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The association between antimicrobials and the antimicrobial-resistant phenotypes and resistance genes of *Escherichia coli* isolated from hospital wastewaters and adjacent surface waters in Sri Lanka

Keerthi S. Guruge [a, b, c, *, 1], Yukino A. Tamamura [a, 1], Prasun Goswami [d], Rumi Tanoue [e], K.B.S.N. Jinadasa [f], Kei Nomiyama [e], Takeshi Ohura [g], Tatsuya Kunisue [e], Shinsuke Tanabe [e], Masato Akiba [a]

[a] National Institute of Animal Health, National Agriculture and Food Research Organization, 3-1-5 Kannondai, Tsukuba, 305-0856, Ibaraki, Japan
[b] Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-oraikita, Izumisano, Osaka, 598-8531, Japan
[c] National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka
[d] Atal Centre for Ocean Science and Technology for Islands, ESSO - National Institute of Ocean Technology, Dollygunj, Port Blair, 744103, Andaman and Nicobar Islands, India
[e] Center for Marine Environmental Studies, Ehime University, 2-5 Bunkyo-cho, Matsuyama, Ehime, 790-8577, Japan
[f] Department of Civil Engineering, University of Peradeniya, Peradeniya, 20400, Sri Lanka
[g] Graduate School of Agriculture, Meijo University, 1-501 Shiogamaguchi, Nagoya, 468-8502, Japan

HIGHLIGHTS

- Clarithromycin was the most abundant antimicrobial found in hospital wastewaters.
- 94% of *E. coli* were fully or intermediately resistant to the tested drugs.
- 61% of the bacterial isolates were categorized as multidrug-resistant.
- *E. coli* isolates frequently harbored bla_{TEM}, bla_{CTX-M}, *tetA*, *qnrS*, and *sul2*.
- PCPs likely facilitated the maintenance and persistence of ARB and ARGs.

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ABSTRACT

The presence of antimicrobials, antimicrobial-resistant bacteria (ARB), and the associated antimicrobial resistance genes (ARGs) in the environment is a global health concern. In this study, the concentrations of 25 antimicrobials, the resistance of *Escherichia coli* (*E. coli*) strains in response to the selection pressure imposed by 15 antimicrobials, and enrichment of 20 ARGs in *E. coli* isolated from hospital wastewaters and surface waters were investigated from 2016 to 2018. In hospital wastewaters, clarithromycin was detected at the highest concentration followed by sulfamethoxazole and sulfapyridine. Approximately 80% of the *E. coli* isolates were resistant, while 14% of the isolates exhibited intermediate resistance against the tested antimicrobial agents. Approximately 61% of the examined isolates were categorized as multidrug-resistant bacteria. The overall abundance of phenotypes that were resistant toward drugs was in the following order: β-lactams, tetracycline, quinolones, sulfamethoxazole/trimethoprim, aminoglycosides, and chloramphenicol. The data showed that the *E. coli* isolates frequently harbored bla_{TEM}, bla_{CTX-M}, *tetA*, *qnrS*, and *sul2*. These results indicated that personal care products were significantly associated with the presence of several resistant phenotypes and resistance genes, implying their role in co-association with multidrug resistance. Statistical analysis also indicated a disparity specific to the site, treatment, and year in the data describing the prevalence of ARB and ARGs and their release into downstream waters. This study provides novel insights into the abundance of antimicrobial, ARB and

* Corresponding author. National Institute of Animal Health, National Agriculture and Food Research Organization, 3-1-5 Kannondai, Tsukuba, 305-0856, Ibaraki, Japan.
E-mail address: guruge@affrc.go.jp (K.S. Guruge).
[1] These authors contributed equally to this work.

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ARGs in Sri Lanka, and could further offer invaluable information that can be integrated into global antimicrobial resistance databases.

1. Introduction

Pharmaceutically active compounds such as antimicrobials and biocides/preservatives (often referred to as personal care products [PCPs]) are introduced into aquatic environments via numerous pathways, including the direct discharge of untreated or treated wastewater from municipal wastewater treatment plants (WWTPs), hospitals, industrial WWTPs, and sewage overflow (Azuma et al., 2016; Coutu et al., 2013; Larsson et al., 2007; Paulshus et al., 2019; Verlicchi et al., 2012). The effluent from WWTPs is considered a major source of antimicrobials found in aquatic ecosystems, and considerable variations in their concentrations have been reported across geographical regions (Reviewed by Tran et al., 2018).

Among all the classes of drugs targeting humans and animals, antimicrobials are indispensable in clinical practice for the control of infectious diseases. However, excessive and inappropriate use of such drugs and their subsequent release into the aquatic environments could accelerate the risk of emergence and proliferation of ARB and the increase in the copy number of ARGs in such bacteria (Reviewed by Bouki et al., 2013; Reviewed by Qiao et al., 2018; WHO, 2014; Zhang et al., 2009). ARB and ARGs are a cross-cutting issues that pose a risk to global health and could hamper the advances made in medical science for the treatment of infectious diseases (Hendriksen et al., 2019). Even very low concentrations of antimicrobials in the environment could facilitate the development and maintenance of bacterial resistance (Gullberg et al., 2011). WWTPs, including hospital WWTPs, have been recognized as reservoirs of resistant bacteria (Galvin et al., 2010; Reviewed by Rizzo et al., 2013) and major routes for the transmission of antimicrobial resistance from man-made sources into the environment (Baquero et al., 2008; Reviewed by Pazda et al., 2019). Considering its close association with humans and ease of isolation and identification, *E. coli* is one of the most widely monitored bacteria in the assessment of antimicrobial resistance in the environment (Akiba et al., 2016; Figueira et al., 2011; Paulshus et al., 2019).

The global consumption of antimicrobials and other pharmaceuticals has continued to increase, and the bulk of this increase has been observed in developing nations (Klein et al., 2018). Global monitoring of antimicrobial resistance (AMR) based on metagenomic analyses of urban sewage has revealed that the abundance of resistance genes is highly correlated with socio-economic, health, and environmental factors, and varies across regions (Hendriksen et al., 2019). However, data regarding the distribution and abundance of ARB and ARGs in the environment at national and global scales are inadequate, which limits the evaluation of the risk of AMR transmission among bacteria (Berendonk et al., 2015). Therefore, it is necessary to investigate the regional differences in the occurrence and distribution of antimicrobials and ARB/ARGs in WWTPs and their receiving waters to facilitate efforts aimed at alleviating the spread of AMR in aquatic environments (Pärnänen et al., 2019; Yuan et al., 2020).

Previously, 16 antimicrobials were detected in surface waters across Sri Lanka. It was reported that certain concentration thresholds of such drugs in urban wastewater canals pose high risks to the ecosystem and suggested that hospital wastewater discharge could be a major source of such antimicrobials (Guruge et al., 2019). Although large-scale urban WWTPs are not well established in major Sri Lankan cities, hospital wastewater is treated before discharge. Nevertheless, precise data on the levels of multiple antimicrobials, ARB, and ARGs in hospital wastewaters in Sri Lanka are limited. Therefore, the present study aimed to investigate 1) the presence of 25 antimicrobials, 2) the prevalence of *E. coli* phenotypes resistant to 15 selected antimicrobials, 3) the prevalence of 20 frequently detected ARGs in *E. coli,* and 4) the effects of multiple antimicrobials on the selective potential of ARB and ARGs in wastewaters collected from three hospitals and a nearby upstream lake and a downstream canal in 2016–2018.

2. Materials and methods

2.1. Sample collection

Samples were collected from five locations, including three hospitals, a lake, and a wastewater canal from Kandy, the second-largest city in Sri Lanka. Details outlining the study area and sample collection are presented in Fig. 1 and Supplementary Table S1.

