



# Occurrences of antibiotic resistant bacteria in a tropical river impacted by anthropogenic activities in Ho Chi Minh City

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## Abstract

Antibiotic resistance causes serious health problems associated with an increase in hospitalization time, treatment cost, and mortality. In this study, the contamination levels of antibiotic resistant bacteria were determined in the Saigon River, which is impacted by different anthropogenic activities in Ho Chi Minh City. Five target antibiotics were supplemented to the R2A medium at the concentration of amoxicillin (9.6 µg/ml) / clavulanic acid (4.8 µg/ml), amikacin (19.2 µg/ml), cefixime (1.2 µg/ml), ciprofloxacin (1.2 µg/ml), and trimethoprim (2.4 µg/ml)/ sulfamethoxazole (45.6 µg/ml). As the results, the antibiotic resistant bacteria were found in all the sampling sites and at the highest concentration at the sites impacted by wastewater from both industrial and residential activities. *Enterobacteriaceae* was the most abundant family with 23.8% of total isolates of the antibiotic resistant bacteria. Human opportunistic pathogens and pathogens such as *Klebsiella pneumoniae* (6.74%), *Enterobacter* sp. (4.15%), *Escherichia coli* (6.63%), *Stenotrophomonas maltophilia* (3.11%), *Aeromonas Caviae* (4.66%) were identified in all surface water samples. Correlation analysis suggested that the pollutions of dissolved salt, organic matter, and wastewaters may facilitate the antibiotic resistant bacteria in the aquatic environments.

**Keywords** Antibiotic resistant bacteria · Surface water · Saigon River · Water quality · Human activities

## Introduction

Antibiotic resistance is considered one of the leading causes of human death and is a global concern (World Health Organization (WHO) 2021). Every year, about 700,000 deaths are

caused by antibiotic resistant bacteria (ARB), and this number was estimated to reach 10,000,000 deaths per year by 2050 (O'Neill 2016; de Kraker et al. 2016). Although antibiotic resistance was initially considered as a nosocomial infection for ill patients, it has been recently recognized as a global health issue due to its wide dissemination in the community (O'Neill 2016). Environmental contamination is believed to contribute to the emergence and spread of antibiotic resistance as the large amounts of antimicrobial agents, pesticides, and heavy metals are released into the environments from major sources such as pharmaceutical industry, livestock, aquaculture, hospital, and domestic wastewater (Berendonk et al. 2015b; Taneja and Sharma 2019). ARB in the environment can come from industrial, agricultural, and residential wastes, as well as from random mutations in bacterial genomes, and horizontal gene transfer of plasmid bearing antibiotic resistant genes (ARGs) under the presence of specific conditions with antimicrobials and detergents acting as selective agents (Berendonk et al. 2015a; Reygaert 2018).

Wastewater is considered an important reservoir of antibiotic resistance as it often contains a high abundance of nutrients, carbon sources, antimicrobial agents, and particles on which bacteria including human pathogens can absorb,

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grow, and maintain for a long time. A study on the hospital wastewater in Singapore reported a high concentration of bacteria resistant to many different antibiotics such as amikacin, clindamycin, erythromycin, ciprofloxacin, tetracycline, meropenem, ceftazidime, vancomycin chloramphenicol and co-trimoxazole (Le et al. 2016). Many isolates of ARB from the hospital wastewater were identified as human opportunistic pathogens and pathogens such as *Pseudomonas* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Acinetobacter* spp., *Aeromonas* spp., *Escherichia coli*, and *Elizabethkingia meningoseptica* (Haller et al. 2018). In another study of surface water collected from a canal receiving wastewater from both domestic and livestock sources, a high concentration of ARB were detected with high levels of resistance rate ranging from 2.14 to 94.44% for sulfamethoxazole, and 0.01–3.38% for erythromycin (Hoa et al. 2011). Wastewater treatment plants (WWTP) play a crucial role in the removal of antibiotic resistance vectors and other contaminants. However, treated water may not be completely free of the contaminants including various antimicrobial agents, bacteria, and genes which can be released to the receiving water bodies such as surface water (e.g., river, lake) (Berendonk et al. 2015b; Manaia 2017; Rizzo et al. 2013; Gao et al. 2012). The presence of antimicrobial agents such as antibiotics, pesticides, and heavy metals, even at a low concentration in the environments can create a selective pressure for the development of antibiotic resistance in the bacterial community (Sandegren 2014).

