

***Escherichia coli* concentrations and antibiotic resistance profile in nearshore waters of Manila Bay, Philippines**

Aaron Jan Palmares*, Jianina Lauryne Morabe, Ma. Liezl Parazo, Mary Isabelle Maddara, Dyve John Rodas, Erica Placino, Reynato Rodriguez III, Cybelle Alyxes Nicolas

Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., 1015, Manila, Philippines

*Corresponding author e-mail: apalmares@feu.edu.ph

Received: 20 June 2025 / Accepted: 6 March 2026

Abstract. This study investigates the extent of microbial contamination and antimicrobial resistance (AMR) in the nearshore waters of Manila Bay, Philippines, a region heavily impacted by urbanization and anthropogenic pollution. Forty water samples were collected from five coastal sites and analyzed for physicochemical parameters, *Escherichia coli* (*E. coli*) concentrations, and antimicrobial susceptibility. Results revealed *E. coli* levels averaging 17,300 CFU/100 mL, which significantly exceeded national water quality standards and indicated severe and sustained fecal contamination. Physicochemical conditions (mean turbidity of 10.8 NTU, pH 7.35, low dissolved oxygen at 1.46 mg/L, and high conductivity at 22,509 μ S/cm) support short-term bacterial survival. Antimicrobial susceptibility testing of 40 *E. coli* isolates showed resistance to at least one antibiotic in 70% of isolates, with 20% classified as multiple antibiotic-resistant (MAR) and 17.5% as multidrug-resistant (MDR). Ampicillin resistance was most common (60%), followed by gentamicin (25%), cotrimoxazole (22.5%), and ciprofloxacin (12.5%). No extended-spectrum beta-lactamase (ESBL)-producing strains were detected. Correlation analyses found significant associations between *E. coli* concentration and turbidity (positive) and conductivity (negative), but no significant link between resistance levels and physicochemical parameters, suggesting resistance development is driven by other environmental or genetic factors. These findings underscore the urgent need for integrated water quality management, stricter pollution control, and enhanced AMR surveillance to protect public health and the marine environment. The study highlights Manila Bay as a critical hotspot for microbial pollution and antibiotic resistance, warranting immediate action and continued monitoring.

Keywords: *Escherichia coli*, antimicrobial resistance, fecal contamination, nearshore waters, Manila Bay

1. Introduction

Manila Bay is a semi-enclosed natural harbor located on the western coast of Luzon Island in the Philippines and plays a crucial role in the country's socio-economic development

and environmental sustainability. It supports a wide array of activities including fisheries, transportation, tourism, and industrial operations. However, its strategic location near Metro Manila, a densely populated urban center, has made it highly vulnerable to pollution and multiple anthropogenic pressures (Vallejo et al., 2019). The continuous influx of domestic sewage, industrial effluents, stormwater runoff, and agricultural discharge has led to significant environmental degradation (Osorio et al., 2021). Among the most pressing concerns are the elevated levels of organic matter, nutrients, and microbial pollution of its waters, which pose serious risks to marine ecosystems, aquaculture, recreational water use, and public health (Enova et al., 2025; Palmares et al., 2024; Sy & Ong, 2023). One of the key indicators of microbial pollution is *Escherichia coli* (*E. coli*), a facultative anaerobic, Gram-negative bacteria commonly found in the intestines of warm-blooded animals. While many strains of *E. coli* are harmless and essential for maintaining gut health, the presence of this bacteria in environmental waters is widely used to signal fecal contamination (Carreon et. al., 2025; Palmares et al., 2025). In particular, pathogenic strains of *E. coli* can lead to severe infections in humans, ranging from gastrointestinal illnesses to urinary tract infections. Beyond its role as an indicator organism, *E. coli* is also a critical subject of concern due to its ability to acquire and transmit antimicrobial resistance (AMR) (Cardos et al., 2023; S. Suzuki et al., 2013). The proliferation of AMR bacteria in aquatic environments has emerged as a global health challenge, driven in part by the unregulated discharge of antibiotics and resistant microorganisms into water systems (Larsson & Flach, 2022). These environmental waters may facilitate the persistence and spread of resistance genes, potentially leading to the emergence of multidrug-resistant pathogens in human and animal populations (Larsson & Flach, 2022). In areas like Manila Bay, where antibiotic use in healthcare, agriculture, and aquaculture can be potentially present, assessing the resistance profiles of *E. coli* isolates can offer insights into the broader AMR burden in the environment (Dela Peña et al., 2022; Yoneda et al., 2022). In response to this concern, the current study investigates both the total coliform levels and the antibiotic resistance profiles of *E. coli* from

