leaking sewers. *Appl Microbiol Biotechnol* **69**, 106–112.
39. Webster LF, Thompson BC, Fulton MH, Chestnut DE, Van Dolah RF, Leight AK, Scott GI (2004) Identification of sources of *Escherichia coli* in South Carolina estuaries using antibiotic resistance analysis. *J Exp Mar Bio Ecol* **298**, 179–195.

Appendix A. Supplementary data

Table S1 Resistance/intermediate/sensitivity to 20 antibiotics of the *E. coli* isolates from the CAS samples (the 1st sampling).

Antibiotic					Percentage of *E. coli* isolate ($n = 100$ for each)								
Group		Name	Abb[b]	Dosage (μg)	Resistance			Intermediate			Sensitivity		
					inf	*eff*	*slu*	*inf*	*eff*	*slu*	*inf*	*eff*	*slu*
Aminoglycoside		Kanamycin	KM	30	8	9	9	12	27	33	80	64	58
		Gentamicin	GM	10	7	5	15	0	0	0	93	95	85
		Tobramycin	TOB	10	1	4	6	3	0	5	96	96	89
		Amikacin	AMK	30	0	0	0	0	0	6	100	100	94
		Avg[a]			4.0	4.5	7.5	3.8	6.8	11.0	92.3	88.8	81.5
Tetracycline		Tetracycline	TC	30	42	37	48	11	3	2	47	60	50
		Doxycycline	DOT	30	25	26	26	10	11	17	65	63	57
		Minocycline	MNO	30	11	8	7	9	5	18	80	87	75
		Avg[a]			26.0	23.7	27.0	10.0	6.3	12.3	64.0	70.0	60.7
Chloramphenicol		Chloramphenicol	CP	30	30	25	20	20	12	5	50	63	75
Quinolone	G[c]-1	Nalidixic acid	NA	30	54	44	39	10	7	4	36	49	57
		Cinoxacin	CIN	100	13	16	24	10	16	12	77	68	64
		Avg[a]			33.5	30.0	31.5	10.0	11.5	8.0	56.5	58.5	60.5
	G[c]-2	Norfloxacin	NFX	10	10	5	20	5	3	2	85	92	78
		Ofloxacin	OFX	5	9	5	23	4	3	2	87	92	75
		Enoxacin	ENX	10	25	13	30	17	9	13	58	78	57
		Ciprofloxacin	CIP	5	12	6	23	23	12	10	65	82	67
		Lomefloxacin	LFX	10	32	23	33	24	27	22	44	50	45
		Fleroxacin	FLX	5	9	8	25	11	12	11	80	80	64
		Avg[a]			16.2	10.0	25.7	14.0	11.0	10.0	69.8	79.0	64.3
	G[c]-3	Levofloxacin	LVX	5	6	7	22	4	2	3	90	91	75
	G[c]-4	Gatifloxacin	GFLX	5	9	9	24	8	2	6	83	89	70
		Sitafloxacin	STFX	5	3	2	12	4	3	5	93	95	83
		Avg[a]			6.0	6.0	19.3	5.3	2.3	4.7	88.7	91.7	76.0
Sulfonamide		Sulfamethoxazole	ST	23.75/	50	51	51	2	3	0	48	46	49
		(trimethoprim/trimoxazole)		1.25	50	51	51	2	3	0	48	46	49

[a]: average value of each group of antibiotics; [b]: abbreviation; [c]: generation; *inf/eff/slu*: influent/effluent/sludges samples.

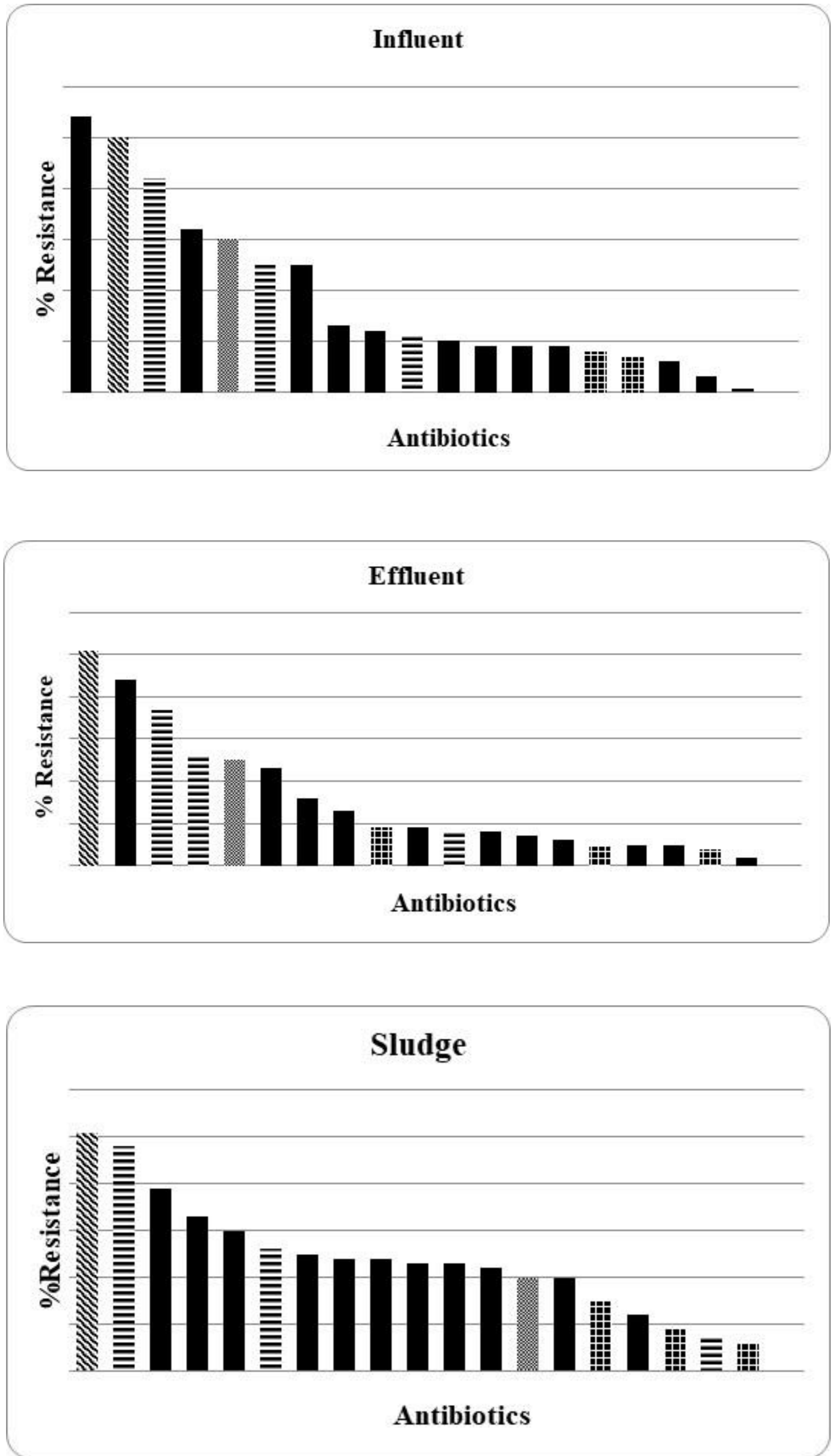

Fig. S1 Antibiotic resistance percentages of 100 *E. Coli* strains in influent, effluent, and sludge.
Antibiotic classes: ▧ Sulfonamides, ≣ Tetracyclines, ■ Quinolones, ■ Chloramphenicol, and ▦ Aminoglycosides.