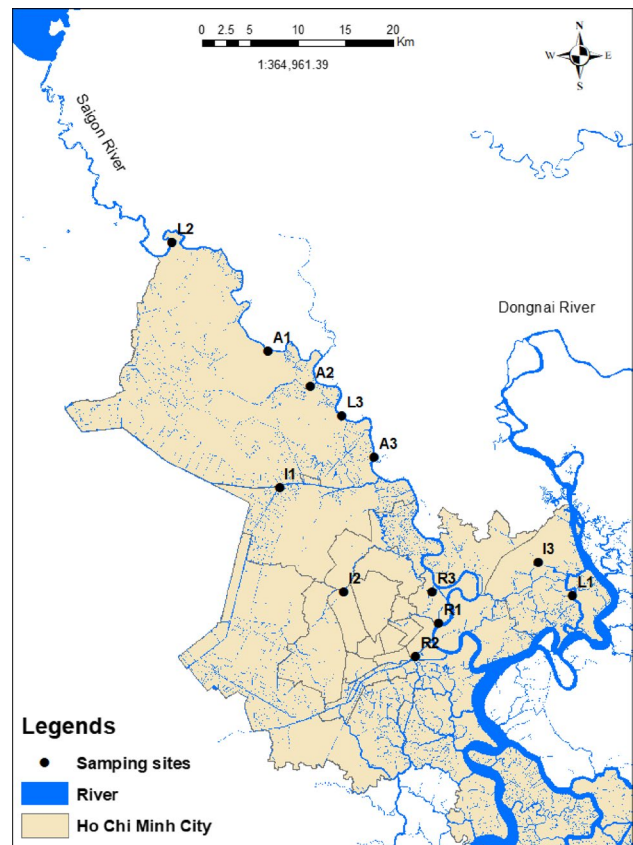
Ho Chi Minh City is the largest city with a high population density in Vietnam (General Statistics Office 2019). To meet the huge demand for food, healthcare, and other services, there are many agriculture activities (e.g., livestock, aquaculture), hospitals, and industrial parks built in the City. However, less than 50% of the domestic wastewater in Ho Chi Minh City is collected and appropriately treated at the centralized wastewater treatment plants (Asia 2013). A majority of the untreated wastewater is released to the city canals and eventually end up at the Saigon River, which could create an environment for the development and spread of antibiotic resistance in the environment.

Until now, there is very little information on the occurrence of ARB in the surface water in the Saigon River. This study is an effort to provide baseline information on the level and bacterial identification of antibiotic resistant bacteria in the surface water along a tropical river impacted by different human activities in a megacity. Correlation analysis was conducted to investigate any potential relationship between water quality parameters and development of ARB in the aquatic environments.

## Materials and methods

### Sampling sites description

Total 12 sampling sites were selected along the Saigon River in Ho Chi Minh City, starting from the Ben Duoc, Cu Chi District and ending at the Truong Phuoc Bridge. The detailed map and descriptions of all the sampling sites are presented in Fig. 1, and Table S1. Briefly, the sampling sites of surface water samples were selected in the areas that represented anthropogenic activities classified based on dominant land uses in Ho Chi Minh City. Three sampling sites were chosen for each area as follows: (i) The residential sites of R1, R2, and R3 were mainly affected by domestic wastewater, (ii) The industrial sites of I1, I2, and I3 were mainly affected by industrial wastewater, (iii) The agricultural sites of A1, A2, and A3 were mainly affected by agricultural activity, iv) The less impacted areas of L1, L2, and L3 were far away from human activities.



**Fig. 1** A map of sampling sites along the Saigon River. A total of 12 sampling sites were selected for surface water collection; 3 sites in the agricultural area (A1, A2, A3), 3 sites in the Industrial area (I1, I2, I3), 3 sites in the residential area (R1, R2, R3); 3 sites in a less impacted area (L1, L2, L3)

## Sample collection

Total 24 samples were collected at 2 time points within a year: the first sampling event in August 2019 during the wet season, and the second sampling event in April 2020 during the dry season. Surface water samples were collected using a 5L plastic bucket, at the depth of 20–30 cm from the river surface. For every sampling site, surface water was taken with three 5L buckets including 1 bucket of water at the middle of the river/canal and 2 buckets of water at the points close to the two banks of the River. The three spatial samples of surface water were mixed into a larger bucket to make up an aggregated sample before the water samples were collected into 1 L sterilized glass bottles. All water samples were kept on ice during shipping to the laboratory and processed for microbiological and other analysis within the same day.

## Quantification of ARB

Heterotrophic bacteria resistant to 5 target antibiotics including Amoxicillin/Clavulanic acid (AMC), Amikacin (AMK), Cefixime (CFM), Ciprofloxacin (CIP), and Sulfamethoxazole/Trimethoprim (SXT) in the surface water samples were quantified using a pour plate method with R2A agar medium (Sigma-Aldrich, Canada). Each antibiotic was supplemented into the agar medium at the concentrations of 20% higher than the susceptible breakpoints published in Clinical and Laboratory Standard Institute guideline (CLSI 2013; Le et al. 2018). The specific concentrations of the target antibiotics were used were as follows: Amoxicillin (9.6 µg/ml, Sigma-Aldrich, US) / Clavulanic acid (4.8 µg/ml, Sigma-Aldrich, US), Amikacin (19.2 µg/ml, Himedia, India), Cefixime (1.2 µg/ml, Himedia, India), Ciprofloxacin (1.2 µg/ml, Himedia, India), sulfamethoxazole (45.6 µg/ml, Himedia, India) / trimethoprim (2.4 µg/ml, Himedia, India). Water samples were diluted in sterile phosphate buffer saline (PBS) into a series of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$ . A volume of 1 ml for each dilution was added into a petri plate containing about 15 ml warm liquid R2A agar supplemented with target antibiotics. Two replicates were performed for each dilution. Then, the plates were incubated at 35 °C for about 20–24 h before counting the bacterial colonies. Only the agar plates with growth colonies ranging from 30 to 300 CFU/ml were taken into account.