various sites along the Manila Bay coastline. Specifically, it aims to determine the concentration of *E. coli* in water samples, evaluate the antimicrobial susceptibility patterns of the isolates, and analyze their correlation to relevant physicochemical parameters such as pH, turbidity, conductivity, and dissolved oxygen. By integrating microbiological, chemical, and antimicrobial resistance data, this research seeks to enhance our understanding of the extent and drivers of microbial contamination in Manila Bay, and to provide evidence-based insights that may inform water quality management, public health policy future environmental monitoring efforts and public health interventions.

2. Materials and Methods

2.1. Sample collection

For this study, five sampling sites were selected along the shores of Manila Bay within Metro Manila, designated as Sites 1 to 5. These sites were located in the coastal areas of Navotas, Manila, Pasay, Parañaque, and Las Piñas, respectively, with a particular focus on non-point source pollution (Figure 1). Site coordinates were approximated using Google Maps. At each of the five sampling locations, four grab water samples (25 mL each) were collected at depths ranging from 1 to 15 cm, yielding a total of 20 samples per batch. Two sampling batches were conducted, one in the first week of March 2025 and another in the first week of April 2025, resulting in a total of 40 grab samples. Additionally, during each visit, a 2-liter water sample was collected from each site using polytetrafluoroethylene (PTFE) containers for physicochemical analysis, amounting to ten 2-liter samples overall.

