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Prevalence of Antibiotic Resistance Genes in the Saigon River Impacted by Anthropogenic Activities

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Abstract: Despite of a high abundance of antibiotics, heavy metals, and organic matters detected in the Saigon River in Ho Chi Minh City, the level and spread of antibiotic resistance genes in this river are poorly understood. In this study, total 10 antibiotic resistance genes (ARGs), including genes conferring resistance to aminoglycosides (*aac(6)-Ib-cr*), β-lactam antibiotics (bla_{CTX-M}, bla_{SHV}, bla_{TEM}), quinolones (*qnrA*, *qnrB*), sulfonamides (*sul1*, *sul2*), trimethoprim (*dfrA*), efflux pump (*oqxB*), and three genes of genetic elements, including integron classes 1, 2, and 3 (*intI1*, *intI2*, *intI3*), are quantified by qPCR. Water samples were collected from the industrial, agricultural, residential, and less impacted areas for the wet and dry seasons. The results present high occurrence rates for 10 ARGs that were observed in all the sampling sites with the following order: *sul1*, *sul2*, *dfrA* > *aac(6)-Ib-cr* > bla_{CTX-M}, bla_{SHV}, bla_{TEM} > *qnrA*, *qnrB*. Although the levels of ARGs and integrons in the dry season were found about to be about one order of magnitude higher than those in the wet season, the exact mechanisms for this are not fully clear. The correlation analysis presented here suggests that the contamination of organic matter and nutrients from agricultural, industrial, and residential activities likely contributes to the prevalence of ARGs, integrons, total bacterial load, and the potential development and spread of antibiotic resistance in the aquatic environments considered here.

Keywords: antibiotic resistance gene; surface water; anthropogenic activity; water quality

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1. Introduction

Antibiotic resistance is one of the largest threats to global health and food security in all parts of the world. Every year, more than 700,000 people die due to antibiotic-resistant infections, and this number could grow to 10 million by 2050 [1]. The main causes of antibiotic resistance are considered to be the overuse of antibiotics or antibiotic misuse in human and animal medicines, livestock, and aquaculture [2–4]. Antibiotics are not completely metabolized in humans and animals after administration, where typically more than 70% of an antibiotic is excreted in an unchanged form via urine and feces [5]. Thus, large amounts of antibiotics and their metabolites can be released to wastewaters, which may enter different environmental compartments and serve as a selective pressure for the proliferation of antibiotic-resistant bacteria [5].

Antibiotic resistance genes (ARGs) are genetic determinants of antibiotic resistance in bacteria, and most of them are found on mobile genetic elements such as plasmids, transposons, and integrons, which can facilitate their migration among various microbes via the horizontal gene transfer phenomenon [6–8]. Recently, ARGs have been considered

as emerging environmental contaminants as they likely persist in a bacterial population, even without the selective pressure of antimicrobial agents in the environments [9,10]. There are growing reports of abundances of ARGs in various environments, including surface water [11,12], drinking water [13], wastewater treatment plants [14–16], soil [17], and sediment [18]. Contamination with ARGs and genetic elements such as integrons in various environments may enable the growth and spread of antibiotic resistance in a bacterial population, which can pose a potential risk to human and animal health in the ecosystem.

Anthropogenic activities such as aquaculture, livestock and poultry farming, chemical/pharmaceutical production, and residential activities generate large amounts of waste that contains various antimicrobial compounds, such as heavy metals, biocides, human/animal medicines, and antibiotics. These wastes can be released, sometimes without any treatments, to the various environments and consequently promote selective pressures for the development of antibiotic-resistant bacteria and genes [19–22]. In addition, with current technology, even in developed countries, the removal capacity in wastewater treatment plants has been reported to not be adequate in regard to eliminating antibiotics and ARGs [23–26]. The situation is even worse in developing countries, such as those in South East Asia, where about 80% of total wastewater is not treated at a centralized wastewater treatment plant and this significantly contributes to the overall pollution of various environments [27].

Vietnam is a large consumer of antibiotics in Asia. In 2015, the total consumption amounts of antibiotics for humans in Vietnam, South Korea, Japan, the Philippines, Mongolia, and Brunei were estimated as 1086, 546.4, 524.9, 260.5, 133.2, and 1.13 tons, respectively [28,29]. For animal uses, the total consumption amount of antibiotics in 2015 in Vietnam was reported at 2751 tons, while that in South Korea was estimated at about 984 tons [29]. In 2019, a review paper reported that Vietnam and China were the two countries that have the highest number of antimicrobial agents used in aquaculture industry, and the antibiotic usages tended to increase over the last decade due to the uncontrolled use of drugs in both countries [30]. Recently, there is growing surveillance reporting the presence of antibiotic residues in surface water in Vietnam. Particularly, in the Mekong River, antibiotics including sulfamethoxazole, trimethoprim, enrofloxacin, and sulfadiazine were found in the canal and river water samples with median values of 21, 17, 12, and 4 ng/L, respectively [31]. In another study on Hau River, a tributary of the Mekong River, 17 antibiotic residues were detected in the surface water with concentrations ranging from 17 to 91 ng/L [32].

In the south of Vietnam, the Saigon River flows through Ho Chi Minh City, the largest city of Vietnam, with a population of approximately 9 million in 2019, and serves as an important water source for the drinking water supply, as well as other human activities in the city. Due to the fast economic development in Ho Chi Minh City during the last few decades, the river has become seriously polluted with various contaminants, such as heavy metals, pharmaceuticals, antibiotics, and organic matter [33–36]. Despite the increasing contamination of various antimicrobial agents in the Saigon River, there is very little information regarding the antibiotic resistance there, especially the prevalence of antibiotic resistance genes in the surface water of Ho Chi Minh City. This study aims to provide baseline data regarding the occurrence of 10 ARGs likely conferring resistance to widely used antibiotics and 3 genetic elements (integron classes 1, 2, and 3) in the Saigon River. The influence of anthropogenic activities in Ho Chi Minh City on the prevalence of ARGs and integrons is investigated here by correlation analysis among the target genes measured at all the sampling sites in the agricultural, residential, and industrial areas. The relative abundances of each target gene in the wet and dry seasons are used to explore the seasonal variations of the prevalence of ARGs and integrons in the surface water.