## Identification of ARB isolates

A total of 193 isolates of heterotrophic bacteria resistant to the target antibiotics were selected randomly for identification using 16S rRNA Sanger sequencing. The number of identified isolates resistant to AMC, AMK, CFM, CIP, and SXT were 48, 41, 42, 24, and 38 isolates, respectively. Each isolate was streaked onto another R2A plate supplemented with the target antibiotic that it exhibited resistance to in order to ensure

growth and confirm its resistance capacity. To ensure purity, only single colony was picked and sub-cultured in R2A broth for storage in 15% glycerol at  $-80$  °C for further analysis. The genomic DNA of each isolates was extracted and used as DNA templates in the 16S rRNA gene PCR using the primers 27F (AGAGTTTGTATYMTGGCTCAG), and 1492R (GGY TACCTTGTTACGACTT) (Heuer et al. 1997). The PCR products were purified using TopPURE PCR/Gel DNA purification kit (ABT, Ho Chi Minh City, Vietnam) before be sent to the 1st Base company (Singapore) for bacterial identification using 16S rRNA Sanger sequencing.

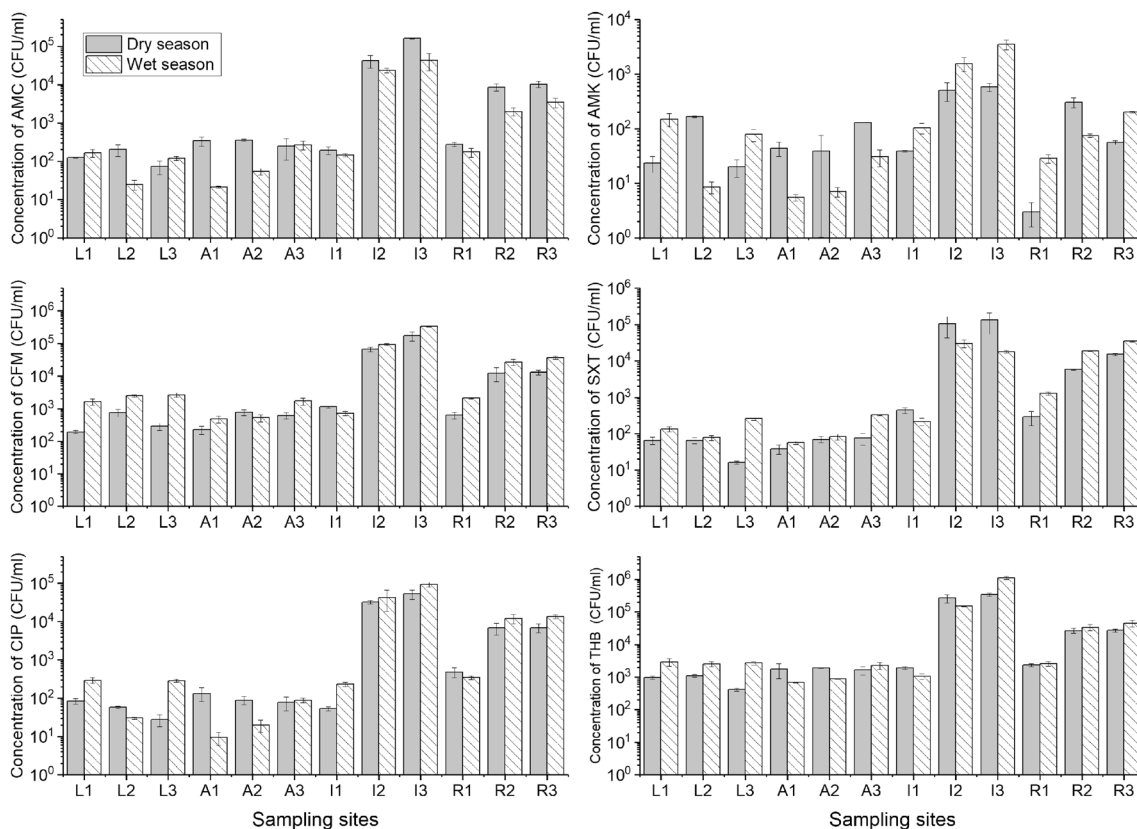
## Data analysis and statistical methods

The concentrations of ARB in surface water samples at each site were determined by enumerating the number of colonies that grew on the R2A agar medium supplemented with the target antibiotics and taking the average of three replicates. Figures were plotted using Origin software version 2019. The statistical differences of the antibiotic resistance rate between the wet and dry seasons were examined using a *t*-test, and *p*-values less than 0.05 were considered as significant differences. Spearman analysis was conducted to investigate correlations between physicochemical parameters and the abundance of ARB for all the surface water samples using the Origin software version 2019. Significant correlations were considered only if *p*-values were less than 0.05.

## Results and discussion

### Occurrence of ARB in the Saigon River impacted by human activities

The occurrences of heterotrophic bacteria resistant to five selected antibiotics including Amoxicillin/Clavulanic acid, Amikacin, Cefixime, Ciprofloxacin, and Sulfamethoxazole/Trimethoprim in the surface water along the Saigon River presented in Fig. 2 and Table S2. On average, the total heterotrophic bacteria in all sampling sites of the river were detected within the range of 420 to  $1.1 \times 10^6$  CFU/ml. Among the 5 antibiotics, the levels of cefixime resistant bacteria (CFM) was found at the highest range of between 200 to  $3.3 \times 10^5$  CFU/ml, followed by Amoxicillin/Clavulanic acid resistant bacteria (AMC) from 21 to  $1.6 \times 10^5$  CFU/ml, Sulfamethoxazole/Trimethoprim resistant bacteria (SXT) from 16 to  $1.3 \times 10^5$  CFU/ml, Ciprofloxacin resistant bacteria (CIP) from 10 to  $9.3 \times 10^4$  CFU/ml, and Amikacin resistant bacteria (AMK) from 6 to  $3.5 \times 10^3$  CFU/ml. On average, the relative resistance rates of heterotrophic bacteria in the Saigon River were varied for both seasons and determined at the highest rate for CFM (42.1–71.6%), followed by SXT (18.2–22.8%), AMC (7.1–21.4%), CIP (11.7–14.0%), and AMK (2.0–3.2%)