Three hospitals were selected in the study area (hospital names and locations have not been disclosed). Hospital 1 (H1) was the largest, with 78 wards for the treatment of in-house patients, followed by Hospital 2 (H2) and Hospital 3 (H3), with 21 and 11 wards, respectively. The sewage/wastewater treatment capacity in H1, H2, and H3 was 600, 450, and 300 m^3/day, respectively. Surface water samples were collected from Kandy Lake and the downstream Mid Canal (Meda Ela), which originates from the Kandy Lake and passes through a densely populated area where several public and private hospitals and clinical laboratories are located in the city. Grab water samples were collected in clean 500 mL polypropylene bottles three times in December 2016, September 2017, and September 2018. Typically, the months from September to December represent periods with relatively low or no rainfall in the sampling area. A total of 34 samples were collected from the treatment plants at the hospitals, Kandy Lake, and the Mid Canal (Supplementary Table S1). All samples were transported to Japan within 48 h of sampling. Soon after arrival, an aliquot of each sample was fixed using 30% sterilized glycerin, mixed well, and stored at −80 °C until further bacterial isolation. The remaining sample was stored at −20 °C for chemical residue analyses. The samples were analyzed in Japan due to inadequate laboratory facilities in Sri Lanka. The microbiological analyses were performed at the National Institute of Animal Health-NARO, Tsukuba, Japan, and the chemical analyses were conducted at the Ehime University, Matsuyama, Japan. The permission for sampling and sample transportation was obtained from the hospital authorities and the Department of Civil Engineering, University of Peradeniya, Sri Lanka. Previously, it was observed that several target compounds were stable during storage and transportation (Prabhasankar et al., 2016), and the sample quality was adequate for conducting both chemical and microbiological analyses (Akiba et al., 2015; Guruge et al., 2019).

2.2. Antimicrobial analyses

Twenty-five antimicrobials (18 antibiotics and seven PCPs) were analyzed in the present study; 13 isotopic internal standards (ISs)

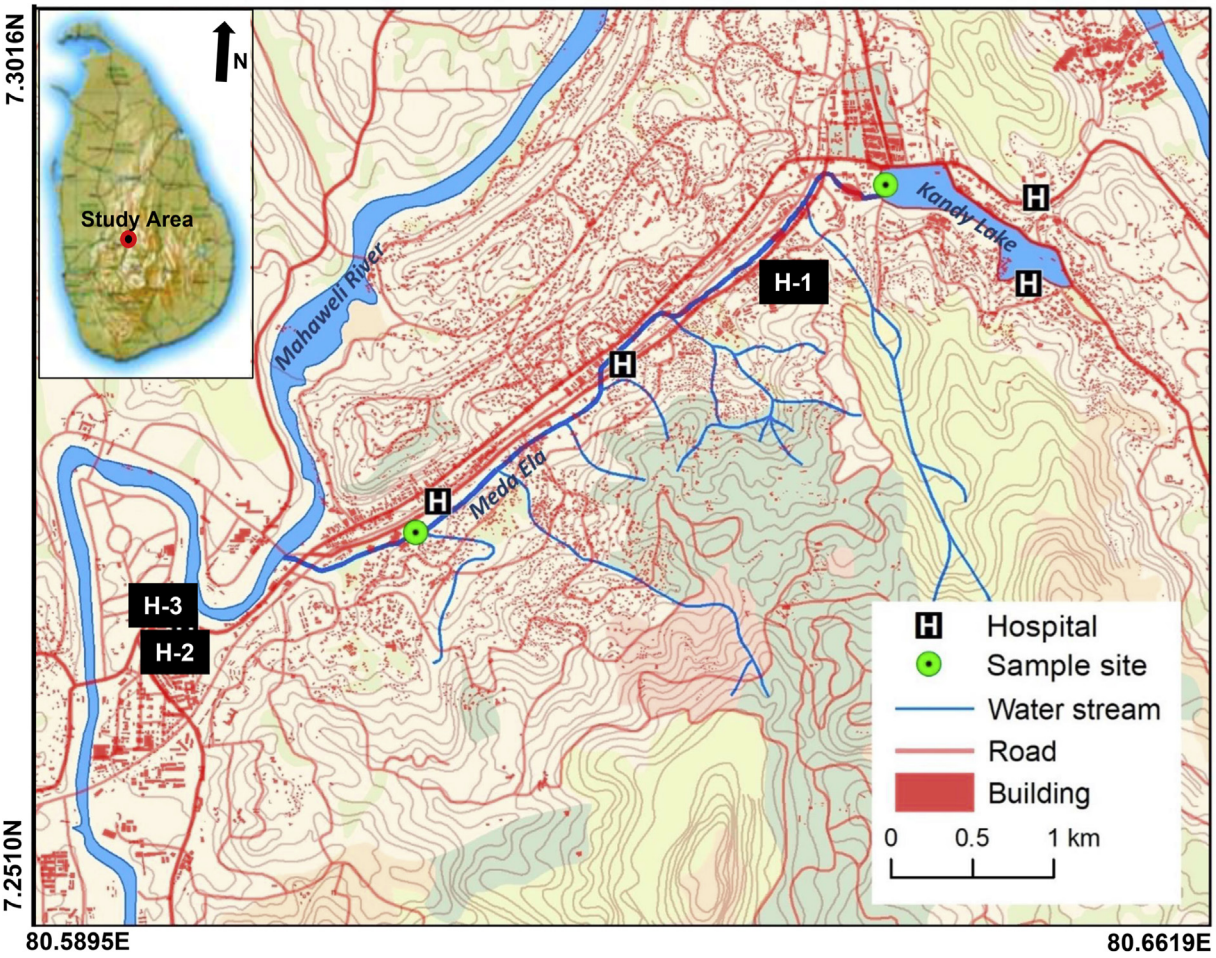

Fig. 1. The location of the sampling sites in Sri Lanka.

were used for quality control and quality assurance of the analytical methods (Supplementary Table S2) (Guruge et al., 2019). High purity (>95%) analytical standards of the target compounds, ISs, organic solvents, and deionized water were purchased from commercial suppliers (Guruge et al., 2019).

2.3. Sample extraction

Samples were extracted according to the methods proposed in a previous study (Guruge et al., 2019). Briefly, all the samples were filtered through glass fiber filters to remove suspended solids. The filtrate (50 mL for surface waters or 5 mL for hospital wastewater) was spiked with internal standards (Supplementary Table S2) and loaded onto a preconditioned Oasis HLB Plus Light cartridge (Waters, Milford, MA, USA). The cartridge was washed with Milli-Q water and vacuum-dried. The analytes retained in the cartridge were eluted, and the eluate was concentrated under nitrogen gas flow. The residue was reconstituted in methanol/Milli-Q water and filtered before analysis.

2.4. Instrumental analysis, quality assurance, and quality control

Identification and quantification of the analytes were performed using an ultra-fast liquid chromatography system (Shimadzu, Japan) coupled with an AB Sciex Qtrap 5500 mass spectrometer (Applied Biosystems, Tokyo, Japan) operating under positive and negative electrospray ionization (ESI) modes with multiple reaction monitoring (MRM). Detailed information on the liquid chromatography and mass spectrometry parameters, chromatographic separation, ion sources, MRM transitions, and data acquisition has been presented in a previous publication (Guruge et al., 2019).

The concentrations of target compounds were determined using the isotope-dilution method (Guruge et al., 2019). IS-corrected (relative) recovery rates ± standard deviation (SD) and method detection limits (MDLs) of the target compounds are listed in Supplementary Table S2. The recovery rates were determined by analyses of surface water samples spiked with native standards at concentrations of 4, 20, and 100 ng/L (in triplicate). MDLs were calculated from the SDs of nine replicate injections of surface water extracts spiked with native standards at low concentrations. During data calculations and statistical analyses, the concentrations below the MDL were considered as zero.

2.5. Isolation and identification of bacteria

The standard operating procedures for bacterial isolation have been described in a previous study (Akiba et al., 2015). Briefly, the frozen water samples were thawed and diluted appropriately with sterilized phosphate-buffered saline. The diluted wastewater samples were plated on chromocult coliform agar (Merck KGaA, Darmstadt, Germany) and incubated at 37 °C under aerobic conditions. The plates were examined after 24 h, following which, violet colonies—positive for both β-galactosidase and β-glucuronidase, indicating the presence of *E. coli*—were randomly

isolated. Up to 10 violet colonies were selected from each sample. Kovacs' indole reagent (Merck KGaA, Darmstadt, Germany) was used for the detection of indole production. PCR was used to confirm the indole-positive isolates as *E. coli* (Iguchi et al., 2015). The isolates were stored in Luria-Bertani broth (Becton, Dickinson and Company, Franklin Lakes, NJ) with 25% glycerol at −80 °C until further use.

