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Original Article

Antimicrobial resistance profiles of presumptive *Escherichia coli* isolated from wild-caught tilapia (*Oreochromis* sp.) in Iloilo River, Iloilo, Philippines

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Abstract: Antimicrobial resistance (AMR) remains a growing global threat to human, animal, and environmental health. Aquatic environments have become important reservoirs and transmission pathways for antimicrobial-resistant bacteria due to increasing anthropogenic pressures. *Escherichia coli*, a common commensal bacterium in humans and animals, is widely used as an indicator organism for AMR surveillance and poses environmental and public health concerns when resistant strains are present. However, wild and apparently healthy fish remain underrepresented in AMR research. In this study, apparently healthy wild tilapia (*Oreochromis* sp.) were collected from the Iloilo River. Gut, kidney, liver, and spleen samples were plated to obtain bacterial isolates. Presumptively identified *E. coli* based on biochemical testing were subjected to antimicrobial susceptibility testing against 11 antibiotics representing seven classes. The highest resistance rates were observed for ampicillin, cefotaxime, and tetracycline. The average multiple antibiotic resistance (MAR) index was 0.09, with four isolates exhibiting multidrug resistance and six identified as potential extended-spectrum β-lactamase (ESBL) producers. Although the MAR index indicates relatively low antimicrobial selective pressure, the detection of multidrug-resistant *E. coli* in wild, apparently healthy fish underscores their potential as sentinels of antimicrobial resistance in the river system. Further studies are warranted to characterize the genetic determinants and resistance mechanisms contributing to environmental AMR.

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Introduction

Antimicrobial resistance (AMR) persists as a global threat to human, animal, and environmental health (Salam et al., 2023). Within the One Health framework, AMR is understood as a consequence of the interconnectedness of these domains (Al-Khalaifah et al., 2025). In particular, aquatic ecosystems play a critical role in the persistence and transmission of antimicrobial-resistant bacteria due to their close interaction with human populations (Popoola et al., 2025). Rivers and estuarine systems receive antibiotic residues and resistant microorganisms from urban runoff, wastewater discharges, agricultural activities, aquaculture effluents, and other anthropogenic sources (Meradji et al., 2025; Naznine et al., 2025).

Among bacterial species investigated for antimicrobial resistance, *Escherichia coli* has been widely utilized as an indicator organism (Poirel et al., 2018). *Escherichia coli* is a commensal bacterium commonly found in the intestinal tract of humans and animals, and its presence in the environment is widely used as an indicator of fecal contamination, particularly in water quality monitoring (Puvača and de Llanos Frutos, 2021; Boubacar Moussa et al., 2025). As such, it reflects the extent of anthropogenic influence in aquatic systems. Moreover, *E. coli* readily acquires and disseminates antimicrobial resistance genes, making it a useful proxy for resistance dynamics in both environmental and public health surveillance (Poirel et al., 2018; Bengtsson-Palme et al., 2023; Thaotumpitak et al., 2024). Importantly, *E.*

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coli can harbor antimicrobial resistance in the absence of clinical disease (Ramos et al., 2020). In line with this, the Philippine National Action Plan on Antimicrobial Resistance (2024-2028) identifies *E. coli* as a priority organism for active AMR surveillance in both healthy and diseased fish (Hart et al., 2023; Inter-Agency Committee on Antimicrobial Resistance, 2024).

Escherichia coli enters aquatic environments through various human activities and can persist in water bodies by associating with sediments and biota (Okeke et al., 2022). In fish, *E. coli* may be present on external surfaces or within the gastrointestinal tract (Guzmán et al., 2004; Cortés-Sánchez et al., 2025). Fish-associated *E. coli* can harbor antimicrobial resistance even when the host shows no clinical signs of disease (Dias et al., 2024). *Oreochromis* sp. (tilapia) are highly adaptable fish commonly found in riverine and can tolerate estuarine systems (Gu et al., 2018; Lutz, 2024). Due to their wide distribution, tolerance to varying environmental conditions, and importance as a food source, tilapia is frequently used in food safety and environmental monitoring studies (Eltholth et al., 2018; Páez-Osuna et al., 2024). While many AMR studies have focused on diseased fish, clinically normal fish can also harbor antimicrobial-resistant bacteria, underscoring their role as silent sentinels of environmental AMR (Ballash et al., 2022).