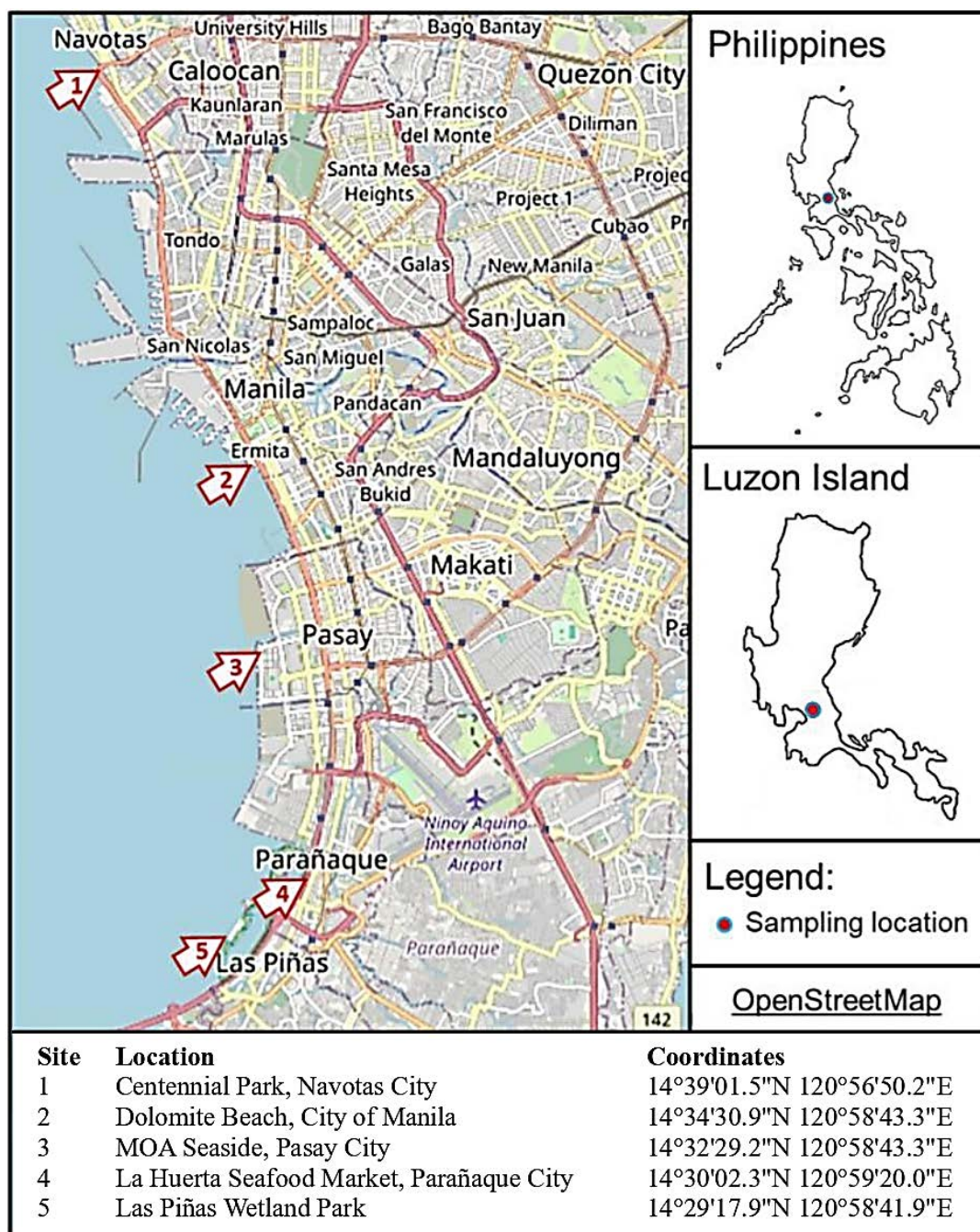


Figure 1. Schematic Diagram of Sampling Points, Collection Sites, and Coordinates.

2.2. Physicochemical testing

Ten water samples, each with a volume of 2 liters, were analyzed to assess various water quality indicators. The procedures applied for these assessments included nephelometric turbidity analysis following Standard Method (SM) 2130 B; pH evaluation via glass electrode in accordance with SM 4500-H⁺ B; dissolved oxygen (DO) determination based on the Azide

Modification outlined in SM 4500-O.C; and electrical conductivity measurement conducted with a conductivity meter as per SM 2510 B (Addisie, 2022; Estabillo et al., 2025).

2.3. Microbial quality testing

To isolate *E. coli* and other coliform bacteria, each water sample was analyzed using 3M Petrifilm™ *E. coli*/Coliform Count (EC) Plates (3M Microbiology, St. Paul, MN), following the manufacturer's guidelines. A 1-mL aliquot from a 1:50 dilution, prepared using sterile 0.85% saline solution, was plated onto each Petrifilm plate. Plates were incubated at 37 ± 0.1 °C for 48 ± 2 hours, with no more than five plates stacked during incubation to ensure uniform temperature exposure. Post-incubation, colonies were enumerated using a digital colony counter (Colony Counter Model CC-1, BOECO, Germany). Colonies appearing blue and producing gas were identified as presumptive *E. coli*, while blue or red colonies with gas production were classified as total coliforms. Colorless colonies were recorded as non-coliform organisms. Colony-forming units (CFU) per mL were calculated by multiplying the colony count by the dilution factor ($\times 50$), and the final CFU per 100 mL was obtained by further multiplying the result by 100 (Azuma et al., 2022).

2.4. Purification of presumptive *Escherichia coli* colonies

To confirm the identity of *E. coli* and evaluate antimicrobial resistance, selected blue colonies with visible gas formation (presumptive *E. coli* colonies) from the Petrifilm EC plates were subjected to further analysis. Between four and five presumptive *E. coli* colonies were streaked onto Eosin Methylene Blue (EMB) agar (HiMedia Laboratories, Mumbai, India) to obtain pure cultures. Plates were incubated at 37 ± 0.5 °C for 24 hours. On EMB agar, typical *E. coli* colonies exhibited a dark purple coloration with a characteristic metallic green sheen, indicative of strong lactose fermentation. Colonies with this morphology were then subcultured onto MacConkey (MAC) agar (HiMedia Laboratories, Mumbai, India) using the isolation streak method and incubated at 37 ± 0.5 °C for 18 hours. On MAC agar, *E. coli* colonies were identified by their

pink color, often accompanied by a surrounding pink halo due to acid production from lactose fermentation (Palmares et al., 2024).