2.6. Antimicrobial susceptibility tests

Antimicrobial susceptibility was assessed by the Kirby−Bauer disk diffusion test using Sensi-Disc susceptibility test discs (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) on Muller-Hinton agar plates (Becton, Dickenson and Company) according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI, 2017). In the present study, the following 15 antimicrobials were tested: ampicillin (AMP; 10 μg), cefazolin (CEZ; 30 μg), cefotaxime (FOX; 30 μg), cefoxitin (CTX; 30 μg), chloramphenicol (CHLP; 30 μg), tetracycline (TC; 30 μg), streptomycin (SM; 10 μg), kanamycin (KM; 30 μg), gentamicin (GM; 10 μg), sulfamethoxazole/trimethoprim (SMXZ/TRI; 23.75/1.25 μg), nalidixic acid (NA; 30 μg), ciprofloxacin (CIP; 5 μg), levofloxacin (LVX; 5 μg), ofloxacin (OFX; 5 μg), norfloxacin (NORF; 10 μg).

2.7. Bacterial DNA extraction and identification of ARGs

DNA from each *E. coli* isolate was extracted using the alkaline-boiling method described previously (Ooka et al., 2009). All DNA samples were stored at −20 °C until further analysis. PCRs were conducted to detect 20 ARGs, including 3 β-lactam (bla_{CTX-M}, bla_{SHV}, and bla_{TEM}), 3 sulfonamide (*sul1*, *sul2*, and *sul3*), 4 trimethoprim (*dfrA1*, *dfrA5/14*, *dfrA7/17,* and *dfrA12*), 2 TC (*tetA* and *tetB*), 2 S M (*aadA* and *strAB*), 1 KM (*aphA1-Iab*), 2 CHLP (*cat* and *cmlA*), and 3 quinolone (*qnrA*, *qnrB,* and *qnrS*) resistance genes. Each gene-specific primer and PCR conditions are listed in the references given in the Supplementary Table S3. The PCR products were analyzed by electrophoresis on 2% agarose gels.

2.8. Statistical analysis

All data were tested for normality and homogeneity of variance and were $\log_{10}$ transformed wherever necessary to meet these criteria. Comparisons of average chemical concentrations among various sample settings were performed using one-way ANOVA, followed by Tukey's multiple comparison test. The correlation between the concentration of antimicrobials and prevalence of antimicrobial-resistant *E. coli* or ARGs was determined by linear regression analysis. In addition, multivariate logistic regression analyses were performed to assess the associations between the concentrations of fluoroquinolones (FQs) and PCPs, locations, treatment process (influent or effluent), and sampling year, while selecting the prevalence of antimicrobial resistance of *E. coli* as the target variable (Akiba et al., 2015). The concentration of FQs and PCPs, location, treatment step and sampling year were designated as the explanatory variables. Similarly, the effects of these explanatory variables on the persistence of resistance genes (target variable) were also studied. For each explanatory variable, H1 (the largest hospital) as the location, influent data, and data obtained in 2016 were used as reference levels to determine the significance. Resistance data were used for all statistical analyses (intermediate resistance data not included). The analysis was conducted with the R Core Team (2019). Differences were considered significant if $p < 0.05$.

3. Results

3.1. Presence of antimicrobials in hospital wastewaters and surface waters

A summary of the concentrations of the detected compounds is presented in Table 1. Among the 25 investigated compounds, 19 were detected in the water samples. Among the sulfonamides, sulfapyridine (SPR) and SMXZ were detected at concentrations of up to 14,120 and 15,300 ng/L in the influent and effluent waters of H1, respectively. However, sulfamethazine (SMT) was detected only in samples obtained from H1 and the Mid Canal, with maximum concentrations of 6.2 and 3.2 ng/L, respectively. The SPR ($p < 0.05$) and SMXZ ($p < 0.001$) concentrations were significantly higher in the influent and effluent waters of H1 than those of other hospitals, Kandy Lake, and the Mid Canal. In addition, SMXZ levels in the H1 effluent were significantly higher ($p < 0.001$) than those in the influent. Among all target compounds, clarithromycin (CLA), a macrolide, was present at the highest concentrations (18,800 ng/L) in the H1 influent. The highest concentrations of the other two macrolides, namely, erythromycin (ERY) and roxithromycin (ROX) were 838 ng/L and 29 ng/L in the H2 and H1 influents, respectively. Among the fluoroquinolones (FQs), CIP was dominant in the H1 influent followed by LVX and NORF with the highest concentrations of 11,300 ng/L, 3760 ng/L, and 144 ng/L, respectively. All three compounds were detected at 100% frequency in H1 and the Mid Canal samples. However, no compound was observed in the samples collected from Kandy Lake. The LVX concentrations in the H1 influent were significantly higher than those in the H3 influent ($p < 0.05$).

The concentrations of TRI, which is often administered in combination with SMXZ, were significantly higher ($p < 0.05$) in the H1 effluent than those in the H1 influent and at all other sites ($p < 0.01$). The highest TRI levels (2440 ng/L) were observed in the H1 influent, while its mean concentrations in the Mid Canal (75 ng/L) increased by two orders of magnitude when compared to the concentrations in Kandy Lake. The highest lincomycin (LIN) concentration of 1316 ng/L was observed in the H1 influent; however, it was not detected in Kandy Lake. Notably, CHLP, a phenicol, was not detected in H1, Kandy Lake, and the Mid Canal samples, and was observed mostly in the effluents of H2 and H3, reaching up to the concentrations of 4100 ng/L and 2115 ng/L, respectively. In addition, CHLP concentrations in the H2 effluent were significantly higher ($p < 0.05$) than those observed in the influent.

The concentrations of PCPs varied considerably across all locations. Triclocarban (TCC) was detected in all samples excluding lake water, with the highest value recorded in the H1 effluent (363 ng/L). The maximum triclosan (TCS) concentration of 77 ng/L was detected in the H3 influent. Among parabens, the maximum concentrations of methyl paraben (MeP), ethyl paraben (EtP), and propyl paraben (PrP) were 468 ng/L, 96 ng/L, and 525 ng/L, respectively, which were detected in H2 influent. While butyl paraben (BuP) concentrations were the highest in H3 influent (11 ng/L). Notably, N,N-diethyl-3-toluamide (DEET) was detected in all Mid Canal samples with the highest concentration of 626 ng/L. Its mean value was one order of magnitude greater than the value observed in the sample collected from the lake.

3.2. Prevalence of antimicrobial-resistant E. coli phenotypes

A total of 297 *E. coli* colonies selected randomly from 30 samples were tested for sensitivity against 15 antimicrobials. The prevalence rates of non-susceptible *E. coli* are listed in Table 2. Eighty, fourteen and six percent of the total isolates exhibited resistance, intermediate resistance, and susceptibility to the tested antimicrobials,

Table 1
Concentrations (ng/L) of antimicrobials measured in the Kandy Lake, hospital influents, hospital effluents and the Mid Canal.