**Fig. 2** The average concentration (CFU/ml) of heterotrophic bacteria resistant to antibiotics in the surface water along the Saigon River in the dry and wet seasons. AMC: Amoxicillin/Clavulanic acid resistant bacteria; AMK: Amikacin resistant bacteria; CFM: Cefixime resist-

ant bacteria; CIP: Ciprofloxacin resistant bacteria; SXT: Sulfamethoxazole/Trimethoprim resistant bacteria; THB: Total heterotrophic bacteria

(Table S3). Cefixime is a cephalosporin antibiotic used to treat infections caused by bacteria such as bronchitis, gonorrhea, and infections of the ear, throat, tonsils, and urinary tract. The high resistance rate for CFM may indicate that heterotrophic bacteria in the surface water may have some intrinsic resistance mechanisms to help them more resistant to cefixime compared to the pathogenic bacteria. For the other antibiotics, our results showed a similar range of resistance rates for environmental bacteria in the surface water in comparison with some other studies in literature. For example, Carmen et al. quantified multidrug resistant bacteria from surface water collected from the Basaseachi waterfall and its main river in Mexico using a selective medium for pathogenic *Enterobacteriaceae*. This study reported that the resistance rate of isolates were determined for sulfamethoxazole-trimethoprim (12.1%), ciprofloxacin (6.1%), and amikacin (3%) (Delgado-Gardea et al. 2016), which were slightly lower than that found in our study for the heterotrophic bacteria in the Saigon River. However, the contamination levels of SXT resistant bacteria in the Saigon River were lower than that in the surface waters of the Jiyun River, China, impacted by livestock production which was detected with the level of 39.09% total *E. coli*

isolates resistant to sulfamethoxazole-trimethoprim (Zhang et al. 2014). In another study in Mafikeng, South Africa, the resistance rates of the heterotrophic bacteria in the surface and drinking water sources were reported from 50 to 80% for amoxicillin, from 0 to 80% for erythromycin, and from 50 to 80% for trimethoprim, which were higher than the resistance rates found in the Saigon River (Mulamattathil et al. 2014).

Figure 2 shows the prevalence of heterotrophic bacteria resistant to the selected antibiotics for the surface water samples in the Saigon River impacted by different human activities. The highest levels of resistant heterotrophic bacteria were found in the industrial sites such as the site I2 ( $51\text{--}10^5$  CFU/ml), and the site I3 ( $59\text{--}3.3 \times 10^5$  CFU/ml) (Table S2). These levels were about three orders of magnitudes higher than those in the less impacted sites (L1, L2, L3), which ranged between 16 to  $2.7 \times 10^3$  CFU/ml (Table S2). On average, although the abundance of ARB in the industrial site I1 was found slightly higher than those in the less impacted sites, this difference was not statistically significant. The sites of I2 and I3 are located at Tham Luong canal in the Tan Binh industrial Park and at the Suoi Cai Bridge in the Saigon Hi-Tech Park, respectively. Both of these sites are large industrial zones with many



manufacturing plants, and are located in a densely populated area in Ho Chi Minh City. The surface water samples from these sites were not only impacted by wastewater from industrial activities but also from untreated wastewater from residential areas, while the site I1 at Tan Phu Trung—Nhi Xuan industrial park is about 20 km away from the central of Ho Chi Minh City. The synergistic effects from both industrial and residential wastewaters was likely a major factor causing the development and spread of antibiotic resistance in the aquatic environments.

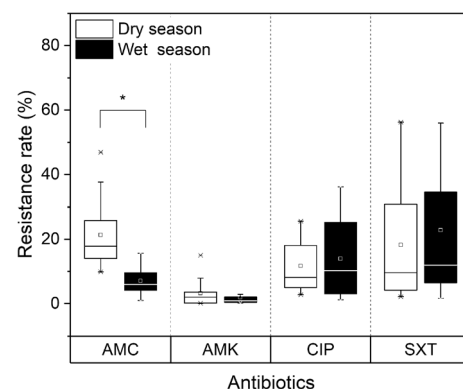
On average, the levels of ARB in the residential sites were ranged from 75 to  $2.7 \times 10^4$  CFU/ml (site R2), and from 56 to  $3.7 \times 10^4$  CFU/ml (site R3), which were lower than those in the industrial sites (I2, I3) and about two orders of magnitude higher than those in the less impacted sites (Fig. 2, Table S2). Site R2 is located at the convergence of many canals including Te canal, Doi canal, Tau Hu canal and Ben Nghe canal, which receives the untreated wastewater from a large residential area including the Districts of 5, 6, and 8; while site R3 is located at the Lang canal which runs through Binh Thanh District, one of the most densely populated residential areas in Ho Chi Minh City. Both sites (R2, R3) are located in the central region of Ho Chi Minh City, where the surface water samples were heavily affected by untreated wastewater and other residential activities. The abundance of ARB at the residential sites of R1 was found from 3.0 to  $2.1 \times 10^3$  CFU/ml, which was significantly lower than that at the sites of R2, and R3. This could be because the site R1 is located at the intersection of Nhieu Loc-Thi Nghe canal and Van Thanh canal. Along this canal, wastewater is collected in a sewage system to a pump station close to the Saigon River. The collected wastewater is regularly pumped out to the river and highly diluted with the river water (Ho et al. 2015).