Rivers serve as interfaces between anthropogenic activities and natural ecosystems, making them important environments for the selection and dissemination of antimicrobial resistance (Nnadozie and Odume, 2019; Grenni, 2022). However, most AMR studies in tilapia have focused on aquaculture settings or market samples, with comparatively fewer investigations targeting wild riverine populations. Baseline data on antimicrobial resistance in wild fish, therefore, remain limited. The Iloilo River is a major urban waterway that traverses residential, commercial, industrial, agricultural, and aquaculture areas in Iloilo City, located in the Central Philippines, supporting local fisheries and livelihoods (Environmental Management Bureau, 2022). Given these anthropogenic pressures, the river represents a relevant site for AMR surveillance. Despite this, published data on antimicrobial resistance in wild fish from the Iloilo River are scarce. Wild, apparently healthy fish remain underrepresented in AMR studies, despite their potential role as asymptomatic carriers of resistant bacteria. Hence, the objective of this study was to characterize the antimicrobial resistance profiles of *E. coli* isolated from wild-caught, apparently healthy tilapia collected from the Iloilo River.

Materials and Methods

Sampling collection: Apparently healthy tilapia (n=27) were collected from three identified sites in the Iloilo River: Site 1 (10°41'44"N, 122°31'19"E), Site 2 (10°42'00"N, 122°32'34"E), and Site 3 (10°42'04"N, 122°33'10"E) (Fig. 1). Apparently healthy fish were qualified as those exhibiting normal body conformation, including intact scales and fins, clear eyes, and absence of visible ulceration, discolorations, or ectoparasites. Samples were transported in sterile plastic bags containing river water to the Fisheries Integrated Laboratory Section of the Bureau of Fisheries and Aquatic Resources, Region 6, for processing and microbiological analysis. Necessary permits were obtained from the relevant offices before collection.

Bacterial isolation: Each fish was aseptically dissected to obtain gut, spleen, kidney, and liver samples, which were briefly placed in sterile normal saline solution (NSS) before plating on eosin methylene blue (EMB) agar and subsequently transferred to *E. coli* (EC) broth. Tubes that tested positive after incubation were plated on EMB plates. Isolated colonies exhibiting a metallic green sheen morphology were stored on nutrient agar slants. For presumptive identification of *E. coli,* the isolates were subjected to the following biochemical tests: indole, methyl red, Voges-Proskauer, and citrate utilization (IMVC) (Dewi et al., 2022; Thaotumpitak et al., 2022; Basnayake et al., 2023). Those that aligned with the expected biochemical profile for *E. coli*: positive for indole and methyl red, and negative for Vogues-

Table 1. Distribution of *Escherichia coli* isolates from the different organs of apparently healthy wild tilapia sourced from the Iloilo River.

Organ	Isolated Colonies	Presumptive *E. coli*
Gut	40	20
Kidney/Liver	9	4
Spleen	7	6
Total	56	30

Figure 1. Map of the sampling sites along the Iloilo River: Site 1 (10°41'44"N 122°31'19"E), Site 2 (10°42'00"N 122°32'34"E), and Site 3 (10°42'04"N 122°33'10"E). Image modified from Google Maps (2026).

Proskauer and citrate, were subjected to antimicrobial susceptibility testing using the disc diffusion method.