Compound	Kandy Lake (n = 3)		Hospital 1 (n = 8)				Hospital 2 (n = 10)				Hospital 3 (n = 10)				Mid Canal (n = 3)	
			Influent		Effluent		Influent		Effluent		Influent		Effluent			
	Range	Det. %[b]	Range	Det. %	Range	Det. %	Range	Det. %	Range	Det. %	Range	Det. %	Range	Det. %	Range	Det. %
	Mean		Mean		Mean		Mean		Mean		Mean		Mean		Mean	
SPR	<MDL[a]-1.7	67	1458–14,120	100	7050–8920	100	24–4660	100	3.6–1536	100	28–1232	100	3.1–752	100	157–262	100
	0.76		6540		8230		1474		1166		555		322		216	
SMT	<MDL		<MDL-2.7	60	1.7–6.2	100	<MDL		<MDL		<MDL		<MDL		1.0–3.2	100
			2.6		3.4										1.9	
SMXZ	0.90–4.2	100	1248–7040	100	10,320-15,3000	100	42–2000	100	5.2–416	100	46–260	100	4.0–642	100	119–450	100
	2.2		4610		13,167		470		210		147		247		244	
TRI	0.59–0.82	100	22–2440	100	1470–2040	100	42–918	100	28–262	100	24–110	100	19–181	100	27–118	100
	0.67		673		1850		253		156		84		73		75	
LIN	<MDL		52–1316	100	184–1256	100	<MDL-596	80	<MDL-1162	100	<MDL-3.8	40	<MDL-3.4	40	15–58	100
			557		896		182		512		1.4		1.4		34	
ERY	<MDL		42–122	100	161–355	100	2.9–838	100	42–165	100	23–760	100	33–455	100	27–31	100
			67		262		181		110		292		225		29	
CLA	0.37–3.1	100	2780–18,800	100	4800–6350	100	636–10,880	100	282–5800	100	486–3560	100	264–948	100	284–388	100
	1.9		6840		5817		5020		3576		1312		612		273	
ROX	<MDL		<MDL-29	40	<MDL		<MDL		<MDL-3.9	40	<MDL-6.2	60	<MDL-5.4	40	<MDL	
			5.7						1.3		2.6		1.9			
CHLP	<MDL		<MDL		<MDL		<MDL-39	40	<MDL-4100	60	<MDL-108	40	<MDL-2115	80	<MDL	
							20		2009		22		428			
NORF	<MDL		<MDL-144	40	9.5–138	100	<MDL		<MDL-47	40	<MDL		<MDL-18	40	<MDL	
			32		53				17				5.9			
LVX	<MDL		85–3760	100	252–1438	100	<MDL-119	80	<MDL-406	80	19–177	100	<MDL-29	80	9.5–33	100
			1414		648		77		151		59		18		18	
CIP	<MDL		214–11,300	100	872–2800	100	116–1516	100	12–1370	100	101–690	100	<MDL-229	100	18–63	100
			3493		1382		660		600		296		108		34	
TCC	<MDL		23–265	100	53–363	100	30–233	100	52–310	100	33–231	100	14–314	100	15–31	100
			99		165		93		120		110		112		23	
TCS	<MDL-5.5	33	<MDL-22	40	17–20	100	<MDL-67	60	<MDL-44	40	<MDL-77	60	<MDL-32	40	<MDL-19	67
	1.9		8.4		18		22		16		27		7.4		12	
MeP	<MDL-42	67	<MDL-163	40	<MDL-237	67	<MDL-468	40	<MDL-29	20	<MDL-275	40	<MDL-73	40	<MDL	
	26		63		148		165		5.7		103		25			
EtP	<MDL-9.3	33	<MDL-19	60	<MDL-22	67	<MDL-96	40	<MDL		<MDL-52	40	<MDL-20	40	<MDL	
	3.1		8.2		14		23				18		6.9			
PrP	<MDL-9.1	33	<MDL-330	80	<MDL-270	67	<MDL-525	60	<MDL		<MDL-380	80	<MDL-10	20	<MDL-60	67
	3.0		111		169		170				142		2.1		22	
BuP	<MDL		<MDL-4.6	40	<MDL-4.5	67	<MDL-10	60	<MDL		<MDL-11	40	<MDL		<MDL	
			1.2		2.1		2.7				3.9					
DEET	<MDL-47	67	<MDL-40	40	<MDL-69	67	<MDL-44	20	<MDL-30	20	<MDL-382	20	<MDL-558	60	145–626	100
	29		13		36		8.7		5.9		76		125		319	

[a] MDL: Method detection limit.
[b] Detection frequency.

Table 2
Prevalence of complete antimicrobial resistance and intermediate resistance phenotypes in *E. coli* observed in hospital wastewaters and surface waters.

Antimicrobial	Kandy Lake [8][a]		Hospital 1 [80]		Hospital 2 [100]		Hospital 3 [79]		Mid Canal [30]	
	Resistant	I. resistant[b]	Resistant	I. resistant	Resistant	I. resistant	Resistant	I. resistant	Resistant	I. resistant
AMP	5 (63)[c]	1 (13)	64 (80)	2 (3)	78 (78)	3 (3)	54 (70)	0	16 (53)	2 (7)
CEZ	3 (38)	5 (63)	44 (55)	24 (30)	67 (67)	23 (23)	45 (57)	20 (25)	9 (30)	18 (60)
CTX	1 (13)	0	32 (40)	9 (11)	57 (57)	12 (12)	35 (44)	3 (4)	1 (3)	10 (33)
FOX	0 (0)	0	8 (10)	5 (6)	12 (12)	16 (16)	17 (22)	2 (3)	1 (3)	0
SM	1 (13)	3 (38)	21 (26)	23 (29)	20 (20)	34 (34)	8 (10)	28 (35)	10 (33)	10 (33)
KM	2 (25)	1 (13)	22 (28)	6 (8)	20 (20)	10 (10)	14 (18)	6 (8)	3 10)	5 (17)
GM	1 (13)	0	6 (8)	0	6 (6)	0	7 (9)	0	1 (3)	0
CHLP	0	0	8 (10)	4 (5)	7 (7)	1 (1)	5 (6)	0	6 (20)	0
TC	4 (50)	0	42 (53)	0	52 (52)	0	37 (47)	0	17 (57)	0
SMXZ/TRI	4 (50)	0	38 (48)	3 (4)	32 (32)	1 (1)	28 (35)	2 (3)	13 (43)	1 (3)
NA	2 (25)	2 (25)	43 (54)	7 (9)	60 (60)	4 (4)	24 (30)	14 (18)	15 (50)	4 (13)
CIP	2 (25)	0	32 (40)	8 (10)	34 (34)	19 (19)	12 (15)	6 (8)	12 (40)	1 (3)
LVX	2 (25)	0	31 (40)	5 (6)	33 (33)	2 (2)	12 (15)	0	11 (37)	1 (3)
OFX	2 (25)	0	33 (41)	3 (4)	34 (34)	1 (1)	12 (15)	1 (1)	12 (40)	0
NORF	2 (25)	0	30 (38)	3 (4)	33 (33)	1 (1)	11 (15)	1 (1)	12 (40)	0

[a] Number of total isolates.
[b] Intermediate resistant.
[c] Number of isolates and percentage of isolates in parenthesis.

respectively. The percentage of isolates exhibiting intermediate resistance against CEZ, SM, KM, and NA was higher than that of isolates exhibiting intermediate resistance against other drugs. When considering only resistant isolates, samples collected from all hospitals demonstrated ≥70% resistance against AMP, with H1 samples showing the highest resistance rate (80%). Upon examining cephem resistance in hospitals, it was observed that resistance against CEZ (55–67%) was dominant when compared to resistance against CTX (40–57%) and FOX (10–22%).

The prevalence of *E. coli* exhibiting resistance against TC and SMXZ/TRI was in the range of 47–53% and 32–48%, respectively. Unique trends of resistance against FQ were observed in the wastewaters and surface waters, where the rates of isolates exhibiting resistance against CIP, LVX, OFX, and NORF were in the range of 25–41%. However, the rates of resistance were almost similar at each site. NA exhibited the highest resistance rates among all quinolones, ranging from 30 to 60% in the hospitals and the Mid Canal. Resistance to aminoglycosides was relatively low. SM and KM resistance rates ranged from 10 to 26% and 18–28%, respectively, while GM resistance rates were the lowest (6–9%) in the hospitals. The CHLP resistance rates were also low at all hospitals (6–10%). In the samples collected from Kandy Lake, resistance was studied only in 8 isolates. Nevertheless, data obtained from Kandy Lake located upstream along the flow of hospital discharges indicated that the lake had the lowest abundance rate of *E. coli* exhibiting resistance against most of the target compounds. During the three incidences of sampling at the Mid Canal, resistance against target drugs ranged from 3 (CTX, FOX and GM) to 57% (TC). The abundance of resistant phenotypes in 297 *E. coli* isolates is shown in Supplementary Fig. S1.

When a microorganism is resistant to at least one agent in three or more antimicrobial classes it can be considered to demonstrate multi-drug resistance (MDR). It was observed that 180 (61%) of the examined isolates demonstrated MDR. The MDR rates in all hospital samples were 69% (H2), 66% (H1), and 47% (H3). The MDR rates in the Mid Canal and Kandy Lake samples were 53% and 63%, respectively. Furthermore, 40%, 32%, and 17% isolates observed in the samples collected from H1, H2, and H3 were resistant toward more than 8 tested antimicrobials, respectively. None of the isolates were resistant to all the 15 targeted antimicrobials (Supplementary Table S4).

3.3. Abundance of antimicrobial-resistance genes

In this study, 20 resistance genes imparting resistance toward antimicrobials from seven drug classes in *E. coli* isolates were analyzed. *QnrA* was not detected in any isolates (Table 3). $Bla_{CTX\text{-}M}$ and *tetA* were the most abundant genes in the isolates observed from the samples collected from H1, followed by *qnrS*, bla_{TEM}, and *sul2*. The highest $bla_{CTX\text{-}M}$ (51%) detection rates were observed in the isolates of samples collected from H2, while bla_{TEM}, *tetB*, *sul2,* and *qnrS* were enriched in over 20% of the isolates. The *tetA* and *qnrS* detection rates were higher than those of $bla_{CTX\text{-}M}$ in the isolates of samples collected from H3. The detection rates of TRI resistant genes showed that *dfrA5/14* was dominant in the isolates of H1 samples, while *dfrA7/17* demonstrated higher detection rates in H3 samples. In addition, all aminoglycoside resistance genes (*aadA*, *strAB*, *aphA1-Iab*) demonstrated higher detection rates in H1 samples than those observed in the samples collected from the remaining two hospitals. However, *dfrA1* and bla_{SHV} were detected only in samples collected from H1 and H2, respectively. Detection frequencies of bla_{TEM} and *tetA* were higher in the samples collected from Kandy Lake and Mid Canal. Notably, bla_{SHV} was observed only in H2 samples, while $bla_{CTX\text{-}M}$ was not observed in the Mid Canal samples.