Agricultural activities such as aquaculture and livestock production are considered as important contributors to the development and dissemination of antibiotic resistance in the environments due to the widespread use of antimicrobials for the treatment and prevention of animal diseases and growth promotion (You and Silbergeld 2014; Hong et al. 2013). In this study, except for the site A3, where the ARB concentration was observed slightly higher than those in the less impacted area, the level of ARB in the sites of A1, and A2 were found in a similar range with those in the less impacted sites (Fig. 2, Table S2). Perhaps, this is because the surface water samples both agricultural activities and the less impacted area were taken at the adjacent sites located in the upstream of the Saigon River. The non-point pollution of ARB by agricultural activities might be diluted by the large volume of the continuous flow of the Saigon River. In addition, the agricultural areas in Cu Chi District have recently been organized as a center of high technology agriculture in Ho Chi Minh City. Many environmentally benign technologies are encouraged in farming practices to reduce the uses

of antimicrobial agents and to control the release of untreated wastewater to the environment.

### Seasonal variation on the antibiotic resistance rate of heterotrophic bacteria

The impact of seasonal variation on the antibiotic resistance rate of heterotrophic bacteria in the Saigon River was reported in Fig. 3 and Table S2. Among the tested antibiotics, the resistance rate to amoxicillin/Clavulanic acid was detected at the range from 0.2 to 15.7% for the wet season, which was significantly lower than that in the dry season from 9.9 to 46.9% ( $p$ -value  $< 0.01$ ), while there were no significant difference found for other target ARB between the dry and set season (Fig. 3, Table S3). Amoxicillin is highly used for both human and veterinary medicines, the presence of amoxicillin residues in the aquatic environments was not well detected due to its high hydrolysis rate of b-lactam ring (Zhang et al. 2015; Gozlan et al. 2013). It is worth noting that it rains almost every day during the wet season from May to October in Ho Chi Minh City. High dilution of amoxicillin residues due to the daily rains in the wet season likely leads to a high degradation of this b-lactam antibiotic, which results in a low level of selective pressure for AMK resistant bacteria in the surface water samples. The resistance rates of the heterotrophic bacteria to the other target antibiotics in all the sampling sites were found in the following order: SXT (1.6–77.5%), CIP (1.2–36.1%), and AMK (0.2–9.7%) for the wet season, and SXT (2.2–56.3%), CIP (2.8–25.6%), and AMK (0.1–15.0%) for the dry season (Table S3). Although the resistance rates to SXT and CIP in the wet season were found slightly higher than those in the dry season, this difference was not significant ( $p$ -value  $> 0.05$ ). Sulfamethoxazole, Trimethoprim and ciprofloxacin are antibiotic drugs highly detected in surface water. In 2019, a study on the antibiotic pollution in the river water impacted by livestock production in China reported that the



**Fig. 3** Seasonal variation on antibiotic resistance rate (%) of heterotrophic bacteria in the surface water. \*signifies a significant correlation in the student  $t$ -test ( $p$ -value  $< 0.01$ )

average level of ciprofloxacin residues in the Yitong tributary was found at 3.3 ng/L for the wet season, and 3.1 ng/L in the dry season, while that in the Yima tributary were found at 3.7 ng/L for the wet season, and 3.0 ng/L in the dry season (Ju et al. 2019). In 2015, Nguyen et al. investigated the occurrences of antibiotics highly used for freshwater aquaculture in the aquatic environment on the Mekong Delta, about 20 km southwest of the Saigon River and found the high persistence of sulfamethoxazole and trimethoprim in the surface water sample, with an average concentration of 21 ng/L for sulfamethoxazole, and 17 ng/L for trimethoprim (Nguyen Dang Giang et al. 2015). The high concentration and persistence of sulfamethoxazole, trimethoprim, and ciprofloxacin in the surface water could be an important selective pressure for the development and dissemination of ARB in the surface water.

### Identification of ARB isolated from the Saigon River

A total of 192 isolates of ARB from all the water samples were selected and streaked on an R2A agar supplemented with the target antibiotics to confirm its resistance and collect pure colonies for bacterial identification using the 16S rRNA gene Sanger sequencing. The results show that these identified isolates belonged to 8 classes, 15 orders, 22 families, 38 genera, and 75 species (Table 1). *Enterobacteriaceae* was found as the most abundant bacterial family with 46 isolates, accounting for 23.8% of total identified isolates. This bacterial family was found in all five groups of ARB with the different proportion as follows: 53%, 31%, 21%, 10% and 5% of the total *Enterobacteriaceae* isolates resistant to SXT, AMC, CIP, CFM, and AMK, respectively. There were 18 identified isolates affiliated to the bacterial family of *Burkholderiaceae*,

and all of these isolates were from only 2 ARB groups including AMC (5 isolates) and AMK (13 isolates) resistant bacteria. *Chromobacteriaceae* was identified for 18 ARB isolates in which 11 isolates were resistant to AMC, 4 isolates were resistant to CFM, and 3 isolates were resistant to SXT. Some major bacterial families were identified in the ARB isolates from the Saigon River as follows: *Xanthomonadaceae* (14 isolates), *Aeromonadaceae* (13 isolates), *Flavobacteriaceae* (13 isolates), and *Bacillaceae* (10 isolates). (Fig. 4).

