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Multiple Antibiotic Resistance in *Escherichia coli* Isolates from Fecal and Water Sources in Laguna Lake, Philippines

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Abstract: Due to the misuse and overuse of antibiotics, antibiotic residues accumulate in natural environments, leading to the development of antibiotic-resistant bacteria (ARBs). The presence of ARBs in bodies of water poses health hazards to the surrounding community. This study focused on Laguna Lake, the largest lake in the Philippines, which serves as a water source for agriculture and domestic purposes. We aimed to detect the presence of antibiotic-resistant *Escherichia coli* from the lake waters and potential reservoirs of resistance as well as determine the multiple antibiotic resistance (MAR) indices of the isolates. *E. coli* (n = 450) was isolated from fecal-associated samples (chicken, cow, pig, human, sewage) and water samples (sites in Laguna Lake and selected river tributaries). The isolates were subjected to an antibiotic resistance assay using VITEK 2®. Among the 16 antibiotics tested, the isolates exhibited varying resistance to 14, but complete susceptibility to amikacin and tigecycline was observed. Isolates were most frequently resistant to ampicillin (196/450, 43.6%). Among fecal-associated samples, chicken isolates exhibited the highest MAR index (0.174), whereas samples from Pila River exhibited the highest MAR index (0.152) among water samples. The results of this study demonstrate the presence of multidrug-resistant *E. coli* in samples collected around Laguna Lake and reveal fecal and sewage sources as potential reservoirs of ARBs in the water body. With this information, the public is urged to use antibiotics responsibly to help mitigate the spread of antibiotic resistance.

Keywords: antibiotic resistance; *Escherichia coli*; Laguna Lake; Philippines; multiple antibiotic resistance (MAR); water quality

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1. Introduction

One of the emerging pollutants in important water bodies used for agriculture are antibiotics that are used for the treatment of bacterial infections in humans and animals. They are also incorporated in commercial livestock and poultry feed at subtherapeutic doses for promotion of growth [1]. This practice is an example of antibiotic misuse, which increases the accumulation of antibiotics in natural environments. Various factors, such as the physicochemical properties, partition characteristics, and environmental behavior of the antibiotic [2], are involved in the degradation of the residues. Usually, partially metabolized antibiotics enter the environment via excretion, such as urine and stool, by humans and animals. In general, 50–80% of total original compounds are excreted as a mixture of compounds that are metabolically conjugated [3]. Antibiotic residues in the environment provide selective pressure that leads to the evolution of antibiotic resistance genes (ARGs). It is established that these ARGs can be shared among bacterial populations through mobile elements, such as plasmids, integrons, and transposons [4], fueling the development of antibiotic resistant bacteria (ARBs). The occurrence of ARBs in the environment might harm human health because of the possible feedback through various channels, such as drinking water, bathing water, and the food chain [5]. Furthermore, increased resistance of bacterial

strains to common antibiotics serves as a challenge to treat bacterial infections effectively [6]. Many studies focused on *Escherichia coli* because it acts as the primary reservoir of ARGs which can easily be transmissible to pathogenic bacteria [7]. Moreover, *E. coli* is an indicator microorganism that can be used in the assessment of the microbiological safety of water [8]. With the increasing occurrence of resistant strains, the burden of antibiotic resistance is a primary issue to address globally [9].

Laguna Lake, the largest inland body of water in the Philippines, is used for various purposes, such as aquaculture, generation of electricity, agriculture, industrial cooling, recreation, domestic water supply, and water source. Rapid urbanization and industrialization have resulted to the deterioration of the lake water. Various chemicals and microbiological pollutants from industrial and agricultural wastewater are present in the lake and pose health risks to the 16 million people residing near the lake's vicinity [10]. Therefore, monitoring these pollutants is imperative for maintaining the cleanliness and good water quality, which directly affect the health of the population residing near the lake. Despite its economic importance, there is a paucity of research on the occurrence of antibiotic resistance in the Laguna Lake watershed and potential reservoirs of ARGs. Previous studies have utilized traditional culture methods, such as disk diffusion and broth microdilution, which are highly tedious and time-consuming, thus hindering the adoption of these methods by monitoring agencies [11–13].

With this research gap, the main objective of this study was to investigate the occurrence of antibiotic resistance in 450 *E. coli* isolates from various sources, such as surface water from selected sites in Laguna Lake and some of its river tributaries and fecal-associated samples from sewage, humans, and livestock using an automated system for antibiotic susceptibility testing. Specifically, the study aimed to (1) determine the antibiogram profile of the isolates, (2) compute the multiple antibiotic resistance (MAR) indices of the isolates, and (3) determine co-occurrence of resistance to different antibiotics. To the best of our knowledge, this is the first comprehensive study investigating the occurrence of antibiotic resistance in surface waters of the lake and potential fecal reservoirs using VITEK 2®. This information can help in understanding the occurrence of antibiotic resistance in aquatic environments, and it can be used to draft policies for the improvement of water quality in Laguna Lake.