2. Materials and Methods

2.1. Sampling Sites

Field samples were collected along the Saigon River and at two sites in the Dong Nai River, from upstream (Ben Duoc Temple, Cu Chi District, Ho Chi Minh City, Vietnam) to downstream of the central area of Ho Chi Minh City (Truong Phuoc Bridge, Ho Chi Minh City, Vietnam). Detailed locations and descriptive information for all sampling sites are mapped in Figure S1 and Table 1, respectively. In total, 12 sampling sites were selected based on the dominant land uses in Ho Chi Minh City. The sites represent four different activities, including three sites in residential areas (R1, R2, R3), three sites in industrial areas (I1, I2, I3), three sites in agricultural areas (A1, A2, A3), and three sites in less impacted areas (L1, L2, L3).

Table 1. Description of all sampling sites in the Sai Gon River.

Human Activities	Sample Label	Description
Less impacted areas (L)	L1	The site is under the Truong Phuoc Bridge, which is close to the BCR water park, which features many water recreational activities such as swimming, bathing, and water playgrounds.
	L2	The site is inside the Ben Duoc Temple, upstream of the Sai Gon River, which is about 5 km from industrial, agricultural, and residential areas in Ho Chi Minh City.
	L3	The site is close to the Hoa Phu pump station, a water source for the Tan Hiep water treatment plant, which supplies drinking water for 11/24 districts in HCMC.
Agricultural areas (A)	A1	The site is close to the High-Tech Agricultural Park in Cu Chi District, Ho Chi Minh City.
	A2	The site is below the Rach Ke Bridge, which is a large canal in an agricultural area.
	A3	The site is located at Binh My, a rural commune of Cu Chi District, where there are more than 20 livestock farms.
Industrial areas (I)	I1	This site is at the Xang Canal, which is an intersection of many canals passing through Tan Phu Trung (542.6 ha, main industrial fields include pharmaceuticals, food, packaging, and mechanical engineering) and Nhi Xuan (211 ha, main industrial fields include garments, food processing, plastic products, packaging, and mechanical engineering) industrial parks.
	I2	The site is located at Tham Luong Canal in the Tan Binh Industrial Park (128.7 ha, main industrial fields are such as packaging, cosmetic, textile, plastic product, food processing, plating and mechanical engineeing).
	I3	The site is under the Suoi Cai Bridge in the Sai Gon Hi-Tech Park (913 ha, main industrial fields include microelectronics, information technology, telecommunications, precision mechanics, automation, biotechnology, pharmaceuticals, new energy, new materials, nanotechnology).
Residential areas (R)	R1	The site is located at the intersection of the Nhieu Loc-Thi Nghe Canal and Van Thanh Canal. The Nhieu Loc-Thi Nghe Canal is about 8.7 km in length and is one of the main drainage canals of Ho Chi Minh City.
	R2	The site is at an intersection of Te Canal, Doi Canal, Tau Hu Canal, and Ben Nghe Canal. These canals directly receive wastewater from a residential area with high population density in Ho Chi Minh City.
	R3	The site is under the Cau Do Bridge, which is close to the Lang canal. This canal passes through Binh Thanh District, which is one of the most densely populated areas in Ho Chi Minh City.

2.2. Sample Collection

Water samples were collected in two sampling events, namely, during August 2019 for the wet season and during March 2020 for the dry season. All samples were taken in the morning from 9 a.m. to 11 a.m. when the temperature of the water samples was

fluctuated from 28.1 to 30.1 °C for the wet season and from 28.1 to 31.1 °C for the dry season (Data not shown). For each sampling site, water samples were taken from three different positions (one at the middle of the canal/river, two at the positions close to two banks of the canal/river) using a 5 L bucket at a depth of 20–30 cm from the water surface. The three spatial samples were mixed well in a 15 L bucket before collection into 1 L bottles for microbiological and physicochemical analysis. Samples were kept on ice during the transportation to the laboratory (within 2 h) and processed for further analysis in the same day.

2.3. DNA Extraction

For qPCR analysis, 100 mL surface water samples were centrifuged using 50 mL falcon tubes at 10,000 rpm for 10 min to collect the bacterial biomass. The total genomic DNA of the surface water was extracted using a TopPURE Genomic DNA Extraction Kit (ABT, Ho Chi Minh City, Vietnam). For each water sample, DNA extractions were taken as duplicates and the two DNA extracts from the same samples were combined for any qPCR analysis. The steps of DNA extraction were performed according to the manufacturer's instructions. The concentration of total genomic DNA extract was measured in the Nanodrop 2000/2000 c spectrophotometer (ThermoScientific, Wilmington, NC, USA). All DNA extracts were stored at −80 °C until qPCR analysis.