The genus-level composition of all identified bacterial isolates resistant to the five target antibiotics is shown in Fig. 4. Among the 38 identified genera, *Klebsiella* was found as the most abundant genus with 20 isolates (10.36% of total isolates). This genus of *Klebsiella* was highly resistant to AMC (8 isolates) and SXT (8 isolates) which were accounted for 16.7% of total identified isolates resistant to AMC and SXT, while a few isolates of *Klebsiella* were resistant to AMK, CFM, and CIP. *Enterobacter* was the second major bacteria genus with 18 identified isolates which were mainly resistant to SXT (7 isolates, 18.4% of total SXT isolates), AMC (5 isolates, 10.4% of total AMC isolates), CIP (3 isolates, 12.5% of total CIP isolates), and CFM (3 isolates, 12.5% of total CFM isolates). The following dominant genera were *Chromobacterium* (6.74%), *Aeromonas* (6.74%); *Bacillus* (5.18%), *Burkholderia* (5.18%), *Chryseobacterium* (5.18%), *Chromobacterium* (5.18%), *Ralstonia* (3.63%), *Aquitalea* (3.63%), *Escherichia* (3.63%), *Pseudomonas* (3.63%), *Flectobacillus* (2.07%), *Dechloromonas* (2.07%), *Sphingobacterium* (2.07%); *Comamonas* (2.07%), *Acinetobacter* (2.07%), *Microbacterium* (2.07%), *Novosphingobium* (2.07%). The

**Table 1** The dominant bacterial species of antibiotic resistant isolates from all the surface water samples in the Saigon River

Species	Number of isolates					Total	Percentage (%)	Cum (%)
	AMC <sup>R</sup>	AMK <sup>R</sup>	CFM <sup>R</sup>	CIP <sup>R</sup>	SXT <sup>R</sup>			
<i>Klebsiella pneumoniae</i>	5	2			6	13	6.7	6.7
<i>Aeromonas caviae</i>	1		1	3	4	9	4.7	11.4
<i>Enterobacter</i> sp.	4			2	2	8	4.1	15.5
<i>Escherichia coli</i>	1			1	5	7	3.6	19.2
<i>Stenotrophomonas</i> sp.	2	1	3	1		7	3.6	22.8
<i>Bacillus</i> sp.			5		1	6	3.1	25.9
<i>Dechloromonas</i> sp.			2		4	6	3.1	29
<i>Ralstonia</i> sp.	1	5				6	3.1	32.1
<i>Stenotrophomonas maltophilia</i>		2	4			6	3.1	35.2
<i>Burkholderia</i> sp.	3	2				5	2.6	37.8
<i>Aquitalea pelogenes</i>			2		2	4	2.1	39.9
<i>Aquitalea</i> sp.	1		2		1	4	2.1	42
<i>Burkholderia cepacia</i>	1	3				4	2.1	44
<i>Chromobacterium</i> sp.	4					4	2.1	46.1
<i>Flectobacillus</i> sp.		2	1	1		4	2.1	48.2
<i>Klebsiella</i> sp.	1		1	1	1	4	2.1	50.3



**Table 2** Spearman coefficient between the physicochemical parameters and the concentration of heterotrophic bacteria resistant to the target antibiotics in surface water of the Saigon River. Significant correlations were only considered and presented as normal format when  $p$ -values were less than 0.05, and as bold format when  $p$ -value were

< 0.01. AMC: Amoxicillin/Clavulanic acid resistant bacteria; AMK: Amikacin resistant bacteria; CFM Cefixime resistant bacteria; CIP: Ciprofloxacin resistant bacteria; SXT: sulfamethoxazole/Trimethoprim resistant bacteria; THB: Total number of heterotrophic bacteria

Physicochemical parameters	AMC	AMK	CFM	CIP	SXT	THB
pH	<b>0.537</b>	0.512	<b>0.573</b>	<b>0.717</b>	<b>0.696</b>	<b>0.628</b>
Temperature		<b>0.608</b>	<b>0.668</b>	<b>0.566</b>	<b>0.588</b>	<b>0.589</b>
Conductivity	0.430	0.419	<b>0.672</b>	<b>0.704</b>	<b>0.763</b>	<b>0.679</b>
Salinity	0.421	0.419	<b>0.672</b>	<b>0.704</b>	<b>0.763</b>	<b>0.675</b>
<i>Turbidity</i>						
DO	<b>-0.620</b>	-0.406				
PO <sub>4</sub>	<b>0.571</b>	0.485	<b>0.561</b>	0.451	<b>0.685</b>	<b>0.561</b>
NH <sub>4</sub>	<b>0.687</b>	<b>0.666</b>	<b>0.663</b>	<b>0.562</b>	<b>0.629</b>	<b>0.637</b>
NO <sub>2</sub>		<b>-0.621</b>	<b>-0.586</b>		<b>-0.544</b>	-0.531
NO <sub>3</sub>						
BOD5	<b>0.618</b>	0.416	<b>0.687</b>	<b>0.650</b>	<b>0.754</b>	<b>0.649</b>
Total coliform	<b>0.780</b>		<b>0.864</b>	<b>0.773</b>	<b>0.836</b>	<b>0.744</b>

bacteria genus of *Ralstonia* was observed as the most dominant bacteria group among the bacteria genera resistant to AMK (17.1%). (Fig. 4).