2. Materials and Methods

2.1. Study Site and Sample Collection

To properly investigate the occurrence of antibiotic-resistant *E. coli* isolates in Laguna Lake, samples were collected from the lake and its tributary rivers as well as point sources such as fecal-associated samples from chicken, cows, pigs, humans, and sewage situated along the lake's vicinity. All samples were collected on a monthly basis from July 2017 to July 2019.

The sampling sites for this study are presented in Figure 1. Water samples were collected from three lake stations and eight river tributaries of Laguna Lake, Philippines having a most probable number (MPN) greater than 5000 MPN/100 mL [14]. The selected lake stations were East Bay (LS2), West Bay (LS5), and South Bay (LS8). The selected river tributaries were as follows: Bagumbayan (TR1), Mangangate (TR2), and Tunasan (TR4) in the National Capital Region; Sapang Baho (TR3) in Rizal; Biñan (TR5), Pila (TR6), San Cristobal (TR7), and Sta. Rosa (TR8) in Laguna (Table 1). Sewage samples were collected from both influent and sludge generated from three different sewage treatment facilities near the lake. The wastewater effluents from these facilities are directly discharged into some of the lake's river tributaries. Animal fecal samples were collected monthly from piggeries, poultry farms, cattle farms, and pastures in the municipalities of Rizal and Laguna, representing the northern and southern areas of the sampling site, respectively. Human fecal samples were collected from healthy volunteers at the Rural Health Units of Cupang and Pila on the month of July 2019.

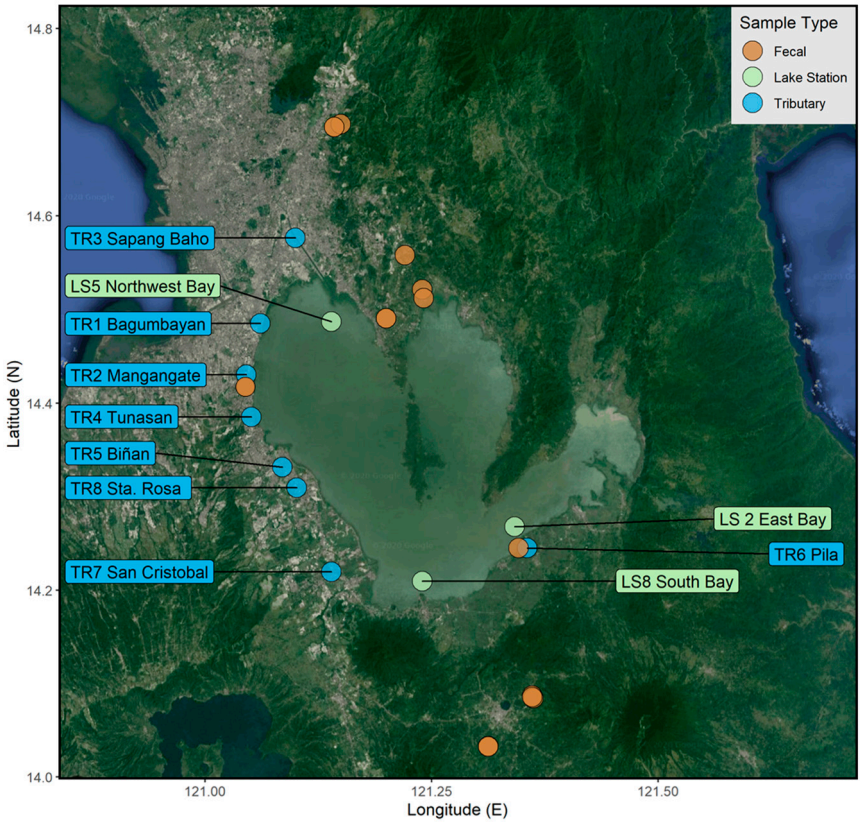

Figure 1. Sites for the collection of various sample types for the assessment of antibiotic resistance in Laguna Lake.

Table 1. List of sampling sites, locations, and number of *E. coli* isolates used in this study.