2.4. Quantification of ARGs

Ten ARGs of interest, including genes conferring resistance to aminoglycosides (*aac(6)-Ib*), β-lactam antibiotics (bla_{CTX-M}, bla_{SHV}, bla_{TEM}), quinolones (*qnrA*, *qnrB*), sulfonamides (*sul1*, *sul2*), trimethoprim (*dfrA*), efflux pump (*oqxB*), 3 genes of genetic elements, including integron classes 1, 2, 3 (*intI1, intI2*, and *IntI3*), and the total bacterial 16S rRNA genes were quantified by qPCR on a Master Cycler Realplex 4S (Eppendorf, Hamburg, Germany). All DNA samples were diluted to 10 ng/μL using a TE buffer (10 mM TrisCl, pH 8.0, 1 mM EDTA) to avoid any inhibitions to qPCR reactions due to high DNA concentrations. A qPCR reaction was prepared for a total volume of 20 μL by adding 8 μL of PCR-grade water, 10 μL SensiFAST SYBR No-ROX (Bioline, Eveleigh, Australia), and 0.5 μL of appropriate forward and reverse primers at 10 μM (GENEWIZ, Tokyo, Japan). The sequences and annealing temperatures of all primers are summarized in Table S1. The qPCR program was initially held at 95 °C for 5 min for the polymerase activation, followed by 40 cycles of 95 °C for 20 s, an optimal annealing temperature for 20 s, then 72 °C for 30 s. Melting curve analysis was performed for 95 °C for 15 s, 60 °C for 15 s, and 95 °C for 15 s. All qPCR reactions for DNA samples were performed in duplicate and included a no template control, positive control using the PCR product confirmed by Sanger sequencing, and a standard curve. A standard curve was built by running the qPCR reaction for a dilution series of the PCR product amplified using the same primers, including 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} ng/μL for DNA templates. Log-transformed values of copy number of the target genes against the CT values followed by the linear regression were used to build the standard curve. Quantifications of any target genes were validated with an average R^2 value of 0.95 and an amplification efficiency range of 85–115%. The limit of detection for each ARG was determined by conducting a qPCR assay for multiple 10-fold dilutions of PCR products until the lowest points were no longer linear with other dilutions (Table S2).

2.5. Data Analysis

All qPCR analyses for the genomic DNA samples were performed in duplicate and the average values are presented in the figures and tables here. The concentrations of all ARGs, integrons, and 16S rRNA genes are expressed as the absolute copy numbers of these genes per 1 mL of the surface water, calculated from the log-transformed gene copy numbers, before normalizing to the DNA extraction yield and the total volume of surface water used for DNA extraction. The relative abundances of the target genes used for

studying the effects of two tropical seasons on the prevalence of antibiotic resistance genes were determined by the ratios of the absolute copy numbers of each gene to the absolute copy number of the 16S rRNA gene (total bacterial load). Both Pearson and Spearman analyses were used to study the correlations between all water quality parameters and ARGs, integrons, and 16S rRNA genes, and significant correlations were only considered if the P-values were less than 0.05. Principal component analysis was conducted to explore any relationship among the water quality parameters, ARGs, and integrons in all the sampling sites in the Saigon River. All the figures, correlation, and multivariate analysis were performed using the software of Origin Lab (Version 2019).

3. Results and Discussion

3.1. Occurrences of ARGs and Integrons in the Saigon River

In this study, 10 ARGs (*aac(6)-Ib-cr*, bla_{CTX-M}, bla_{SHV}, bla_{TEM}, *qnrA*, *qnrB*, *oqxB*, *sul1*, *sul2*, and *dfrA*), 3 integrons (*intI1*, *intI2*, and *IntI3*), and 16S rRNA genes were quantified by qPCR with surface water samples from the Saigon River (Figure 1). The quantities of total bacterial 16S rRNA genes in the surface water range from 1.6×10^4 to 1.6×10^8 copies/mL, which is about from 1 to 4 orders of magnitude more than the abundance of other tested ARGs and integrons. The abundance of 16S rRNA in the Saigon River was found in a similar range to that in the Ter River in Spain (1.20×10^7 copies/mL) and in the Sundarijal, Thapathali, and Chovar Rivers in Nepal (7.9×10^7 copies/mL) [37,38]. In general, all the target ARGs and integrons were detected in all the sampling sites along the Saigon River in both tropical seasons, and the abundances of these ARGs had the following order: sulfamethoxazole-trimethoprim resistance genes (*sul1*, *sul2*, *dfrA*) > aminoglycosides resistance gene (*aac(6)-Ib-cr*) > β-lactam resistance genes (bla_{CTX-M}, bla_{SHV}, bla_{TEM}) > fluoroquinolones resistance genes (*qnrA*, *qnrB*) (Figure 1, Table S3).

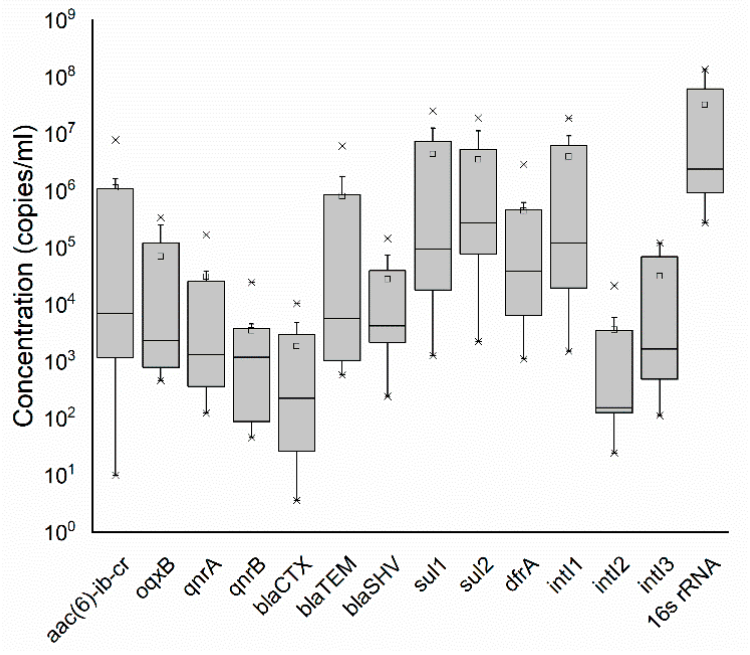

Figure 1. Concentrations (copies/mL) of the target ARGs, integrons, and 16S rRNA gene in the surface water samples of the Saigon River at upstream and downstream locations of Ho Chi Minh City. The lower and upper "×" symbols in each box indicate the 1% and 99% percentiles of data points. The squares in the boxes indicate the mean values of the data points.