Among the total 75 bacteria species identified for all antibiotic resistant isolates, 16 bacterial species were dominant and accounted for more than 50% of the total ARB isolates, as shown in Table 1. *Klebsiella pneumoniae* was the most prevalent bacteria resistant to the target antibiotics with 13 isolates, about 6.74% of total identified isolates from all the surface water samples; followed by *Aeromonas caviae* (9 isolates, 4.66%), *Enterobacter* sp. (8 isolates, 4.15%), *Escherichia coli* (7 isolates, 6.63%), *Stenotrophomonas* sp. (7 isolates, 6.63%); *Bacillus* sp (6 isolates, 3.11%), *Dechloromonas* sp. (6 isolates, 3.11%), *Ralstonia* sp. (6 isolates, 3.11%), *Stenotrophomonas maltophilia* (6 isolates, 3.11%); *Burkholderia* sp. (5 isolates, 2.59%). Many of the ARB isolates were identified as human pathogens and opportunistic pathogens. For example, *Klebsiella pneumoniae* can cause bronchitis, thrombophlebitis, and urinary tract infection (Paczosa and Meccas 2016); *Aeromonas caviae* was reported to cause *Aeromonas* bacteremia in Japan (Kimura et al. 2013); Many strains of *Enterobacter* sp. such as *E. aerogenes* and *E. cloacae* can cause opportunistic infection of urinary and respiratory tracts in the immunocompromised people (Davin-Regli and Pagès 2015). Some strains of *Escherichia coli* can cause serious food poisoning, gastroenteritis, urinary tract infections, and neonatal meningitis in humans (Clements et al. 2012). The high prevalence of bacterial pathogens and opportunistic pathogens identified in the surface water along the Saigon River suggested the contamination of wastewater from intensive anthropogenic activities in the agricultural, industrial and residential areas in Ho Chi Minh City. The antimicrobial resistant strains of these

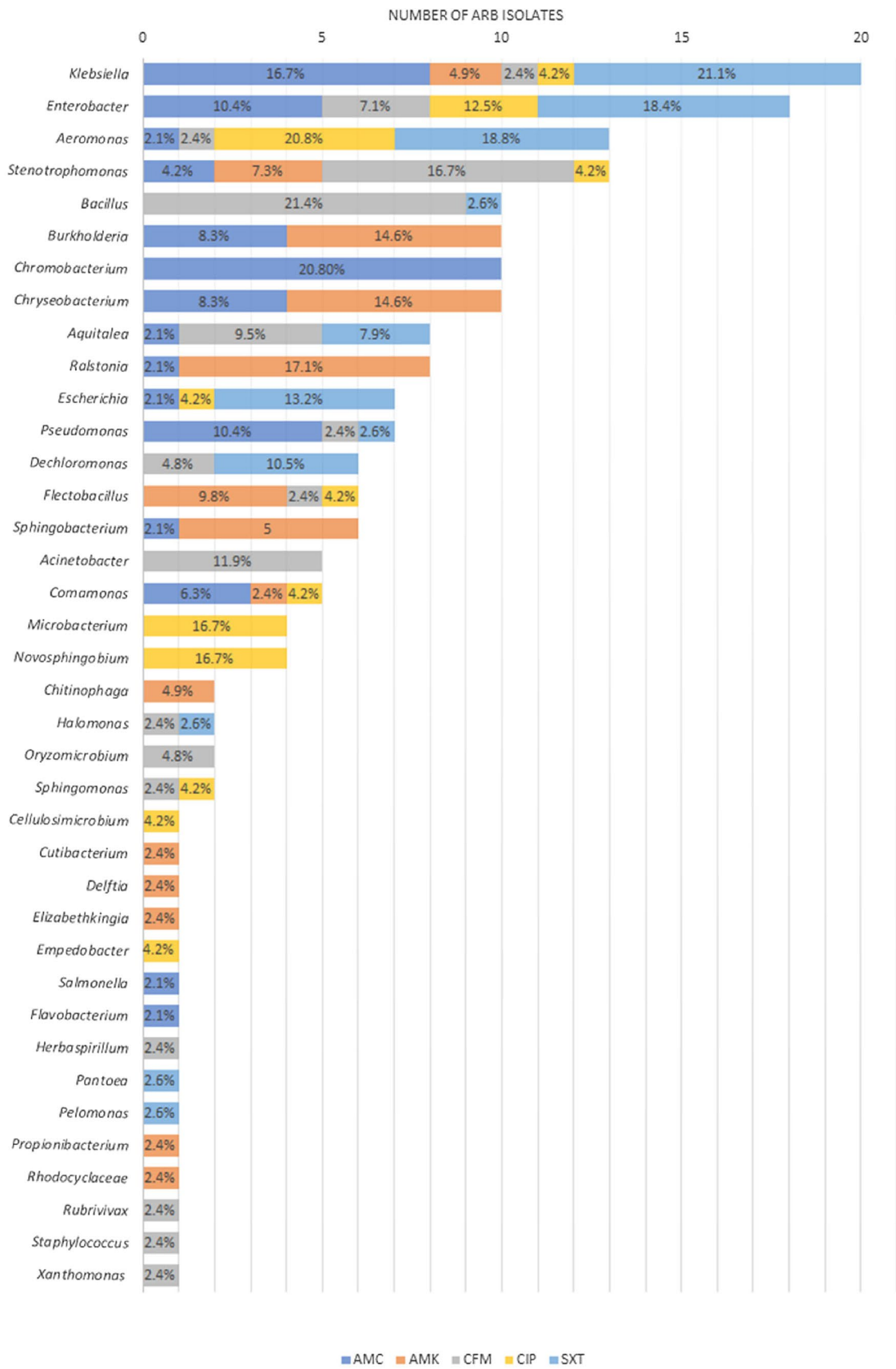
pathogens in the surface water may disseminate the antibiotic resistance across the microbial community in the aquatic environment and pose a risk threatening the animal and human health in the ecosystem.