Antimicrobial susceptibility testing: The isolates were tested against 11 antibiotics from seven classes: amoxicillin-clavulanate (30 ug), ceftazidime (30 ug), meropenem (10 ug), tetracycline (30 ug), ciprofloxacin (5 ug), co-trimoxazole (25 ug), amikacin (30 ug), imipenem (10 ug), cefotaxime (30 ug), ampicillin (10 ug), and gentamicin (10 ug). Zone diameters were measured after incubation and compared with the Clinical and Laboratory Standards Institute (CLSI) (2025) standard breakpoints to classify each isolate as susceptible or resistant to each antibiotic tested. The multiple antibiotic resistance (MAR) index for each isolate was calculated as the ratio of the number of antibiotics to which the isolate was resistant to the total number of antibiotics tested. An isolate was classified as: multidrug-resistant (MDR) if it was resistant to antibiotics from at least three classes, antimicrobial-resistant (AR) if it was resistant to at least one antibiotic, and fully-susceptible (FS) if it was not resistant to any antibiotic. In addition, isolates resistant to at least one cephalosporin (cefotaxime or ceftazidime) were classified as potential extended-spectrum beta-lactamase (ESBL) producers.

Results

Fifty-six (56) isolates showed a green metallic sheen morphology on selective culture media, with 40 isolated from the tilapia gut, 9 from the kidney and liver, and 7 from the spleen (Table 1). Thirty (30) were presumptively identified as *E. coli* based on biochemical tests (Table 2). These isolates were subjected to antimicrobial susceptibility testing.

As shown in Figure 2, the majority of isolates were susceptible to the antibiotics tested. All isolates were sensitive to imipenem. Relatively higher resistance rates were observed for ampicillin (33.3%), tetracycline (16.7%), and cefotaxime (16.7%). Resistance was also noted for ceftazidime (10.0%) and ciprofloxacin (10.0%). Additionally, some isolates displayed intermediate resistance to tetracycline, ciprofloxacin, amikacin, and gentamicin.

As shown in Table 3, of the 30 isolates, four were classified as multidrug-resistant (MDR) with the

Table 2. Biochemical characterization of the bacterial isolates recovered from the different organs of the apparently healthy wild tilapia sourced from the Iloilo River.

Isolate[a]	IMVC Result[b]	*E. coli* ID	Isolate[a]	IMVC Result[b]	*E. coli* ID	Isolate[a]	IMVC Result[b]	*E. coli* ID
01-G	+ + – –	+	20-G	– + – +	–	39-G	+ + – –	+
02-G	+ + – –	+	21-G	– + – +	–	40-G	+ + – –	+
03-G	– + – +	–	22-G	+ + – +	–	41-K	– – – +	–
04-G	– – – –	–	23-G	+ + – –	+	42-K	+ + – +	–
05-G	+ – – +	–	24-G	+ – – +	–	43-K	+ – – –	–
06-G	+ + – –	+	25-G	+ – – +	–	44-K	+ + – –	+
07-G	– – – +	–	26-G	+ + – –	+	45-K	+ + – –	+
08-G	+ + – –	+	27-G	+ + – –	+	46-K	+ + – –	+
09-G	+ + – –	+	28-G	+ + – –	+	47-K	+ + – –	+
10-G	+ – – +	–	29-G	+ + – –	+	48-K	+ + – –	+
11-G	– – – +	–	30-G	+ – – +	–	49-K	+ + – –	+
12-G	+ + – –	+	31-G	+ + – –	+	50-S	+ + – –	+
13-G	+ – – –	–	32-G	+ + – –	+	51-S	+ + – –	+
14-G	– – – –	–	33-G	+ + – –	+	52-S	+ + – +	–
15-G	+ – – +	–	34-G	+ + – –	+	53-S	+ + – –	+
16-G	+ + – +	–	35-G	+ + – –	+	54-S	– + – +	–
17-G	– + – +	–	36-G	+ + – –	+	55-S	+ + – –	+
18-G	– + – +	–	37-G	– + – +	–	56-S	+ + – –	+
19-G	– + – +	–	38-G	– – – –	–			

[a] Isolate Nomenclature: Isolate Number-Organ Source; G, gut; K, kidney/liver; S, spleen. [b] IMVC, indole-methyl red-Vogues-Proskauer-citrate; +, positive; -, negative