The *sul1*, *sul2*, and *dfrA* genes, which confer resistance to co-trimoxazole (sulfamethoxazole and trimethoprim), were among the most abundant ARGs with the ranges of 3.4×10^2–4.4×10^7 copies/mL, 0.85×10^2–3.7×10^7 copies/mL, and 2.7×10^2–0.5×10^7 copies/mL respectively (Figure 1, Table S3). This is similar to some recent studies that have reported

sulfamethoxazole resistance genes (e.g., *sul1* and *sul2*) to be among the most prevalent ARGs in aquatic environments [39–41]. Sulfamethoxazole and trimethoprim have been widely used in combination with human and veterinary medicines to treat infectious diseases for more than 60 years, as the drugs are efficacious, cost-effective, and feature well-defined adverse effects. These drugs are considered to be first-line therapeutics for several infections [42,43]. In Vietnam, abundances of sulfamethoxazole and trimethoprim residues were widely detected in aquatic environments as these antibiotics are the major veterinary medicines used in shrimp farming for the treatment of infectious diseases, such as "white spots" and "white feces" in shrimp [31,44]. In a recent survey on a river in the Netherlands, the presence of sulfamethoxazole resistance genes, including *sul1* and *sul2,* in the surface water receiving the treated effluent of a local wastewater treatment plant were found to range between 10^3 to 10^6 copies/mL and 10^3 to 5×10^5 copies/mL, respectively [38]. These levels of *sul1* and *sul2* were slightly lower than those in our study due to the fact that the surface water in the Saigon River is impacted by untreated wastewater from various anthropogenic activities in Ho Chi Minh City.

Extended-spectrum β-lactamases (ESBLs) have received increasing concern because they can induce resistance to many broad-spectrum β-lactam antibiotics, including penicillin, monobactams, and cephalosporin [45]. Recently, the presence of β-lactam-resistant bacteria and genes has been reported in various environmental samples locations, including soil, sediment, reservoirs, rivers, domestic wastewater, and hospital wastewater [11,46–48]. In this study, 3 genes encoding β-lactamase, including $bla_{CTX\text{-}M}$, bla_{SHV}, and bla_{TEM}, were quantified in the range of 0–1.9×10^4 copies/mL, 0–1.4×10^5 copies/mL, and 2.2×10^2–1.1×10^7 copies/mL, respectively (Figure 1, Table S3). On average, the level of bla_{TEM} in the Saigon River was found to be about one to two orders of magnitude higher than that of $bla_{CTX\text{-}M}$ and bla_{SHV}. This is consistent with some recent studies on ESBL genes in surface water, reporting that the bla_{TEM} is one of the most frequently ESBL genes found in surface water samples, as well as in bacterial isolates from aquatic environments [37–39,47,49].

Quinolones are broad-spectrum antibiotics that are widely used in human and veterinary medicines for the effective treatment of serious infections. Recently, many studies have reported a rapid development of quinolone resistance in bacterial isolates from various environmental matrices, including sewage, surface water, and agricultural soil [49–51]. In this study, the concentrations of quinolone resistance genes *qnrA* and *qnrB* in the surface water of the Saigon River were found range from 74 to 2.6×10^5 copies/mL and 6 to 2.7×10^4 copies/mL, respectively (Figure 1, Table S3). These levels of *qnrA* and *qnrB* in the Saigon River were observed in a similar range to those in the water samples collected from the Xiaoqing River and a wastewater treatment plant in Jinan, China, which were reported to range from 0 to 2.5×10^5 copies/mL (*qnrA*) and from 0 to 3.0×10^5 copies/mL (*qnrB*) [49].

Aminoglycoside antibiotics are usually used in the treatment of healthcare-associated infections. They disrupt bacterial protein synthesis by binding to the A-site of 30S rRNA [52]. One of the main mechanisms of aminoglycoside resistance in bacteria is believed to be caused by three main aminoglycoside-modifying enzymes, such as aminoglycoside acetyltransferases (AAC), aminoglycoside phosphotransferases (APH), and aminoglycoside nucleotidyltransferases (ANT), especially for aminoglycoside acetyltransferases (AAC) enzymes as they are the most prevalent aminoglycoside-modifying enzymes with a large number of variants [53,54]. In this study, the concentration of the *aac(6)-ib-cr* was found from 81 to 1.5×10^7 copies/mL in the surface water and varied largely in the sampling sites along the Saigon River (Figure 1, Table S3). This level of *aac(6)-ib-cr* was slightly lower than that detected in the water samples in the Xiaoqing River (from 0 to 5×10^7 copies/mL) and that in a wastewater treatment plant in Jinan, China (from 0 to 1.5×10^8 copies/mL) [49]. In addition, the abundance of *aac(6)-ib-cr* in the Saigon River was observed on a similar level with the mobile genetic element of integron class 1 gene *intI1* (from 1.5×10^3 to 2.7×10^7, Figure 1, Table S3). This is consistent with some recent reports that the *aac(6)-Ib* genes are often found on the plasmid, as well as being associated with a genetic element, which can

facilitate wide dissemination of this ARG in a bacterial community via horizontal gene transfer [55–57].

Efflux pumps are transport proteins capable of moving toxic substances (e.g., antibiotics and heavy metals) out of the bacterial cells and they play a major role in both intrinsic and acquired multidrug resistance [58,59]. In a recent study, Wei Hou et al. reported a high abundance and diversity of genes encoding efflux pumps in nine lake and reservoirs in the north of China and suggested that efflux pumps are likely a predominant resistance mechanism in natural water bodies [60]. In this study, the occurrence of the efflux pump gene *oqxB* in the surface water was found to range from 53 to 4.6×10^5 copies/mL (Figure 1, Table S3). On average, the level of *oqxB* genes was lower than that of *sul1*, *sul2*, *dfrA*, *intI1*, bla_{TEM} and *aac(6)-Ib-cr,* but higher than the levels of bla_{CTX}, bla_{SHV}, *qnrA*, *qnrB*, *intI2*, and *intI3* in the Saigon River.