### Relationship between ARB and water quality parameters

Spearman analysis was performed to investigate the potential correlation between the concentration of antibiotic resistant heterotrophic bacteria and water quality parameters for the surface water along the Saigon River (Table 2). The water quality parameters measured for all sampling sites were referred to our previous studies (Le Thai-Hoang et al.). The results showed that significant correlations ( $p$ -value < 0.01) were found between the total coliforms and concentration of heterotrophic bacteria resistant to almost the target antibiotics, except for AMK. This indicated that these ARBs might be driven from the same origin contaminated with human and animal waste which may associate with many different pathogenic organisms. This was consistent with our data in the previous section reporting many antibiotic resistant isolates identified as human pathogens and opportunistic pathogens in all the surface water samples along the Saigon River.

Among the physicochemical parameters, turbidity, and NO<sub>3</sub><sup>-</sup> nitrogen were not found to have any significant correlation with the presence of antibiotic resistant bacteria in the water samples. The dissolved oxygen (DO), and NO<sub>2</sub><sup>-</sup> nitrogen were found to be negatively correlated with AMK, AMC, and AMK, CFM, SXT, and THB, respectively (Table 2). There are many factors affecting the level of DO







**Fig. 4** The genus-level composition of all bacterial isolates resistant to the five target antibiotics. The length of the bars represents the total number of ARB isolates for each bacterial genera. The percentages on the bar present the proportion of bacteria exhibiting resistance to the target antibiotics

and  $\text{NO}_2^-$  nitrogen in the aquatic environment, especially the nitrification process catalyzed by the ammonia-oxidizing bacteria and nitrate-oxidizing bacteria. It is unclear whether these parameters affected on the presence of the target ARB in the surface water. The pH and temperature values of all surface water samples were detected in the range from 5.23 to 7.25, and from 28 to 31 °C (Data not shown), which are still below the Vietnam national regulation on the surface water quality (Bộ Tài nguyên và Môi trường 2015). Within this neutral range, the significant correlation between total ARB and pH, and temperature were found and likely indicated a favorite physical condition for the growth of the total bacteria as well as the resistant bacteria in the surface water. In addition, conductivity and salinity parameters were also observed to have significant correlations with the concentration of total heterotrophic bacteria and ARB, which suggested that the pollution of dissolved cations and anions in the surface water may stimulate the growth of total bacteria and resistant bacteria in the surface water. The phosphate ( $\text{PO}_4^{3-}$ ), ammonium ( $\text{NH}_4^+$ ) and Biochemical oxygen demand (BOD5) are important parameters that indicated water quality of the river. The high levels of nitrogen and phosphorus indicate the pollution of nutrient contents in the water bodies, which can be contributed by various human activities with domestic, industrial, and agricultural wastewater. The correlation analysis for all the surface water in the Saigon River showed that the concentrations of heterotrophic bacteria resistant to all target antibiotics were significantly correlated with both nutrient contents of phosphate  $\text{PO}_4^{3-}$ , ammonium  $\text{NH}_4^+$ , and BOD5 (Table 2). This result suggested that the contamination of organic matters in the Saigon River may play an important role to develop and disseminate the ARB in the surface water of the sampling sites.

## Conclusion

Antimicrobial resistance has been considered as the main threat to human life and negative impacts on the global economy as it causes a serious health problem associated with an increase in hospitalization time, treatment cost, and mortality. ARB are increasingly found in the community and can be acquired from many different routes of transmission such as between people, animals, food, and water in the environment. This study found that heterotrophic bacteria resistant to the target antibiotics including AMC, AMK, CFM, CIP, and SXT were detected in all the sampling sites along the

Saigon River. The industrial and residential activities were likely major factors contributing to the overall contamination of the ARB in the surface water in Ho Chi Minh City. The surface water samples collected at the industrial (I2, I3) and residential (R2, R3) areas were found significantly higher than those at the agricultural and less impacted areas. The levels of ARB at the sites I2 and I3 were detected as the highest concentration ranging from  $10^2$  to  $3.3 \times 10^5$  CFU/ml, because these sites were located in the large industrial zone in the central of Ho Chi Minh City and receive both negative impacts from industrial and residential activities. Many water quality parameters were found to have significant correlations with the level of total heterotrophic bacteria and resistant bacteria, especially conductivity, salinity, phosphate, ammonium, BOD5, and total coliform. Pollution of both dissolved salts, organic matters, and wastewater from human activities were important factors facilitating the development and spread of ARB in the aquatic environments. *Enterobacteriaceae* was the most abundant bacterial family, account for more than 23% of total isolates of the antibiotic resistant heterotrophic bacteria in the Saigon River, and showed resistance to all 5 target antibiotics. Among the antibiotic resistant *Enterobacteriaceae*, many bacterial species were identified as human pathogens and opportunistic pathogens such as *Klebsiella pneumoniae* (6.74%), *Enterobacter* sp. (4.15%), *Escherichia coli* (6.63%), *Stenotrophomonas maltophilia* (3.11%), *Aeromonas Caviae* (4.66%). These antibiotic resistant and opportunistic pathogens were detected not only in the highly contaminated sites but also in the less impacted sites along the Saigon River. The contamination of ARB in the surface water can be widely detected in the aquatic environment and pose a potential risk threatening the animal and human health in the ecosystem.

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**Availability of data and materials** Data and material are not shared.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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