Sample Type	Name of Site	Latitude	Longitude	*E. coli* Isolates
Lake Stations	East Bay (LS2)	14.267958	121.341787	28
	West Bay (LS5)	14.486943	121.139375	28
	South Bay (LS8)	14.209662	121.239543	28
Tributaries	Bagumbayan River (TR1)	14.484974	121.060856	28
	Mangangate River (TR2)	14.430198	121.045141	28
	Sapang Baho River (TR3)	14.576433	121.099633	28
	Tunasan River (TR4)	14.385343	121.050977	28
	Biñan River (TR5)	14.3317	121.085	28
	Pila River (TR6)	14.2453	121.3554	28
	San Cristobal River (TR7)	14.2197	121.1392	28
	Sta. Rosa River (TR8)	14.3094	121.101	28
Fecal	Cattle	-	-	28
	Poultry	-	-	28
	Swine	-	-	28
	Human	-	-	28
	Sewage	-	-	30
Total				**450**

For water and sewage samples, 1 L was obtained and transferred to a sterile wide-mouth Nalgene water bottle (ThermoFisher Scientific, Rochester, NY, USA). Fecal samples were collected using a spatula and then transferred to stool containers. All samples were stored on ice and transported to the Pathogen-Host-Environment Interactions Research Laboratory, University of the Philippines Diliman for processing within 24 h of sample collection.

2.2. Sample Types and Origins of *E. coli* Isolates

The isolation of 1408 thermotolerant *E. coli* was described elsewhere [15,16]. A subset of 450 *E. coli* isolates were subjected to antibiotic resistance profiling against 16 antibiotics using VITEK 2®. The types and origins of the *E. coli* isolates are listed on Table 1. The majority (n = 308) of the isolates were obtained from environmental (i.e., surface water) samples. Specifically, 28 isolates were studied from each of the 11 water sampling points, including three stations in Laguna Lake and eight points in various river tributaries. The remaining isolates (n = 142) were obtained from fecal-associated samples, with 28 samples collected from each of four host sources (chicken, cow, pig, and human). Lastly, 30 isolates were obtained from sewage samples.

2.3. Antibiotic Resistance Assay (ARA) Using VITEK 2®

In total, 450 thermotolerant *E. coli* isolates were tested for this study. The isolates were cultured in 1 mL of sterile tryptic soy broth (TSB; BD Difco, East Rutherford, NJ, USA) at 37 °C for 18–24 h. To further ensure the identity of the isolates, the cultures were subcultured on eosin methylene blue agar (EMBA; BD Difco, East Rutherford, NJ, USA) plates at 37 °C for 18–24 h. Isolates that produced green metallic colonies were considered as positive for *E. coli*. To isolate individual colonies, they were further subcultured on tryptic soy agar (TSA; BD Difco, East Rutherford, NJ, USA) plates using the three-way streak technique. The cultures were incubated at 37 °C for 18–24 h in preparation for the ARA using VITEK 2® (BioMérieux, Salt Lake City, UT, USA).

The protocol used for the ARA was based on the manufacturer's recommendations, as described previously [17]. Each card tested against 16 antibiotics, specifically amikacin (AMK), ampicillin (AMP), ampicillin–sulbactam (SAM), aztreonam (ATM), cefazolin (CFZ), cefepime (FEP), ceftriaxone (CRO), ciprofloxacin (CIP), ertapenem (ETP), gentamicin (GEN), meropenem (MEM), nitrofurantoin (NIT), piperacillin–tazobactam (TZP), tigecycline (TGC), tobramycin (TOB), and trimethoprim–sulfamethoxazole (SXT). The phenotypic profiles of the isolates for every antibiotic were recorded as resistant, intermediate, or susceptible based on the updated breakpoints from the Clinical Laboratory Standards Institute. Resistant and intermediate profiles were subsumed as resistance to an antibiotic for the data analyses.

2.4. MAR Indexing and Statistical Analysis

Generation of bar graphs and Chi-square tests were performed using Microsoft Excel. $p < 0.05$ denoted statistical significance. MAR indices were calculated using the formula reported by Blasco et al. [18] as follows:

$$\text{MAR Index} = a/b,$$

where a is the number of antibiotics that the isolate shows resistance against, and b is the total number of antibiotics tested against the isolate. MAR indices per sample type were computed by averaging the MAR indices of the isolates belonging to each category.

Associations between different antibiotic resistance phenotypes of *E. coli* isolates were assessed using Fisher's exact test with Bonferroni correction for multiple testing. Amikacin, tigecycline, cefepime, meropenem, and ertapenem were excluded from this analysis because fewer than 1% of samples exhibited resistance to these antibiotics.