Integrons are genetic elements that are widely known to play a major role in the dissemination of antibiotic resistance by capturing and expressing exogenous DNA/genes [61,62]. In this study, the integron class 1, 2, and 3 genes (*intI1*, *intI2*, and *intI3*) were detected in all the sampling sites in the Saigon River and ranged from 1.5×10^3 to 2.7×10^7 copies/mL, from 3 to 3.5×10^4 copies/mL, from 3 to 1.9×10^5 copies/mL, respectively (Figure 1, Table S3). Among these target integrons, the *intI1* gene was found as the most prevalent gene and was highly detected in all the surface water samples. In a recent study on the three rivers in Nepal (the Sundarijal, Thapathali, and Chovar Rivers), the abundance levels of the *intI1* gene were measured in the range of 15–5×10^6 copies/mL, which is slightly lower than that in the Saigon River in Ho Chi Minh City [37]. The level of the *intI1* gene was observed to associate with the abundance of ARG over time in anthropogenically-impacted rivers and to significantly correlate with human pathogens in a wastewater treatment plant [14,60,63,64]. The high abundance of three integrons (*intI1*, *intI2*, and *intI3*), and the target ARG genes found in the Saigon River impacted by various human activities likely indicates potential widespread antibiotic resistance across the bacterial community in the aquatic environment via horizontal gene transfer.

3.2. The Abundance of ARG Genes in the Saigon River Impacted by Anthropogenic Activities

Figure 2 shows changes in the concentrations of ARGs, integrons, and 16S rRNA in total across the 12 sampling sites along the Saigon River as impacted by different anthropogenic activities in Ho Chi Minh City. In general, the levels of ARGs and integrons were found at the highest level in industrial sites (I1, I2, I3), followed by residential (R1, R2, R3), agricultural (A1, A2, A3), and less impacted sites (L1, L2, L3). It is worth noting that the industrial and residential sites were closer to the central of Ho Chi Minh City, downstream of the Saigon River, while the agriculture and less impacted sites were located far away from the city and upstream of the Saigon River. This reflects the impacts of anthropogenic activities along the river, consequently increasing the contamination of ARGs from upstream to downstream of the River [65]. This level order was found to be similar to the abundance trend of total bacterial 16S rRNA measured in the industrial (8.4×10^7 copies/mL), residential (4.3×10^7 copies/mL), agricultural (1.3×10^6 copies/mL), and less impacted areas (6.3×10^5 copies/mL). It was suggested that the concentration of antibiotic resistance genes and integrons were mainly associated with the abundance of total bacteria including antibiotic-resistant bacteria in the surface water. Perhaps, the environmental conditions supporting the growth of total bacteria can also facilitate the development and spread of the ARG/integron-carrying bacteria in the aquatic environments.

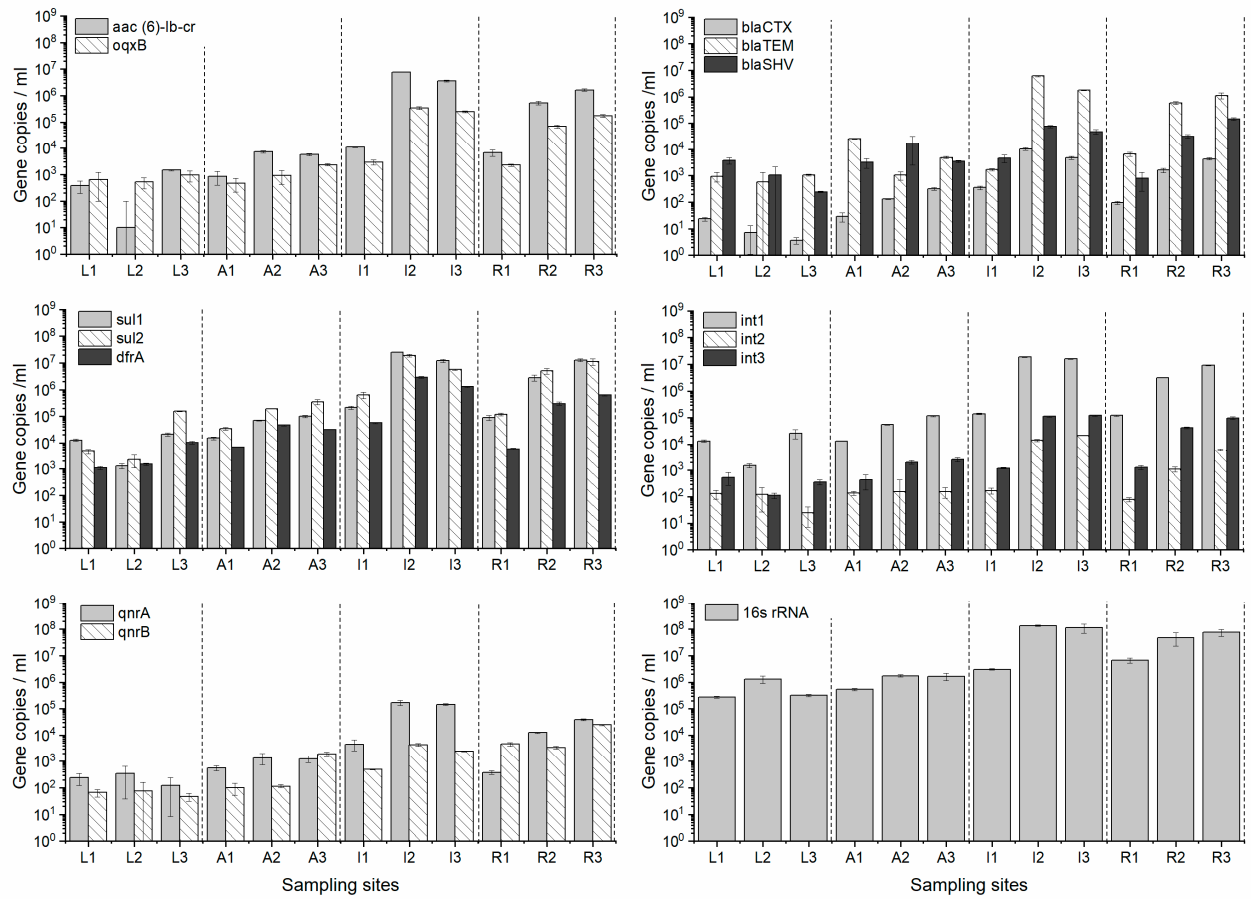

Figure 2. Concentration (copies/mL) of the ARGs, integrons, and the 16S rRNA gene in the surface water of the 12 sampling sites along the Saigon River impacted by different anthropogenic activities.