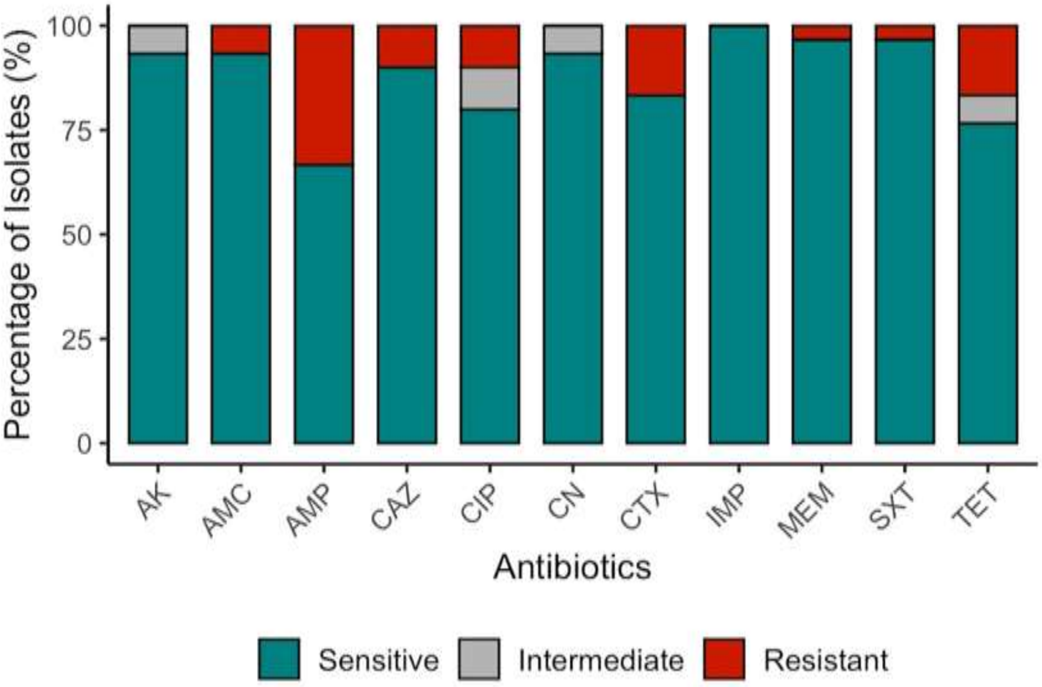

Figure 2. Antibiogram reflecting the susceptibility of *Escherichia coli* isolates against the tested antibiotics: AK, amikacin; AMC, amoxicillin-clavulanate; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CN, gentamicin; CTX, cefotaxime; IMP, imipenem; MEM, meropenem; SXT, co-trimoxazole; TET, tetracycline.

highest having a multiple antibiotic resistance (MAR) index of 0.45. Eight isolates exhibited resistance to at least one antibiotic class, while 18 were fully susceptible to all antibiotics tested. This corresponds to a 13.3% prevalence of multidrug resistance among *E. coli* isolates. Six isolates were resistant to the representative cephalosporins cefotaxime and ceftazidime, indicating potential production of extended-spectrum beta-lactamases (ESBLs). Two of the MDR isolates exhibited similar resistance profiles

Table 3. Antimicrobial resistance profiles of the *Escherichia coli* isolates.

No. of tested *E. coli* isolates	Antimicrobial Resistance Profile*	MAR Index	Classification
2	AMC-CAZ-CIP-CTX-AMP	0.45	Multidrug-resistant (MDR) (13.3%)
1	CIP-CTX-AMP	0.27	
1	SXT-CTX-AMP	0.27	
4	TET-AMP	0.18	Antimicrobial-resistant (AR) (26.7%)
1	CTX-AMP	0.18	
1	MEM-AMP	0.18	
1	TET	0.09	
1	CAZ	0.09	
18	No resistance	0.00	Fully Susceptible (FS)
Total	**Average MAR Index**	**0.09**	

*AK, amikacin; AMC, amoxicillin-clavulanate; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CN, gentamicin; CTX, cefotaxime; IMP, imipenem; MEM, meropenem; SXT, co-trimoxazole; TET, tetracycline.

to amoxicillin-clavulanate, ceftazidime, ciprofloxacin, cefotaxime, and ampicillin. The most common resistance profile involved both tetracycline and ampicillin. MAR index values ranged from 0 to 0.45, with an average of 0.09.