3. Results

3.1. Antibiogram Profiles

The antibiogram profiles (resistant, intermediate, or susceptible) of the 450 isolates against 16 antibiotics are presented in Table 2. Antibiotic resistance was observed in both fecal-associated and environmental samples. All 450 isolates exhibited complete susceptibility to amikacin and tigecycline, but varying resistance to 14 of the antibiotics. For brevity, succeeding data analyses subsumed the occurrences of resistant and intermediate phenotypic profiles as antibiotic resistance. The highest rates of resistance were observed in

ampicillin (AMP; 43.6%), ampicillin/sulbactam (SAM; 33.1%), trimethoprim (SXT; 28.2%), cefazolin (CFZ; 12.9%), ciprofloxacin (CIP; 7.8%), and gentamicin (GEN; 4.2%).

Table 2. Antibiogram of *E. coli* isolates (n = 450) displaying specific phenotype profile (resistant, intermediate, or susceptible) against 16 types of antibiotics.

Name of Antibiotic	Resistant *n* (%)	Intermediate *n* (%)	Susceptible *n* (%)
Ampicillin	178 (39.6)	18 (4.0)	254 (56.4)
Trimethoprim/Sulfamethoxazole	127 (28.2)	0	323 (71.8)
Ampicillin/Sulbactam	52 (11.5)	97 (21.6)	301 (66.9)
Cefazolin	52 (11.6)	2 (0.4)	396 (88.0)
Ciprofloxacin	29 (6.4)	6 (1.3)	415 (92.2)
Gentamicin	18 (4.0)	1 (0.2)	431 (95.8)
Tobramycin	6 (1.3)	10 (2.2)	434 (96.4)
Nitrofurantoin	6 (1.3)	39 (8.7)	405 (90.0)
Ceftriaxone	5 (1.1)	1 (0.2)	444 (98.7)
Aztreonam	5 (1.1)	0	445 (98.9)
Cefepime	4 (0.9)	0	446 (99.1)
Piperacillin/Tazobactam	2 (0.4)	3 (0.7)	445 (98.9)
Ertapenem	2 (0.4)	0	448 (99.6)
Meropenem	2 (0.4)	0	448 (99.6)
Amikacin	0	0	450 (100.0)
Tigecycline	0	0	450 (100.0)

The occurrence of antibiotic resistance in environmental samples against the top six antibiotics is presented in Table 3. Results show that *E. coli* isolates from lake and river samples were mainly resistant to AMP (28.6–71.4%), SXT (14.3–50%), SAM (14.3–46.4%), CFZ (3.6–46.4%), and CIP (3.6–28.6%). GEN resistance (3.6–7.1%) was only observed in river samples. The highest occurrence of AMP, SXT, and SAM was recorded in Pila River, Laguna (TR6).

Table 3. Number (and percentage) of environmental isolates resistant to six antibiotics by sample site (n = 308).

	Number (Percentage) of Antibiotic Resistant *E. coli* Isolates					
Site	**AMP**	**SXT**	**SAM**	**CFZ**	**CIP**	**GEN**
LS2	10 (35.7)	7 (25.0)	8 (28.6)	6 (21.4)	0	0
LS5	11 (39.3)	4 (14.3)	8 (28.6)	13 (46.4)	0	0
LS8	9 (32.1)	5 (17.9)	4 (14.3)	7 (25.0)	1 (3.6)	0
TR1	8 (28.6)	5 (17.9)	7 (25.0)	0	1 (3.6)	0
TR2	11 (39.3)	6 (21.4)	7 (25.0)	3 (10.7)	2 (7.1)	1 (3.6)
TR3	10 (35.7)	8 (28.6)	10 (35.7)	3 (10.7)	8 (28.6)	2 (7.1)
TR4	11 (39.3)	4 (14.3)	8 (28.6)	3 (10.7)	2 (7.1)	0
TR5	19 (67.9)	8 (28.6)	11 (39.3)	8 (28.6)	3 (10.7)	1 (3.6)
TR6	20 (71.4)	14 (50.0)	13 (46.4)	6 (21.4)	0	2 (7.1)
TR7	15 (53.6)	10 (35.7)	13 (46.4)	1 (3.6)	2 (7.1)	2 (7.1)
TR8	13 (46.4)	11 (39.3)	11 (39.3)	1 (3.6)	1 (3.6)	0
Total	137 (44.5)	82 (26.6)	100 (32.5)	51 (16.5)	20 (6.5)	8 (2.6)

LS2, East Bay; LS5, West Bay; LS8, South Bay; TR1, Bagumbayan; TR2, Mangangate; TR3, Sapang Baho; TR4, Tunasan; TR5, Biñan; TR6, Pila; TR7, San Cristobal; TR8, Sta. Rosa; AMP, ampicillin; SXT, trimethoprim–sulfamethoxazole; SAM, ampicillin–sulbactam; CFZ, cefazolin; CIP, ciprofloxacin; GEN, gentamicin.