In comparison to the less impacted sites, the concentrations of ARGs and integrons in the industrial and residential sites were found to be significantly higher, i.e., about two to four orders of magnitude higher (Figure 2, Table S3). In particular, the average concentrations of *aac(6)-Ib-cr* (3.8×10^6 copies/mL), bla_{TEM} (5.2×10^3 copies/mL), and *sul1* (1.7×10^7 copies/mL) in the industrial areas were found about three orders of magnitude more than those in the less impacted sites, while the average concentrations of $bla_{CTX\text{-}M}$ (5.2×10^3 copies/mL), *qnrA* (1.1×10^5 copies/mL), *qnrB* (2.4×10^3 copies/mL), *sul2* (8.5×10^6 copies/mL), *dfrA* (1.4×10^6 copies/mL), *oqxB* (2.0×10^5 copies/mL), *intI1* (1.2×10^7 copies/mL), *intI2* (1.2×10^4 copies/mL), and *IntI3* (7.9×10^4 copies/mL) were about two orders of magnitude higher than those in the less impacted sites (Figure 2, Table S3). It is worth noting that less than 50% of the total municipal wastewater in Ho Chi Minh City is collected and treated properly at a centralized wastewater treatment plant. The untreated wastewater in the city is released to the main canals before entering the tributaries of the Saigon River. Consequently, all surface water samples collected in the central area of Ho Chi Minh City may be contaminated by the untreated wastewater originating from various sources, such as households, urban runoff, hospitals, and commercial/industrial activities. For the surface water in industrial sites, the water samples collected in the main canals passing through the industrial areas could be mainly impacted by diverse types of wastewater from the various manufacturing industries. Per site, these industries include the following: garments, food processing, plastic products, packaging, and mechanical engineering (site I1); packaging, cosmetics, textiles, plastic products, food processing, and plating and mechanical engineering (site I2); microelectronics, telecommunications, precision mechanics, biotechnology, pharmaceuticals, new materials, and nanotechnology (site I3) (Table 1). Among the three sampling sites in the industrial area (I1, I2, I3), site I2 in

the Tan Binh Industrial Park and site I3 in the Saigon Hi-Tech Park were located in Tan Binh District and District 9, which are areas with a high population density in Ho Chi Minh City, while site I1, in the Tan Phu Trung Industrial Park, is located in Cu Chi District, which is quite far away from the urbanized area of Ho Chi Minh City (Table S3). The surface water samples at sites I2 and I3 were not only influenced by the industrial wastewater, but also by the domestic wastewater from the surrounding residential activities in comparison to site I1. As per the results, all target ARGs, integrons, and total bacterial 16S rRNA values were quantified at the highest levels in industrial sites I2 and I3 as compared to the other sites (Figure 2, Table S3). In a recent study, Agramont et al. investigated the contamination of ARGs and class 1 integrons in water and sediment samples that were impacted by human wastewater and mining activities. The authors found that fecal pollution was likely a major driver of ARG and *intI1* genes in comparison to the heavy metal pollution [66]; however, it was still unclear if there were any synergistic effects from both fecal pollution and heavy metals on the overall levels and spreads of ARGs and integron genes in the considered aquatic environments.

Among the three sampling sites in the residential area, site R1 was found to be less contaminated with ARGs and integrons. This is because site R1 was located at the Nhieu Loc–Thi Nghe Canal, where all domestic wastewater along this canal, originating from seven districts in Ho Chi Minh City, is collected in a sewage system to a pump station close to the Saigon River. A large volume of the collected wastewater from this canal is pumped out to the Saigon River and is highly diluted with river water. This may lead to a significantly lower level of ARGs and integrons at residential site I1 than residential sites I2 and I3 (Figure 2, Table S3). In comparison to the industrial and residential activities, agricultural activities in Ho Chi Minh City showed less impact on the contamination of antibiotic resistance genes. In general, all ARGs and integrons in the surface water samples of the agricultural areas were found to be slightly higher, ranging from zero to one order of magnitude in comparison to the less impacted areas (Figure 2, Table S3). It can be explained that the agricultural activities in Cu Chi District have recently been well organized and have applied new technologies to reduce the use of chemical insecticides and fertilizers in farming practices, as well as controlling the release of agricultural wastes and untreated wastewaters to the surrounding environment. This may help to reduce the non-point pollution of antimicrobial compounds and antibiotic resistance factors via agricultural activities to the Saigon River.

3.3. Seasonal Variation of ARGs and Integrons in the Saigon River

The relative abundances of ARGs and integrons were calculated by normalizing the absolute copy numbers of each target gene with that of the 16S rRNA gene and were used for studying the impact of tropical seasons on contamination of antibiotic resistance in the Saigon River. In general, almost all of the ARGs and integrons in the dry season were detected at significantly higher levels than those in the wet season (Figure 3, Table S4). The levels of *aac(6)-Ib-cr*, bla_{CTX}, bla_{SHV}, bla_{TEM}, *sul2*, *dfrA*, *intI2*, and *oqxB* in the dry season were found to be one to two orders of magnitude higher than those in the wet season, while the levels of *qnrA*, *qrnB*, *sul1*, *intI1*, and *intI3* in the dry season were observed to be less than one order of magnitude higher than those in the wet season. One of the major environmental factors contributing to the overall antibiotic resistance, including antibiotic-resistant bacteria and its genetic determinants (ARGs), is the presence of antimicrobial agents (e.g., antibiotics, heavy metals, biocides) in the aquatic environments, which are contaminated by both point and non-point pollutions from different anthropogenic activities [67]. The abundances of those selective agents for the development of antibiotic resistance in the Saigon River can be highly diluted in the wet season by the daily rains in Ho Chi Minh City.