It was observed that 112 (38%) and 105 (35%) of all isolates were enriched in bla_{TEM} and $bla_{CTX\text{-}M}$ (Supplementary Fig. S2) genes, respectively. Tetracycline resistance genes, namely, *tetA* and *tetB*, were detected in 91 (30%) and 58 (20%) isolates, respectively, while sulfonamide resistance genes, *sul1* and *sul2* were observed in 60 (20%) and 76 (26%) isolates, respectively. Quinolone resistance gene, *qnrS,* was observed in 80 (27%) isolates. However, the detection rates of aminoglycoside resistance genes, namely, *aadA* and *strAB,* were 16% and 20%, representing 49 and 59 isolates, respectively. Trimethoprim and chloramphenicol resistance genes were detected with a lower frequency.

3.4. Relationships among resistance rates of E. coli phenotypes and other variables

The manner in which potentially confounding variables, i.e., location, treatment process, and sampling year, were associated with the prevalence of *E. coli* isolates resistant toward a selected

Table 3
Prevalence of antimicrobial resistance genes in *E. coli* isolated in hospital wastewaters and surface waters.

Gene	Kandy Lake (8)[a]		Hospital 1 (80)		Hospital 2 (100)		Hospital 3 (79)		Mid Canal (30)	
	Number of detection	Detection %	Number of detection	Detection %	Number of detection	Detection %	Number of detection	Detection %	Number of detection	Detection %
blaCTX-M	1	13	30	38	51	51	23	29	0	0
blaSHV	0	0	0	0	7	7	0	0	0	0
blaTEM	5	63	23	29	48	48	21	27	15	50
sul1	1	13	21	26	17	17	16	20	5	17
sul2	1	13	22	28	27	27	20	25	6	20
sul3	0	0	15	19	9	9	1	1	9	30
dfrA1	0	0	4	5	0	0	0	0	0	0
dfrA5/14	2	25	14	18	9	9	13	16	1	3
dfrA7/17	0	0	6	8	9	9	15	19	1	3
dfrA12	1	13	5	6	2	2	1	1	0	0
tetA	4	50	30	38	17	17	26	33	14	47
tetB	0	0	11	14	32	32	12	15	3	10
aadA	1	13	19	24	15	15	4	5	10	33
strAB	1	13	21	26	16	16	15	19	6	20
aphA1-Iab	2	25	15	19	9	9	10	13	2	7
Cat	0	0	2	3	5	5	5	6	1	3
cmlA	0	0	6	8	4	4	0	0	5	17
qnrB	2	25	4	5	9	9	17	22	5	17
qnrS	1	13	26	33	22	22	26	33	5	17

[a] Number of isolates.

antimicrobial was investigated. The multivariable logistic regression analysis showed that the occurrence of phenotypes resistant toward CTX in H2 samples was significantly higher ($p < 0.01$). However, the presence of phenotypes resistant toward GM in H2 samples was significantly lower ($p < 0.05$) compared to that in observed the H1 samples (Fig. 2). In contrast, the occurrence of phenotypes resistant toward GM ($p < 0.01$), CIP ($p < 0.01$), LVX ($p < 0.01$), OFX ($p < 0.001$) and NORF ($p < 0.01$) in H3 samples were significantly lower than those observed in H1 samples.

When data obtained from the samples collected before and after treatment at the hospitals were compared (treatment process), the occurrence of SMXZ/TRI-resistant phenotypes in the effluents was slightly higher than that observed in the influents ($p < 0.05$) (Fig. 3A). In contrast, the occurrence rates of phenotypes resistant toward CEZ and CTX in the effluents were significantly lower ($p < 0.001$) than those observed in the influents. The analysis demonstrated that in the samples collected in 2018, the prevalence of phenotypes resistant toward AMP ($p < 0.01$), CHLP ($p < 0.05$), TC ($p < 0.05$), SMXZ/TRI ($p < 0.01$), NP ($p < 0.01$), CIP ($p < 0.01$), LVX ($p < 0.01$), OFX ($p < 0.01$), and NORF ($p < 0.01$) was significantly lower than that observed in 2016 (Fig. 3B).

A similar statistical analysis was conducted to study the effects of the aforementioned variables on the abundance of resistance genes in *E. coli* isolates. When compared to the H1, occurrence of bla_{TEM} ($p < 0.01$) and *tetB* ($p < 0.05$) was significantly higher while occurrences of *tetA* ($p < 0.05$) and *qnrB* ($p < 0.01$) were significantly lower in H2 wastewaters (Fig. 4). Likewise, the occurrences of *sul3* ($p < 0.05$), *aadA* ($p < 0.05$) and *qnrB* ($p < 0.01$) were significantly lower in the H3 samples compared to those in the H1 samples. Interestingly, the occurrence rates of bla_{TEM} ($p < 0.01$), *sul3* ($p < 0.05$), and *cmlA* ($p < 0.05$) in the Mid Canal samples were also significantly higher than those observed in the H1 wastewaters.

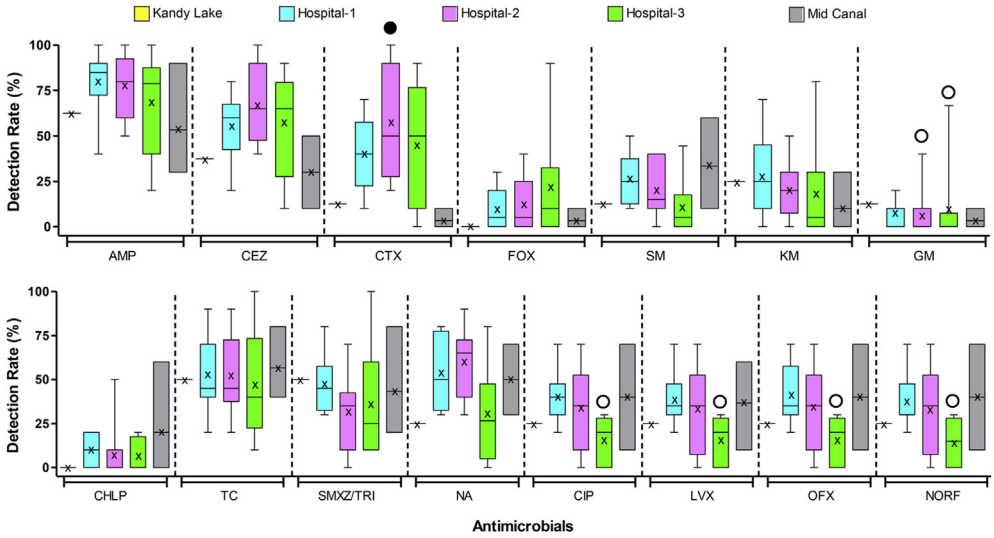

Fig. 2. Box-and-whisker plots showing the detection rates of *E. coli* phenotypes resistant toward antimicrobials observed in the Kandy Lake, hospitals, and Mid Canal. Plots include the minimum and the maximum concentration, the median (–), the mean (x), and the first (5%) and third quartiles (95%). Significant differences (○ negative, ● positive, p values are noted in the text) between H1 and the other locations were estimated by multivariate logistic regression analysis.