Discussions

The study recovered antimicrobial-resistant *E. coli* from various organs of wild, apparently healthy tilapia from the Iloilo River. The MAR index averaged 0.09, which is lower than the generic threshold of 0.20 used to indicate a high risk of antimicrobial resistance. While the majority of isolates were susceptible, multidrug-resistant and cephalosporin-resistant isolates were documented. Even with a low MAR value, the results confirm the presence of AMR in wild freshwater fish, indicating environmental contamination. In contrast to other studies, this research used apparently healthy fish to detect resistant *E. coli*, reinforcing the notion that AMR does not require clinical disease to be harbored by hosts. Fish can act as asymptomatic reservoirs, reflecting upstream anthropogenic influences such as wastewater effluents, agricultural runoff, and urban discharges, supporting the One Health framework (Al-Khalaifah et al., 2025). The resistance observed in these fish is representative of environmental AMR contamination rather than therapeutic antibiotic use, as often seen in aquaculture settings.

The low mean MAR index suggests that *E. coli* populations associated with wild tilapia from the Iloilo River are not subjected to acute selection pressure, and in the context of environmental AMR surveillance, it reflects background contamination from diffuse and chronic anthropogenic inputs rather than intensive and acute antibiotic use as observed in hospital and aquaculture isolates (Hart et al., 2023; de Heredia et al., 2025). This is further supported by the predominance of fully susceptible isolates, with only a minority resistant to antimicrobials. An important caveat in interpreting the MAR index is that it reflects only phenotypic resistance and cannot detect genotypic resistance; silent or emerging resistances may be underestimated. Some species may also have intrinsic resistances. For instance, in the study of Wu et al. (2025) on antimicrobial-resistant *E. coli* isolates from the urbanized Maozhou River in China and its groundwater with testing 20 antibiotics from 11 classes, the average MAR index for the sampled river water and groundwater only ranged from 0.07 to 0.25, while genotypic resistance index, tested through conventional primer-based polymerase chain reaction (PCR) for resistance genes, ranged from 0.04 to 0.12. This provides evidentiary support for the discordance between phenotypic and genotypic antimicrobial resistance, and the MAR index captures only half the picture of the bacterial isolates' resistance. The researchers considered the Maozhou River at low risk of contamination and spreading antibiotic resistance genes (ARGs) due to background antibiotic contamination and not intensive use- the same case for the Iloilo River in this study. Nonetheless, the presence of resistant isolates indicates their persistence in the river system. Even in the absence of overt selection pressure, wild fish can harbor resistant bacteria, underscoring their role as sentinels of baseline AMR contamination in aquatic environments such as the Iloilo River. For instance, the study by Gufe et al. (2025) shows that isolates from wild tilapia in polluted natural lakes in Zimbabwe exhibited higher levels of resistance and residue than those from aquaculture ponds, highlighting environmental pollution as a driver of AMR. Anthropogenic activities, including urban wastes, increase the abundance of ARGs in the receiving sediments and waters (Kümmerer, 2009; Marathe et al., 2013). DENR-EMB reported that the Iloilo River is exposed to commercial and industrial waste from malls and food processing industries, according to its 2020 annual assessment report. Moscoso et al. (2025) reported that commercial establishments are willing to pay penalties rather than install a functioning sewage treatment facility, suggesting complacency among major contributors to AMR.