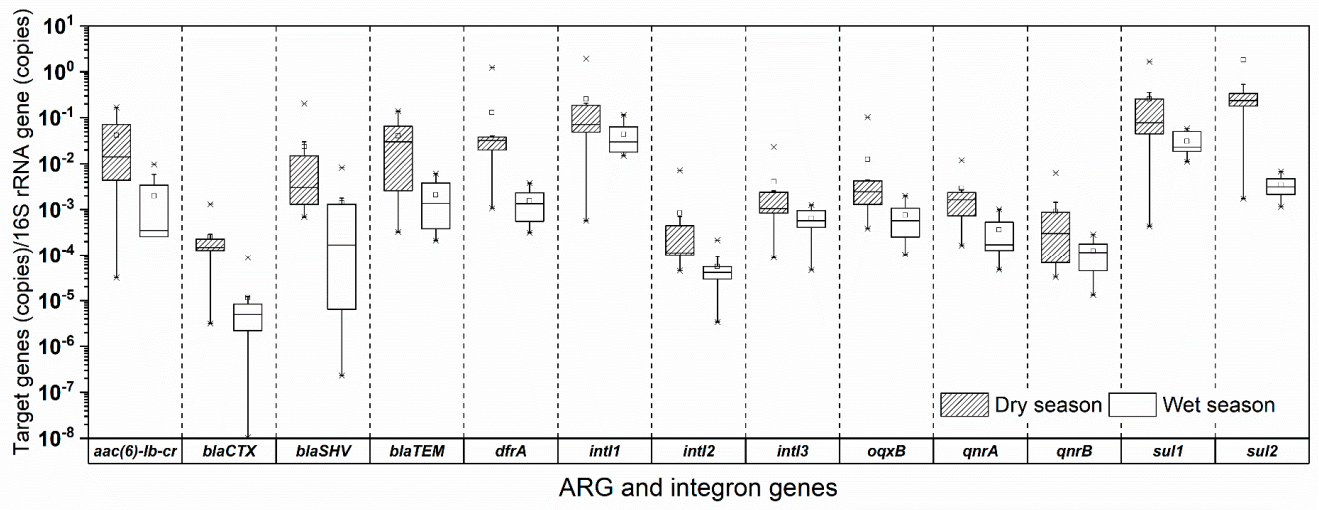

Figure 3. Seasonal variations in the relative abundance of ARGs and integrons in the Saigon River in the wet and dry seasons. The lower and upper "×" symbols in each box indicate the 1% and 99% percentiles of data points. The squares in the boxes indicate the mean values of the data points.

3.4. Correlation between the Water Quality Parameters and the Abundance of ARGs, Integrons, and 16S rRNA Gene

Both Pearson and Spearman analyses were conducted to investigate any correlations between all water quality parameters and the ARGs, integrons, and 16S rRNA genes in the Saigon River. The water quality parameters for all the sampling sites have been reported in our previous study [68]. Significant positive correlations were found between BOD5, pH, and temperature with all ARGs, integrons, and 16S rRNA gene samples (Tables S5 and S6). The pH and temperature values of all the surface water samples were detected in the range of 6.1–7.14 and 28.1–31.1 °C, respectively (data not shown), which were the neutral conditions for bacterial growth, while the high BOD5 values indicated organic contamination in the surface water. These strong correlations indicated that organic contamination could facilitate the growth of total bacteria, including the ARG/integron-carrying bacteria in surface water in the Saigon River. In addition, positive correlations between the nutrient values of NH_4^+, PO_4^{3-}, NO_2^- with *aac(6)-In-cr*, bla_{CTX}, bla_{TEM}, *qnrA*, *intI2*, *intI3*, and *oqxB*, as well as a negative correlation between DO value with *aac(6)-In-cr*, bla_{CTX}, *qnrA*, *sul1*, *intI1*, *intI2*, *intI3*, and *oqxB*, indicated that the nutrient contamination from the wastewaters of different human activities in the surface water could influence the prevalence of ARGs and integrons (Tables S5 and S6). The correlation analyses in this study also found the co-occurrence of the ARGs and integrons in the Saigon River, which is similar to the recent study on the Svartan River in Sweden, which reported a co-occurrent relationship for *aac(6)-Ib-cr*, $bla_{CTX\text{-}M\text{-}9}$, $bla_{OXA\text{-}10}$, $bla_{OXA\text{-}2}$, and *qnrS* in the surface samples impacted by the effluent of a wastewater treatment plant [15].

Multivariate analysis was performed with the data for all sampling sites in the Saigon River to explore any potential relationships among the water quality parameters, the target ARGs, integrons, and human activities (Figure 4). Two principal components were extracted and cumulatively explained 60.2% of the total variance of all parameters. The first principal component (PC1) explained 48.5% of the total variance and was slightly correlated with BOD5, where all ARGs, integron, and 16S rRNA genes had a positive loading value greater than 0.2, except for bla_{SHV}, and *qnrB*. The positive correlation between PC1 with the sampling sites at the industrial (I2, I3) and residential areas (R2, R3) indicated that the domestic and industrial wastewater may have important impacts on the contamination of organic matter, ARGs, integrons, and the total bacterial load in the surface water (Figure 4). The second principal component (PC2) described 12.7% of the total variance and was correlated with DO, conductivity, and salinity with positive loading values greater than 0.3. The negative correlation between PC2 with all the sampling sites suggested low water

quality in the aquatic environment after being contaminated by agricultural, industrial, and residential activities. Our correlation and multivariate analyses found a similar trend with a recent study on the prevalence of ARGs in a river in the Netherlands, for which some target ARGs, including *sul1*, *sul2*, and *intI1*, were highly correlated, while some ARGs, such as *ermB*, and *tetW*, were observed as positively correlated with NH4+ but negatively correlated with DO in the river water impacted with the effluent from a nearby wastewater treatment plant [14].