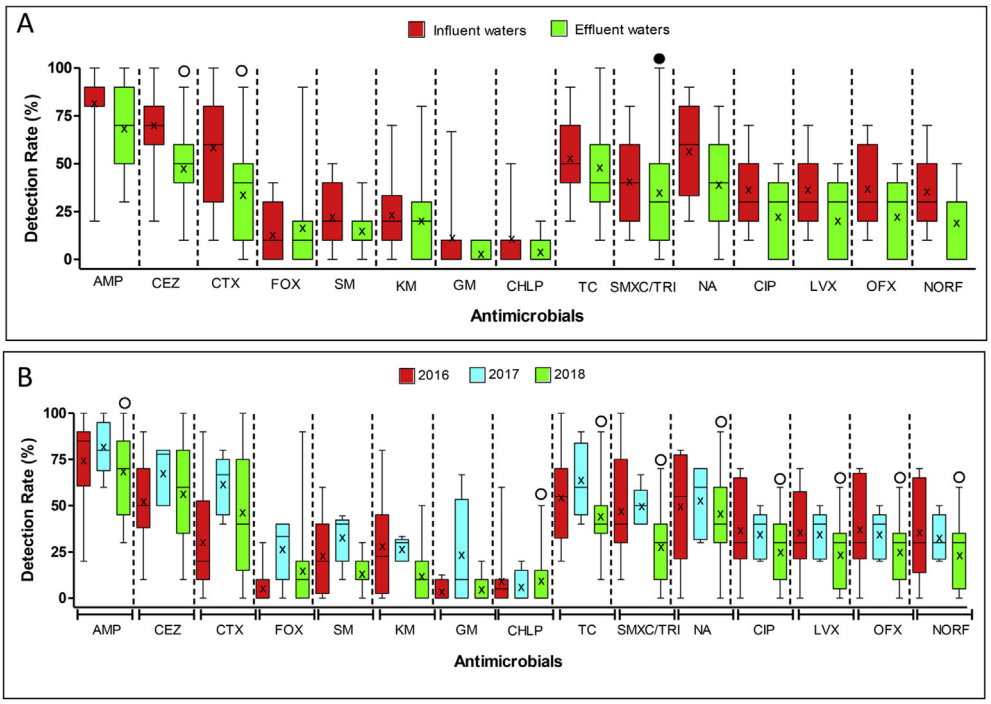

Fig. 3. Box-and-whisker plots showing the detection rates of *E. coli* phenotypes resistant toward antimicrobials in the treatment step (A) and from 2016 to 2018 (B). Plots include minimum and maximum concentrations, the median (–), the mean (x), and the first (5%) and third quartiles (95%). Significant differences (○ negative, ● positive, p values are noted in the text) between the influent and effluent (A) and between 2016 and the other two years (B) were estimated by multivariate logistic regression analysis.

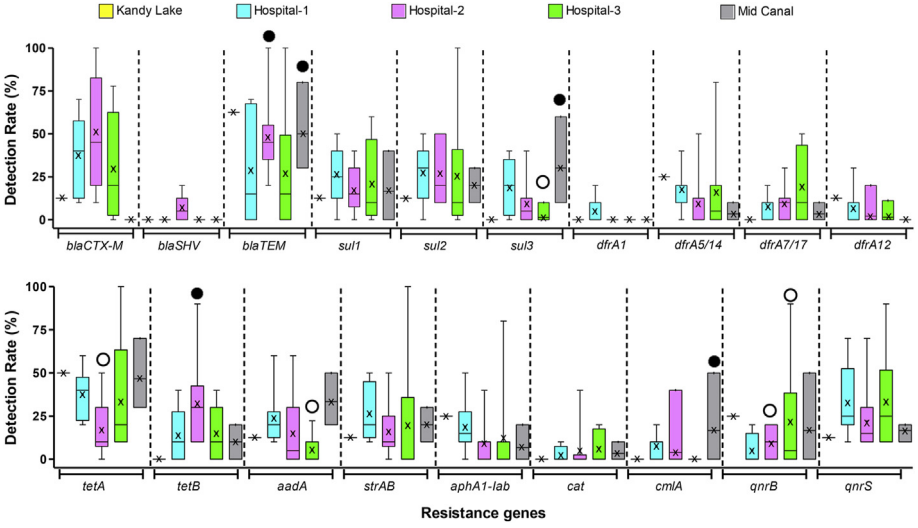

Fig. 4. Box-and-whisker plots showing the detection rates of resistance genes in *E. coli* in Kandy Lake, hospitals, and Mid Canal. Plots include the minimum and maximum concentrations, the median (–), the mean (x), and the first (5%) and third quartiles (95%). Significant differences (○ negative, ● positive, p values are noted in the text) between H1 and the other locations were estimated by multivariate logistic regression analysis.

The treatment process influenced the presence of resistance genes in which the rates of *dfrA5/14*, *tetA*, *strAB*, *aphA1-lab*, and *qnrB* were significantly higher ($p < 0.05 \sim p < 0.01$) in the effluents. However, the rates of $bla_{CTX\text{-}M}$ were significantly lower ($P < 0.05$) in effluents than those observed in the influents (Fig. 5A). Moreover, the prevalence of resistance genes was also influenced by the sampling year. In the year 2017, *sul2* and *strAB* demonstrated higher occurrence rates ($p < 0.01$) while *tetB* ($p < 0.05$) and *qnrB* ($p < 0.001$) showed lower rates than those observed in 2016 (Fig. 5B). In 2018, occurrence rates for *aadA* ($p < 0.05$) and *qnrB* ($p < 0.001$) were lower than those observed in 2016.

Further investigations on how the concentrations of antimicrobials are associated with the prevalence of resistant phenotypes and resistance genes by performing multivariable logistic regression analysis were conducted. Nevertheless, with respect to the confounding explanatory variables, no significant correlation was observed between the antibiotic concentrations and the selection of resistant phenotypes of *E. coli*. In contrast, concentrations of PCPs showed a significant positive correlation with the occurrence of phenotypes resistant to several tested antimicrobials (Table 4). For

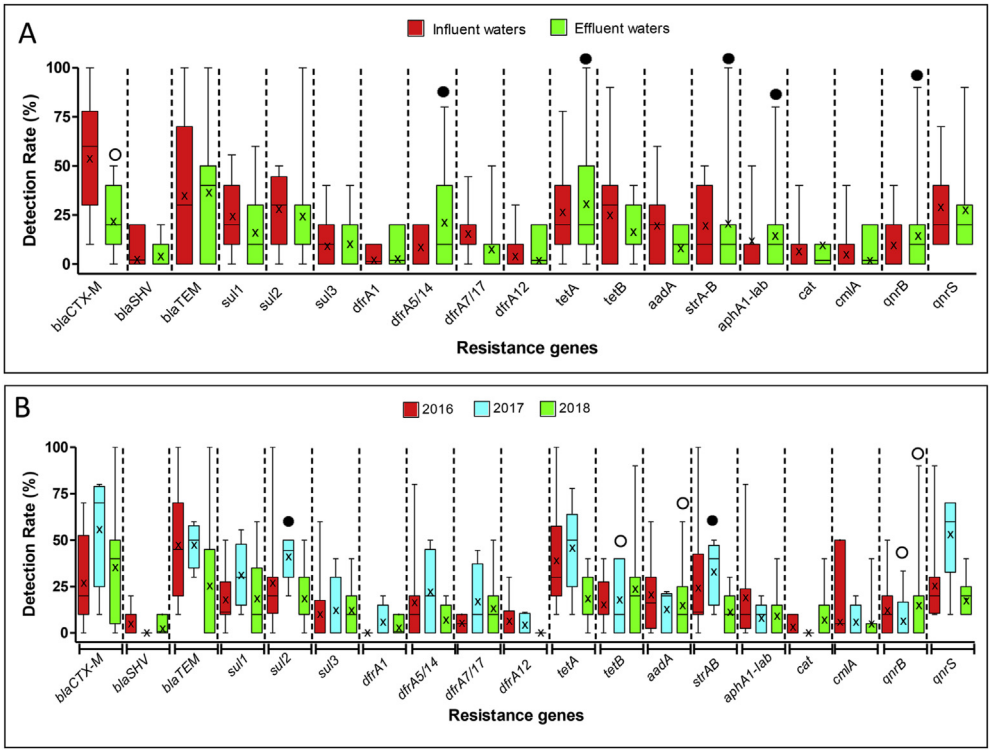

Fig. 5. Box-and-whisker plots showing the detection rates of resistance genes in *E. coli* in the treatment step (A) and during 2016–2018 (B). Plots include the minimum and maximum concentrations, the median (–), the mean (x), and the first (5%) and third quartiles (95%). Significant differences (○ negative, ● positive, p values are noted in the text) between the influent and effluent (A) and between 2016 and the other two years (B) were estimated by multivariate logistic regression analysis.

Table 4
Associations (p) between concentration of antimicrobials and the prevalence of antimicrobial-resistant phenotypes and resistance genes.