Focusing on the observed resistance patterns, ampicillin and tetracycline resistance are commonly reported in aquatic and agricultural environments and are linked to widespread, long-term antimicrobial use (Wahome et al., 2014; Wu et al., 2025). Resistance to clinically important antimicrobials, including cephalosporins and fluoroquinolones, is also epidemiologically relevant (Chen et al., 2020). *Escherichia coli* with cephalosporin resistance was detected more frequently in tilapia than in other species (Thaotumpitak et al., 2024). Complete susceptibility to imipenem suggests no strong selection pressure for last-resort drugs in this aquatic environment. Although the detection of MDR isolates was relatively low (13.3%), this remains concerning. Similarly, cephalosporin-resistant isolates that may harbor ESBL genes pose a risk because of the potential circulation of ESBL-associated resistance genes, which serve as an early warning signal (Marathe et al., 2016). Thaotumpitak et al. (2024) investigated isolates from tilapia that contained the *bla*CTX-M gene (2.7% prevalence), responsible for cephalosporin resistance (3.9%). The *bla* genes (beta-lactamase genes) encode bacterial enzymes that break down β-lactam antibiotics (Haberecht et al., 2019; Thaotumpitak et al., 2024), including cephalosporins, and make β-lactamases the most common cause of β-lactam resistance (Hinthong et al., 2024; Thaotumpitak et al., 2024). Moreover, the *bla*CTX-M is known to be associated with various mobile genetic elements, one of which is integrons (Naseer and Sundsfjord, 2001; Marathe et al., 2016), and also commonly found in plasmids (Haberecht et al., 2019; Thaotumpitak et al., 2024), implicating their capability to be involved in horizontal gene transfer and suggesting that tilapia can become a reservoir of pathogens and resistance factors that are clinically relevant (Marathe et al., 2016). Moreover, intermediate resistance was observed to tetracycline, ciprofloxacin, and aminoglycosides, suggesting early stages of resistance development under continued selective pressure. Such intermediate resistance can serve as a sentinel marker for emerging AMR trends.

Compared with the studies of Debnath et al. (2025), Devadas et al. (2025), Tate et al. (2022), Mumbo et al. (2023), and Chouhan et al. (2025), which primarily sampled aquaculture-raised, market-sourced, or diseased fish, the markedly lower MAR indices and reduced MDR prevalence observed in this study suggest that antimicrobial resistance in wild riverine tilapia is driven predominantly by environmental exposure rather than by clinical or aquaculture-associated selective pressures. The novelty of this study lies in its focus on wild, apparently healthy tilapia in an urban river system, which are not commonly included in previous sampling frames. The results contribute valuable baseline data for AMR surveillance in the Iloilo River and support national AMR monitoring efforts, particularly the Philippine National Action Plan on AMR, which identifies *E. coli* as a priority organism for surveillance in healthy tilapia.

For future studies, increasing sample size, conducting longitudinal and seasonal sampling, and including environmental samples such as water and sediment will strengthen surveillance. This study relied on phenotypic identification without molecular confirmation; this can be addressed in future work by molecular detection of AMR and ESBL genes. Overall, this study serves as a starting point for expanded environmental AMR surveillance, highlighting the importance of wild, apparently healthy fish as bioindicators. As one of the few studies documenting AMR in wild-caught and clinically normal fish, it underscores the need to broaden research beyond farmed and cultured species, particularly as wild fish and their habitats are subject to urban runoff, domestic wastes, and other anthropogenic pressures.

Conclusion

Taken together, this study provided baseline data on the occurrence of antimicrobial-resistant presumptive *E. coli* in wild, apparently healthy tilapia from the Iloilo River. Although the mean MAR index and bacterial population composition indicate low risk and prevalence of antimicrobial resistance, the presence of resistant isolates indicates background contamination in the urban water system. This suggests that wild fish, even in the absence of clinical disease, can be reservoirs of AMR. The observed resistance patterns reflect diffuse, rather than acute, antimicrobial selective pressure. Importantly, the recovery of candidate ESBL producers highlights the potential dissemination of clinically relevant resistance traits in the environment. Intermediate resistance also indicates potential development of resistance, underscoring the need for continued surveillance to detect emerging trends before resistance becomes widespread in the environment. By focusing on wild-caught, apparently healthy tilapia from a riverine system, this study addressed a gap in current AMR research, which has largely focused on aquaculture settings and diseased hosts; it also supports the inclusion of environmental and wildlife components in the concerted surveillance efforts under the One Health framework. Continued monitoring and elucidation of resistance determinants can improve understanding of the dynamics and spread of antimicrobial resistance in aquatic environments.

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