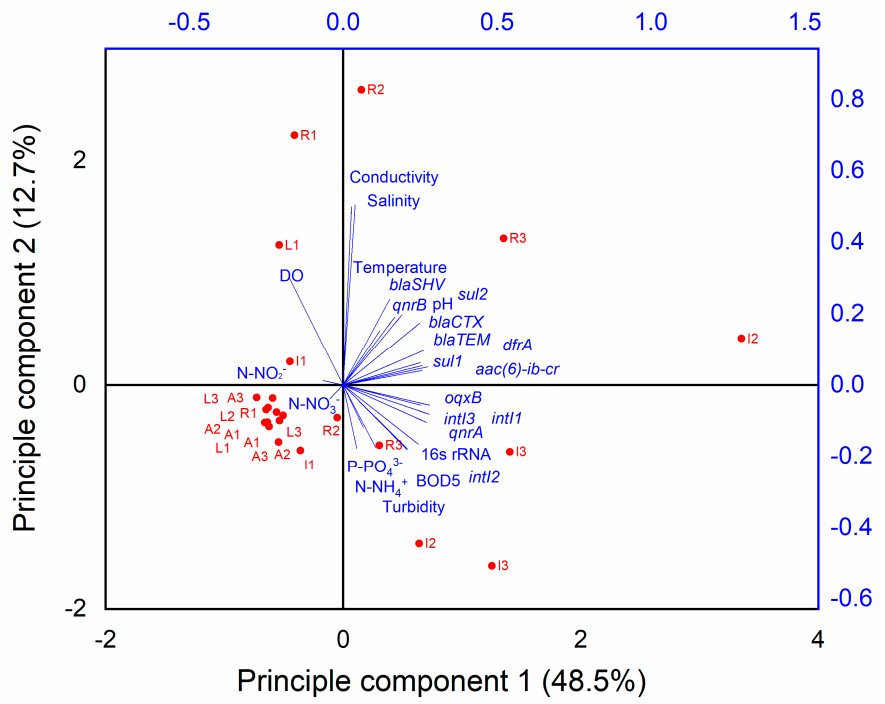

Figure 4. PCA analysis for all water quality parameters, ARGs, integrons, and 16S rRNA genes in all sampling sites along the Saigon River.

4. Conclusions

Antibiotic resistance genes and integrons are important genetic determinants and elements of antimicrobial resistant bacteria as they can migrate among different microorganisms via horizontal gene transfer. In this study, the high occurrences and wide distributions of 10 ARGs were observed in the surface water in the Saigon River with the following order: sulfamethoxazole-trimethoprim resistance genes (*sul1*, *sul2*, *dfrA)* > aminoglycosides resistance gene (*aac(6)-Ib-cr*) > β-lactam resistance genes (bla_{CTX-M}, bla_{SHV}, bla_{TEM}) > fluoroquinolones resistance gene (*qnrA*, *qnrB*). Among three genetic elements, integron class 1 (*intI1*) was widely detected in all sampling sites at the highest level (1.5×10^3–2.7×10^7 copies/mL) as compared to integron class 2 (*intI2*) (3–3.5×10^4 copies/mL) and integron class 3 (*intI3*) (3–1.9×10^5 copies/mL). Our results indicate that the anthropogenic activities in Ho Chi Minh City significantly contribute to the abundance of ARGs, integrons, and total bacteria load in the Saigon River, especially in the areas in proximity to industrial and residential areas. Sampling sites I2 and I3 were influenced by wastewater from both industrial and residential activities and demonstrated the highest prevalence of ARGs and integrons with about two to three orders of magnitude more than those in the less impacted sites, while the agricultural activities in Cu Chi district contributed less in terms of the overall abundance of ARGs and integrons in the Saigon River. Further research is needed to clarify if there are any synergistic effects in the surface water receiving both industrial and residential wastewaters on the proliferation and spread of antibiotic-resistant bacteria and genes in the aquatic environments considered here. Although the overall abundances of ARGs and integrons in the dry season were observed to be about one order

of magnitude higher than those in the wet season, increasing sampling with long-term monitoring is necessary to fully understand the exact mechanisms. In general, the correlation analysis here has suggested the co-occurrence of many ARGs and integrons, as well as significant correlations between these genes with BOD5, NH_4^+ (positive correlation), and DO (negative correlation) in the surface water influenced by different human activities. The levels of organic matter and nutrients in wastewaters from agricultural, industrial, and residential activities in a megacity may not only have negative impacts on the water quality of the surface water, but also contribute to the prevalence of ARGs, integrons, the total bacterial load, and the potential development and spread of antibiotic resistance in aquatic environments.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13162234/s1. Figure S1: Map of all the sampling sites along the Saigon River in Ho Chi Minh City. Table S1: Detailed information of RT-PCR primers for quantification of the ARGs, integrons, and 16S rRNA gene. Table S2: Limit of detection values for all target ARGs in qPCR. Table S3: The concentrations (copies/mL) of the ARGs, integrons, and 16S rRNA genes in the 12 sampling sites of the Saigon River as influenced by different anthropogenic activities such as less-impacted areas (L), agricultural (A), industrial (I), and residential (R) activities during the wet and dry seasons. <LOD: Less than the limit of detection, Table S4: The relative abundance of the ARGs and integrons in the 12 sampling sites of the Saigon River as influenced by different anthropogenic activities such as less-impacted areas (L), agricultural (A), industrial (I), and residential (R) activities during the wet and dry seasons. Table S5: The correlation coefficient values of Pearson and Spearman analyses between water quality parameters and the absolute abundance (copies/mL) of all ARGs, integrons, and 16S rRNA genes in the surface water. The correlation is considered significant only if the *p*-value was less than 0.05 and highlighted with bold format. Table S6: The *p*-values of the Pearson and Spearman analyses between water quality parameters and ARGs, integrons, and 16S rRNA genes in the surface water.

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