Antimicrobial	Resistant phenotype						Resistance gene									
	CEZ	CTX	FOX	KM	GM	SMXZ/TRI	bla_{CTX-M}	bla_{TEM}	*sul1*	*sul2*	*sul3*	*dfrA7/17*	*tetA*	*tetB*	*strAB*	*qnrB*
Σ Fluoroquinolone								<0.01								
DEET	<0.05											<0.05				
TCS					<0.01				<0.05							<0.001
MeP	<0.05	<0.05	*<0.05*[a]				<0.05			*<0.01*					*<0.05*	
EtP			<0.05			<0.05			<0.05	<0.001			<0.05			<0.05
PrP										<0.01					<0.01	
BuP										*<0.01*		<0.05				
TCC		<0.05		*<0.05*						*<0.01*	<0.05			<0.01	*<0.01*	<0.01

[a] Italics indicate negative correlation.

instance, concentrations of DEET and TCS were positively associated with the rates of CEZ and GM resistance, respectively. The MeP concentration was positively correlated with the occurrence of cephalosporin resistance (CEZ and CTX). In addition, EtP levels were correlated significantly with the presence of FOX and SMXZ/TRI resistant isolates. The TCC concentrations demonstrated positive correlations with the prevalence of CTX-resistant *E coli*. In contrast, MeP and TCC demonstrated weak and negative associations with FOX and KM resistance, respectively. Moreover, the total concentration of FQs demonstrated a significant positive correlation with the rates of bla_{TEM} (Table 4). The presence of all seven PCPs was positively correlated with the enrichment of several genes. Conversely, negative associations were also noted between MeP and *sul2*, MeP and *strAB*, BuP and *sul2*, TCC and *sul2*, and TCC and *strAB* (Table 4).

4. Discussion

4.1. Occurrence of antimicrobials in hospital wastewater and adjacent surface water

The concentrations of targeted compounds detected in the hospitals were several folds higher than those previously reported in two municipal sewage treatment plants (Samaraweera et al., 2019). These results indicate that hospital effluents require more consideration as antimicrobial pollution sources in Sri Lanka. The maximum concentration of SMXZ detected in the effluent of H1 was higher than the maximum concentrations reported in hospitals and WWTP wastewater in Asia, Europe, and North America (Cardenas et al., 2016; Kleywegt et al., 2016; Tran et al., 2018). The maximum SPR concentration detected in the H1 influent was

higher than those reported in the UK (Petrie et al., 2015). The frequency of the detection of SMXZ and SPR observed in the present study indicated their regular use in hospitals. In contrast, SMT was detected at low frequency, with several-folds lower levels when compared to the levels reported in Asia, North America, and Europe (Tran et al., 2018). The level of CLA was higher than the levels reported globally, including in Canada and Japan (Tran et al., 2018; Azuma et al., 2016; Kleywegt et al., 2016). Previously, the presence of CLA and CHLP was not detected in a municipal WWTP (Samaraweera et al., 2019), suggesting that these drugs might primarily be used in clinical settings in Sri Lanka. Among the FQs, CIP levels detected in the current samples were higher than those reported for the Asian region but lower than those reported for North America and Europe (Tran et al., 2018; Ratola et al., 2012).

Biocides were detected at low frequencies at all the sampling sites. The TCC and TCS levels were lower than those reported for WWTPs in Asia and other parts of the world, including India, Korea, and the UK (Tran et al., 2018; Balakrishna et al., 2017; Petrie et al., 2015). The concentrations of all parabens detected in the present study were lower than the concentrations in WWTPs in the UK (Kasprzyk-Hordern et al., 2009). DEET is used in insect repellents. Hence, the high DEET pollution in the Mid Canal emphasizes the fact that increased amounts of DEET may largely stem from untreated domestic waste released from densely populated areas in the vicinity. Domestic WWTPs also demonstrated higher TCC detection rates when compared to the detection rates in other treatment plants (Subedi et al., 2014), suggesting that the frequency of the use of such biocides in medical institutions is lower than that in domestic settings.

Although a wide range of antimicrobials have been detected in Kandy Lake and the Mid Canal, their concentrations typically did not exceed the concentrations reported in other studied (Bu et al., 2013; Patel et al., 2019; Tran et al., 2019). The presence of various antimicrobials in hospital effluents at high concentrations might be a major source of downstream contamination, considering that the drugs have been detected widely in urban canals and other surface waters in Sri Lanka (Guruge et al., 2019). The mean levels of all the chemicals measured in the two sites were several-fold higher than the levels reported based on samples collected in 2013 (Guruge et al., 2019). These results demonstrate an increase in aquatic pollution in the study area.

4.2. Prevalence of E. coli with AMR phenotypes

AMR *E. coli* strains have been studied extensively in wastewater and surface waters, and the antimicrobial resistance associated with the antimicrobials targeted in the present study is common in urban settings. The previously reported rates of resistance in *E. coli* toward aminopenicillins, sulfonamides, and tetracyclines are higher than those toward aminoglycosides (reviewed by Rizzo et al., 2013; Rosas et al., 2015), which is consistent with the findings of the present study. In contrast, a recent study demonstrated that *E. coli* isolated from a hospital effluent demonstrated up to 100% resistance toward FQs, TC, and SMXZ (Kumar et al., 2020). These results indicate significant discrepancies based on various locations in Sri Lanka. The data suggest that the resistance of extended-spectrum β-lactamase (ESBL) - producing *E. coli* was dominant when compared with other resistant phenotypes in hospital wastewaters. These findings might reflect the prevalent use of β-lactamase drugs in the hospitals and are consistent with the results of a previous study conducted with samples collected from Southern India. The rates of resistance toward AMP, CFZ, and CTX were the highest, while CHLP demonstrated the lowest resistance rates in the samples collected from Southern India (Akiba et al., 2016). Similarly, resistance toward AMP was prominent, while the least prevalent resistance phenotypes were observed toward CHLP and GM in the USA, Sweden, and Norway (Flach et al., 2018; Kappell et al., 2015; Paulshus et al., 2019). In addition, a recent study involving 10 major European countries revealed that the prevalence of *E. coli* resistance toward AMP was higher than the prevalence of *E. coli* resistance toward CFZ, CTX, and GM, while resistance toward AMP varied significantly among regions in clinical and urban wastewaters (Huijbers et al., 2020). In contrast, the highest resistance in *E. coli* isolated from WWTPs and hospital wastewater was observed toward SMXZ in Portugal and Vietnam (Figueira et al., 2011; Lien et al., 2017). These results were inconsistent with those reported by Korzeniewska et al. (2013), where the prevalence of CTX - and GM-resistant phenotypes was prominent in Polish hospital wastewaters.

The presence of ARB might differ across different hospitals and ward types, and ARB concentrations in treatment plants of hospitals with clinical isolation wards could be higher than the concentrations in other mixed WWTPs (Le et al., 2016). In line with previous reports, a significant variation in the presence of resistant phenotypes was observed among hospitals. Especially, selection for SMXZ/TRI and FQs was low in H3, which was the smallest among the three hospitals. Le et al. (2018) reported that both CAS and membrane bioreactor (MBR) systems could effectively reduce rates of ARB. Therefore, treatment with CAS and MBR systems could be effective at H3, where a lower number of isolates that were resistant toward SMXZ/TRI and FQs were observed compared to those noticed in H1 and H2 samples (with the availability of only CAS-treatment). In this study, a significant annual variation in prevalence rates among resistant isolates and genes in wastewaters and surface waters was observed. This might be an outcome of the combined effect of variation in the operational parameters, such as sludge removal, rainfall, and hydraulic load (Pallares-Vega et al., 2019) and the type and number of patients treated at the hospitals during the sampling. Hence, further monitoring studies should be performed.

Most *E. coli* strains isolated in the present study showed resistance toward multiple antimicrobials, indicating that favorable selection conditions for ARB, are prevalent in the urban streams located adjacent to hospital effluent discharge sites in Sri Lanka. The presence of *E. coli* with MDR phenotypes has been reported in similar environmental settings, which is significantly higher in hospital effluents when compared to the abundance in domestic/municipal wastewaters (Akiba et al., 2015; Korzeniewska et al., 2013; Paulshus et al., 2019). A previous study reported that *E. coli* isolated from the Tama river were primarily resistant to SMXZ/TP, AMP, and TC, which is surrounded by the densely urbanized Tokyo Metropolitan area (Ham et al., 2012). In the Tama River, only 4% of the isolates demonstrated resistance to six out of the 12 antimicrobials tested. However, 32% of the isolates were resistant to seven or more antimicrobials in the samples collected from Mid Canal, Sri Lanka. In contrast, urban waterways demonstrated higher occurrence rates of MDR *E. coli* phenotypes when compared to human-derived sources such as domestic sewage and effluents of clinical origin (Kappell et al., 2015). Overall, the present data along with the globally published data obtained from other regions indicate that the abundance of AMR *E. coli* phenotypes in hospital wastewaters and adjacent surface waters is influenced significantly by the wastewater treatment methods and pharmaceuticals. The prevalence of phenotypes resistant toward 11–12 drugs in the downstream Mid Canal raises concerns on water quality since the canal discharges water directly to the Mahaweli River, which is the largest river in the country, and the major source of drinking for Kandy, the second-largest city in Sri Lanka.