The occurrence of antibiotic resistance in fecal-associated samples is shown in Table 4. Chicken fecal samples displayed the highest resistance to AMP, SXT, SAM, CIP, and GEN but not CFZ. All isolates that exhibited resistance to cefazolin were obtained from human fecal or sewage samples. Isolates from human samples exhibited varying degrees of resistance to all six antibiotics. Furthermore, isolates from sewage samples exhibited resistance to all antibiotics excluding CIP. The rates of resistance to AMP, SXT, SAM, and CIP differed among fecal-associated samples based on the Chi-square test results ($p < 0.05$).

Table 4. Prevalence of antibiotic resistance in *E. coli* isolates from fecal-associated samples ($n = 142$).

	Number (Percentage) of Antibiotic Resistant *E. coli* Isolates					
Fecal Source	**AMP**	**SXT**	**SAM**	**CFZ**	**CIP**	**GEN**
Chicken	19 (67.9)	19 (67.9)	16 (57.1)	0	12 (42.9)	3 (10.7)
Cow	9 (32.1)	6 (21.4)	9 (32.1)	0	0	2 (7.1)
Pig	12 (42.9)	10 (35.7)	11 (39.3)	0	1 (3.6)	2 (7.1)
Human	10 (35.7)	2 (7.1)	6 (21.4)	2 (7.1)	2 (7.1)	2 (7.1)
Sewage	9 (30)	8 (26.7)	7 (23.3)	1 (3.3)	0	2 (6.7)
Total	59 (41.5)	45 (31.7)	49 (34.5)	3 (2.1)	15 (10.6)	11 (7.7)

AMP, ampicillin; SXT, trimethoprim–sulfamethoxazole; SAM, ampicillin–sulbactam; CFZ, cefazolin; CIP, ciprofloxacin; GEN, gentamicin.

A comparison of the occurrence of antibiotic resistance between fecal-associated and environmental isolates is presented in Figure 2. Fecal isolates exhibited higher rates of resistance to SXT (33.0%), SAM (37.5%), CIP (13.24%), and GEN (7.8%). Based on the Chi-square test results ($p < 0.05$), the rates of resistance to CFZ and GEN significantly differed between fecal and environmental samples.

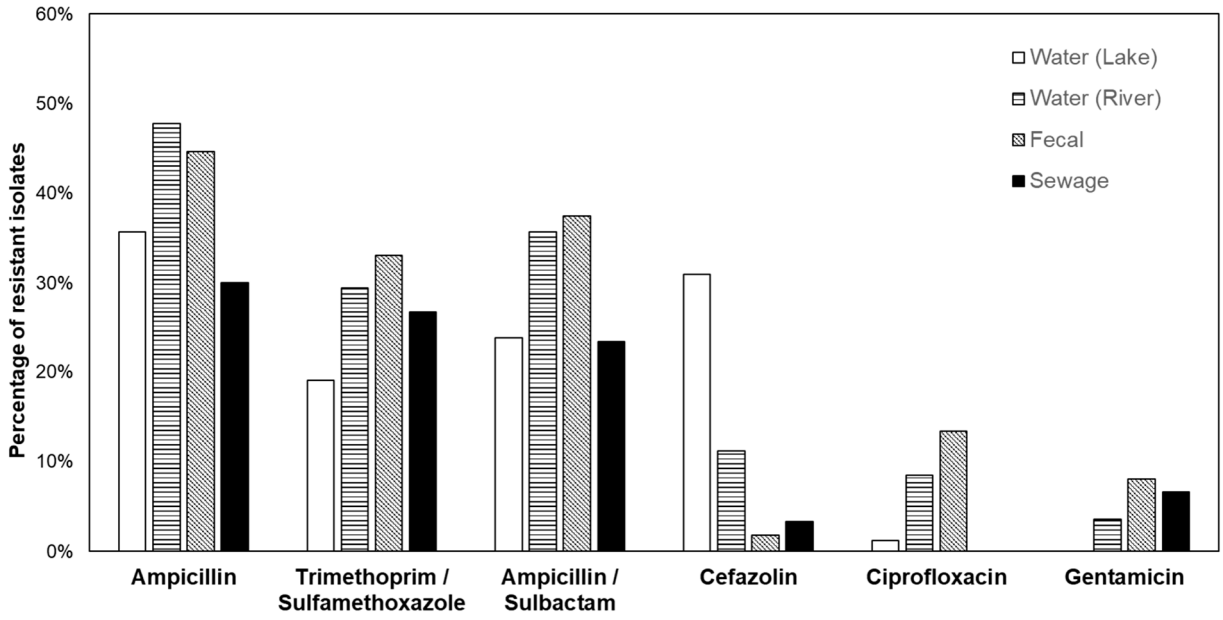

Figure 2. Occurrence of antibiotic resistance between fecal-associated and environmental isolates.