4.3. Prevalence of resistance genes in E. coli

In this study, 19 genes imparting resistance toward drugs belonging to seven major antimicrobial classes were detected. The presence of bla_{TEM} encoding β-lactamase was dominant in H2 hospital wastewaters and the Mid Canal. This gene was widely observed in WWTPs globally, from Asia, Europe, and America (Pazda et al., 2019). Resistant *E. coli* strains harboring multiple resistance genes (MRGs) have been reported in the samples obtained post-treatment from hospital wastewater (Lien et al., 2017; Yuan et al., 2020). The abundance of *sul* and *tet* genes in treated water in WWTPs remained consistently high in the final effluents and sludge (Wang et al., 2015). In contrast, the diversity of ARGs and their transfer potentials in *E. coli.* Could be largely source-dependent; hospital sources could show less potential compared to sources such as livestock and municipal wastewater (Yuan et al., 2020). The collated data demonstrated that the frequency of resistance genes was significantly altered before and after treatment, with potential enrichment of several gene determinants during treatment processes, especially for tetracyclines, streptomycin, and quinolones. All hospitals investigated in the study had activated sludge treatment processes, while only H3 had an additional MBR treatment. Biswal et al. (2014) reported that the activated sludge treatment had no effect on the removal of ARG carrying *E. coli*, and instead, could increase ARG against several types of drug classes. In addition, all hospital treatment plants selected in the present study chlorinated the wastewaters. However, chlorination may not eliminate ARGs effectively (Yuan et al., 2015). This study revealed that approximately 61% of the *E. coli* isolates harbored MRGs in all samples, while the occurrence rate in downstream samples was 53%, indicating that the conditions in the study area remained favorable for the enrichment of MRGs. Therefore, further surveillance studies should be performed at the national level to identify and collect data for understanding the distribution of the MRGs in aquatic environments and their potential impact on public health.

4.4. Correlation among antimicrobials, resistant phenotypes, and resistant genes

This is the first comprehensive study to examine the effects of a wide range of antimicrobials used in clinical settings on the phenotype and genotype of *E. coli* in Sri Lanka. However, statistical analysis showed that the concentrations of the 12 antibiotics detected in the samples did not significantly facilitate the resistance selection of *E. coli*. Antibiotics may not directly select their own resistance mechanisms owing to the high abundance of genes conferring cross-resistance to many compounds (Murray et al., 2019). Likewise, apart from the presence of high concentrations of antibiotics, the complexity of wastewater treatment facilities and physicochemical parameters, and changes in the bacterial taxonomic composition might influence the selection of the ARGs (Bengtsson-Palme et al., 2016; Wang et al., 2015). The findings of the present study demonstrated that the total FQ concentration was significantly associated with the enrichment of bla_{TEM}. Exposure to CIP, the primary FQ detected in all samples, significantly increased the presence of genes resistant to several drug classes other than quinolone genes (Kraupner et al., 2018; Murray et al., 2019). In addition, 1000 ng/L of CIP demonstrated the lowest observable effect for quinolone resistance selection in complex bacterial communities (Kraupner et al., 2018). A similar average concentration was detected in hospital wastewaters, implying that current discharge levels could increase the risk of AMR selection and influence the microbial community in the surrounding environment.

Certain active substances in PCPs used in various settings can lead to an increased presence of ARB since bacteria exhibit similar mechanisms of resistance to both biocides and antibiotics, which would result in the co-selection of AMR (reviewed by Ortega-Morente et al., 2013, Murray et al., 2019; SCENIHR, 2019). However, the presence of biocides and their influence on AMR co-selection in wastewaters are not well documented. Remarkably, EtP detected in the present study were positively correlated with the prevalence of SMXC/TRI resistant phenotype and its respective genes (*sul2* and *sul3*), demonstrated the potential of EtPs for the co-selection of AMR. In addition, exposure to MeP also demonstrated the co-selection potential of cephalosporin resistance phenotypes in combination with their relevant ESBL-producing gene bla_{CTX-M}. The data further indicated that EtP and PrP concentrations were significantly positively correlated with the occurrence of aminoglycoside and quinolone resistance genes, suggesting that parabens may facilitate the co-selection of AMR. TCS concentrations also demonstrated significant co-selection of aminoglycoside resistance and enrichment of sulfonamide and quinolone resistance genes. Exposure to TCS might lead to increased TCS resistance and frequently result in cross-resistance to a wide range of antimicrobials with a spread of MDR in microbial communities in the environment (Carey and McNamara, 2014; Fujimoto et al., 2018). The potential of DEET-related resistance in bacterial communities is not well known. The positive correlations observed between DEET levels, cephalosporin resistance, and the TRI-resistance gene indicate that DEET should be investigated further in AMR studies. TCC could select for a multidrug efflux pump encoded by the *mexB* gene in a mixed microbial community (Carey et al., 2016). Significant positive correlations were noted between TCC levels and enrichment of resistance genes to multiple drug classes such as sulfonamide-, tetracycline- and FQs. In some cases, exposure to biocides could significantly reduce ARGs including efflux genes in conjunction with the associated bacterial community (Murray et al., 2019). Likewise, the present data demonstrated that PCPs such as MeP, BuP, and TCC, can negatively affect the abundance of ARGs, which is indicative of their multifaceted resistance mechanism. Collectively, focusing on the presence of PCPs at low concentrations in the environment and their potential effects on the co-selection of resistance along with gene enrichment in the bacterial community would enhance the knowledge regarding the relationships between co-exposure to multiple antimicrobials.

In this study, it was not possible to analyze the parameters of water quality, such as biological oxygen demand, chemical oxygen demand, and total suspended solids in water samples. Consequently, their potential effects on the prevalence of ARB and ARGs have not been explored.

5. Conclusions

To the best of our knowledge, this is the first three-year in-depth analysis of antimicrobials, AMR phenotypes, ARGs specifically in *E. coli* present in the samples collected from hospital wastewaters and surface waters in Sri Lanka. The concentrations of antimicrobials detected in wastewater were proportional to hospital capacity. Several-fold increases in antimicrobial concentrations were observed downstream when compared to the concentrations upstream, indicating that hospital effluent was considerably influenced by downstream contamination. This study demonstrated that most *E. coli* isolates demonstrated MDR and the rate of prevalence of isolates resistant toward β-lactams, tetracyclines, sulfonamides/trimethoprim, and quinolones were relatively high. However, such rates were relatively low toward aminoglycosides and phenicols. In addition, the occurrence of resistance genes in *E. coli* isolates demonstrated an increased possibility of carrying *bla*-genes, *tetA*, *qnrS, and sul2*. The statistical analysis showed that

the concentrations of PCPs likely facilitated the maintenance and persistence of ARB and ARGs. These results implied that further studies should be performed on how the presence of multiple antimicrobials and their collective cocktail-effects in wastewater could implicate the role in the co-selection of resistance. The data obtained in this study on the current status of antimicrobials, ARB, and ARGs in hospitals and neighboring aquatic environments in Sri Lanka provide invaluable information at the national level and will facilitate long-term monitoring programs. Hospital effluents in Sri Lanka require further treatment with appropriate technologies, and centralized WWTPs are required in cities to prevent ecological damage and public health risks in the country.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.130591.

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Author declaration

Keerthi S. Guruge: Conceptualization; Funding acquisition; Supervision; Writing. Yukino A. Tamamura: Microbiological analysis, Validation, Formal analysis. Prasun Goswami: Visualization; Formal analysis. Rumi Tanoue: Chemical analysis, Formal analysis. K. B. S. N. Jinadasa: Investigation, Sampling. Kei Nomiyama: Methodology Takeshi Ohura: Funding acquisition. Tatsuya Kunisue: Resources. Shinsuke Tanabe: Supervision. Masato Akiba: Methodology.

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