3.2. MAR Index and Co-Occurence of Antibiotic Resistance

Figure 3 presents the average MAR indices computed for each sample type, which ranged from 0.058 to 0.174. Among all samples, chicken isolates had the highest MAR value (0.174), followed by Pila River (TR6-0.152) and Biñan River (TR5-0.136).

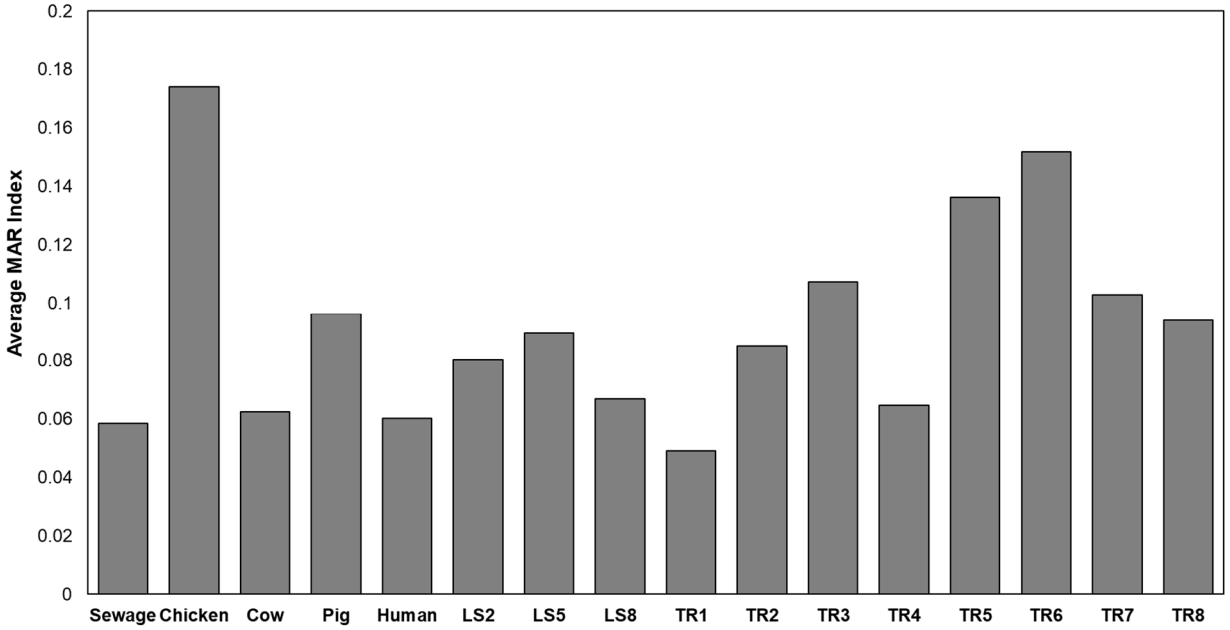

Figure 3. Average MAR indices of sewage, fecal, lake and tributary river samples. LS2, East Bay; LS5, West Bay; LS8, South Bay; TR1, Bagumbayan; TR2, Mangangate; TR3, Sapang Baho; TR4, Tunasan; TR5, Biñan; TR6, Pila; TR7, San Cristobal; TR8, Sta. Rosa; AMP, ampicillin; SXT, trimethoprim–sulfamethoxazole; SAM, ampicillin–sulbactam; CFZ, cefazolin; CIP, ciprofloxacin; GEN, gentamicin.

The co-occurrence of resistance to different antibiotics was tested using Fisher's exact test (Table 5). Aside from co-resistance exhibited by antibiotics belonging to the same class, penicillin resistance was associated with resistance to aminoglycosides, fluoroquinolones, nitrofurantoin, and trimethoprim (all $p < 0.001$).

Table 5. Tests for associations between antibiotic resistance phenotypes in *E. coli* isolates. Symbols represent levels of significance by *p*-values.

Antibiotic	β-Lactams						Non-β-Lactams				
	Penicillin			Cephalo-Sporin		Mono-Bactam	Aminoglycoside		Fluoroqui-Nolone	Others	
	AMP	**SAM**	**TZP**	**CFZ**	**CRO**	**ATM**	**GEN**	**TOB**	**CIP**	**NIT**	**SXT**
AMP											
SAM	***										
TZP	-	-									
CFZ	***	-	-								
CRO	-	-	-	***							
ATM	-	-	-	***	***						
GEN	***	***	-	-	-	-					
TOB	***	***	-	-	-	-	***				
CIP	**	***	-	-	-	-	***	***			
NIT	***	***	-	***	-	-	-	-	-		
TMP	***	***	-	-	-	-	***	***	***	-	

AMP, ampicillin; SAM, ampicillin–sulbactam; TZP, piperacillin–tazobactam; CFZ, cefazolin; CRO; ceftriaxone; ATM, aztreonam; GEN; gentamicin; TOB; tobramycin; CIP, ciprofloxacin; NIT, nitrofurantoin; SXT, trimethoprim–sulfamethoxazole; -: $p \geq 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

4. Discussion

Waterborne diseases caused by pathogens such as *E. coli*, *Salmonella*, *Vibrio*, and *Shigella* result in 5 million deaths annually worldwide [6]. Adding to this growing concern is the occurrence of ARBs and ARGs in aquatic environments [19–21], leading to the increase

in global antibiotic resistance among pathogenic bacteria. It is even more alarming when ARBs and ARGs reach an aquatic environment that is used for irrigation, aquaculture, farming, and domestic purposes, such as the case of Laguna Lake [10]. Vital et al. [13], established the presence of multidrug resistant *E. coli* in nearby surface waters used for irrigation, as well as soil and vegetables in urban farms in Metro Manila. According to their report, 34% of water samples were resistant to ampicillin, which may suggest horizontal transmission of ARBs from water to soil and vegetables through irrigation [13]. In this study, we report the resistance rates of *E. coli* isolates to ampicillin at 39.6% (Table 1), similar to the results of other studies [11,22]. The occurrence of ampicillin-resistant *E. coli* in water samples collected in similar sites from Laguna Lake was reported by Salvador-Membreve and Rivera [11]. Positive detection of ARGs such as *strA*, bla_{SHV}, bla_{TEM}, and *tetA* from the same isolates suggests that ARGs contribute to the presence of ARBs in Laguna Lake.

In addition, the observation of ampicillin–sulbactam resistance (11.5%) corroborates a report by Chen et al. [4]. The rates of trimethoprim–sulfamethoxazole (28.2%) and ciprofloxacin (6.4%) resistance were also similar to previous findings [22,23]. By contrast, complete susceptibility of resistant isolates to ciprofloxacin was reported by Li et al. [24]. The low rate of gentamicin-resistant *E. coli* was also in line with previous research [22,24]. Conversely, Talukdar et al. [23] reported a lower rate (1%) of gentamicin resistance. The contrasting results may be attributable to the difference in geographical locations and antimicrobial usage and practices. Another possible mechanism of resistance to antibiotics may be due to microbial adaptation to heavy metals. Tolerance to heavy metals such as cadmium and lead have been linked to an increase in antibiotic resistance [12]. In a study by Ntabugi et al. [12], heavy-metal-tolerant bacteria from San Cristobal River (TR7 in this study) had a 66–75% resistance rate against ampicillin and 50–53% against ciprofloxacin. In contrast, we report the resistance rates in isolates from San Cristobal River to be 53.6% against ampicillin and 7.1% against ciprofloxacin. It is possible that plasmids conferring metal tolerance may also synergistically increase antibiotic resistance [12].

4.1. High-Risk Sources of Fecal Contamination

The Southeast Asian region has been reported as a major reservoir of antibiotic resistance as well as a center of emerging infectious diseases [25–27]. In developing countries such as the Philippines, misuse of antibiotics is specifically problematic because antibiotics are widely used in both humans and animals. In animal husbandry and aquaculture, subtherapeutic doses of antibiotics are administered as a preventive measure against the onset of diseases [28,29] opposed to the treatment of bacterial infections. Similar problems have occurred in developed countries such as the United States. According to the US Food and Drug Administration [29], 80% of the total antimicrobial consumption in some countries is linked to animals raised for human consumption. Alarmingly, the global rate of antibiotics use is two-fold higher in animals than in humans [30]. Thus, it is not surprising that fecal material from farm animals harbors ARBs and ARGs, and it is intuitive that *E. coli* isolates of animal origin exhibited higher rates of antibiotic resistance than isolates of human origin [31]. This could explain why *E. coli* isolates derived from sewage and human fecal samples displayed lower rates of resistance than isolates from the fecal samples of farm animals.

Aminopenicillins, gentamicin, and trimethoprim/sulfamethoxazole are antibiotics typically used in livestock production in the Philippines [32]. In this study, fecal samples exhibited the highest percentage of gentamicin- (7.7%), trimethoprim- (31.7%), and ampicillin/sulbactam-resistant (34.5%) isolates among different sample types. This demonstrates that livestock waste may be potential reservoirs of ARGs and ARBs, which may eventually contaminate surface waters through runoffs. Fecal contamination and ARGs have been previously shown to be correlated in urban streams [33], which emphasize the role of sewage and agricultural contamination in the spread of antibiotic resistance in water bodies [34,35].

4.2. Multidrug Resistance in E. coli

By definition, multidrug resistance describes the resistance of bacterial strains to three or more antibiotic categories [36]. The results of this study revealed that among the samples, the highest MAR indices were recorded for chicken fecal isolates and water samples from Pila River (Figure 3). This may be attributable to the high use of antibiotics in the poultry industry for growth promotion and disease prevention instead of infection treatment [37]. Additionally, chicken fecal contamination in Pila River was previously associated with the numerous chicken and duck farms in the area [16,38].

In general, samples with a MAR index exceeding 0.2 carry a high risk of contamination [39]. Although no MAR indices from this study exceeded this threshold, the findings call attention to the indiscriminate use of antibiotics in both humans and animals.

In addition to multidrug resistance, we found significant associations between different resistance phenotypes (Table 5). Cross-resistance and cross-sensitivity in *E. coli* from clinical isolates stem from simultaneous exposure to several drugs, resulting in co-selection [40]. Several ARGs are known to occur in the same plasmid or mobile genetic elements, which would confer the associated resistance phenotypes in the organism [4,41]. Another possible explanation is that resistance in these isolates was conferred by genes and other determinants that cover a wide range of antibiotics [42]. Analysis of ARGs in *E. coli* from Laguna Lake previously revealed an association of β-lactamase genes (*bla*) with tetracycline resistance genes (*tetA*) [11]. This further supports our findings of multidrug resistance in *E. coli* from Laguna Lake.

Overall, the findings of this study in terms of antibiotic resistance are in line with other reports. This is alarming because the presence of ARBs in aquatic environments can increase antibiotic resistance in bacteria other than *E. coli*, such as *Salmonella* and *Campylobacter.* The regular and continuous use of antibiotics in aquaculture is one of the main factors driving the increasing incidence of ARBs in aquatic environments [43]. Furthermore, *E. coli* is a well-documented bacterium that has antibiotic-resistant gene pools, which can be transferred into the normal flora of humans and animals through horizontal gene transfer. These ARG pools exert strong selective pressure, which promotes the emergence and spread of resistance in *E. coli* strains. Eventually, this may render antibiotics ineffective.

The occurrence of these ARBs and ARGs in aquatic environments poses a threat to the fields of public health, drug discovery, and environmental management. Thus, the continuous monitoring of antimicrobial resistance in our water systems is recommended. In this study, we utilized an automated system for antibiotic susceptibility testing that is less time-consuming and more efficient compared to traditional culture methods such as disk diffusion and broth microdilution assays. We demonstrate its potential to be adapted in current monitoring practices conducted by environmental agencies. Furthermore, these results highlight the importance of mitigating and regulating the use of antibiotics, particularly in agriculture and clinical settings. We hope this can encourage regulatory bodies to draft appropriate policies for antimicrobial resistance surveillance and prevention.

5. Conclusions

The presence of antibiotic-resistant *E. coli* in fecal and water samples from Laguna Lake, Philippines indicates the presence of fecal contamination in this body of water. Our results revealed a high occurrence of resistance to ampicillin, ampicillin–sulbactam, trimethoprim–sulfamethoxazole, cefazolin, ciprofloxacin, and gentamicin in *E. coli* isolates. The presence of multiple antibiotic-resistant phenotypes suggests the increasing occurrence of antibiotic resistance in aquatic environments. In particular, isolates from chicken fecal samples (0.17) and Pila River samples (0.15) exhibited the highest MAR indices, which may be attributable to the heavy use of antibiotics in the poultry industry. Finally, statistical analyses of the results using Fisher's exact test identified significant associations in resistance between different antibiotics. ARGs are known to occur in the same plasmid or mobile genetic elements, which may cause resistance to multiple antibiotics.

The increasing occurrence of antibiotic resistance in aquatic environments should raise public health concerns, especially because Laguna Lake is used for irrigation, aquaculture, and industrial purposes. The high rate of antibiotic resistance from the lake and fecal point sources suggests that they may be important reservoirs of multidrug-resistant bacteria. Unless antibiotic use in humans and animals is controlled and strictly regulated, antibiotic resistance will continue to spread. Thus, the public is urged to use antibiotics responsibly to help mitigate the spread of antibiotic resistance. Furthermore, it is suggested to develop surveillance programs to monitor the presence of ARBs and ARGs in Laguna Lake and other major bodies of water in the country.

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