2.5. Identification and antimicrobial susceptibility testing

Presumptive *E. coli* colonies grown on MacConkey (MAC) agar were emulsified in 0.5% sodium chloride solution to prepare bacterial suspensions. The turbidity of each suspension was standardized to a 0.55 McFarland equivalent using the VITEK DensiCHEK™ (bioMérieux). Species identification was performed using the VITEK 2 system (bioMérieux, Durham, NC, USA) with the ID-N261 identification card, following the manufacturer's instructions. Antimicrobial susceptibility testing (AST) was conducted using the VITEK 2 AST-N261 card and software version 9.03.3, which also included the Extended-Spectrum Beta-Lactamase (ESBL) detection assay specific for *E. coli*. A total of seventeen antibiotics spanning seven antimicrobial classes or subclasses were tested. These included β -lactams such as penicillins [amoxicillin/clavulanic acid (AMC), ampicillin (AMP), piperacillin/tazobactam (TZP)], cephalosporins [cefepime (FEP), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), cefuroxime (CXM), cefuroxime axetil (CXM-AX)], and carbapenems [ertapenem (ETP), imipenem (IMP), meropenem (MEM)]. Other antibiotic classes tested were aminoglycosides [amikacin (AMK), gentamicin (GEN)], quinolones [ciprofloxacin (CIP)], polymyxins [colistin (CST)], and sulfonamides [cotrimoxazole (SXT)]. Isolates were classified as susceptible, intermediate, or resistant based on their minimum inhibitory concentrations (MICs), interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines. Additionally, further AST was performed using Sensititre™ plates (Thermo Fisher Scientific, Dardilly, France) to test thirteen more antibiotics from five antimicrobial classes or subclasses: penicillin [(ampicillin/sulbactam (SAM), ticarcillin/clavulanic acid (TIC-CLA)], cephalosporins [cefixime (CFM), cefotaxime (CTX), ceftazidime/avibactam (CZA), ceftolozane/tazobactam(C/T)], monobactam [aztreonam (ATM)], carbapenems [doripenem

(DOR), imipenem/relebactam (IMIREL), meropenem/vaborbactam (MEMV)], aminoglycosides [tobramyxin (TOB)], and quinolones [(levofloxacin (LEV), norfloxacin (NOR))]. These results were interpreted following the EUCAST guidelines (Bonnin et al., 2022).

2.6. Data Analysis

The mean concentrations of *E. coli*, coliforms, and non-coliforms were calculated based on their site of collection. Similarly, descriptive data for water quality parameters such as turbidity, pH, DO, and conductivity were also computed. The isolates were also categorized based on their levels of antimicrobial resistance: antibiotic-resistant (AR) if resistant to 1 or 2 antimicrobials, multiple antibiotic-resistant (MAR) if resistant to ≥ 3 antimicrobials, and multidrug-resistant (MDR) if resistant to at least one antimicrobial in ≥ 3 different classes or subclasses. They were also further determined if they were an ESBL producer. All statistical analyses were performed using SPSS version 30.0 (SPSS Inc., Chicago, IL, USA). The relationships between physicochemical parameters and the minimum inhibitory concentrations (MICs) of *E. coli* were assessed using Spearman's rank correlation. A p-value of ≤ 0.01 was considered statistically significant.

3. Results and Discussion

3.1. The physicochemical characteristics of water

The water samples from Manila Bay exhibited an average turbidity of 10.8 NTU, a pH of 7.35, dissolved oxygen (DO) of 1.46 mg/L, and conductivity of 22,509 $\mu\text{S}/\text{cm}$ (Table 1). These values characterize an environmental profile that, while mixed, presents moderately favorable conditions for the short-term survival and persistence of *E. coli* in the coastal waters of Manila Bay. Firstly, the moderate turbidity suggests the presence of suspended particles such as silt, organic matter, or detritus. These particulates can enhance *E. coli* survival by providing protection from ultraviolet (UV) radiation and by offering surfaces for bacterial attachment.

These particles may also create micro-niches rich in nutrients, further prolonging bacterial viability (Smith et al., 2008). Secondly, although the DO level is extremely low and hypoxic by aquatic life standards, *E. coli* is a facultative anaerobe and is capable of surviving, and even proliferating, under low-oxygen or anaerobic conditions. Low DO often reflects elevated organic pollution, typically linked to sewage inputs, fecal contamination, or urban runoff, which are likely sources of *E. coli* in these waters. The hypoxic condition may also indicate poor circulation near the shoreline, especially in semi-enclosed areas like bays, where stagnation exacerbates oxygen depletion in bottom and near-shore zones (Jacinto et al., 2011; Vallejo et al., 2019). Thirdly, the elevated conductivity suggests moderately high salinity, characteristic of brackish or estuarine waters. While *E. coli* is naturally adapted to low-salinity environments such as freshwater and the intestinal tract, elevated salinity imposes osmotic stress that can reduce bacterial viability over time (Agwu et al., 2017). Nevertheless, *E. coli* can persist in the short term, particularly when shielded by organic matter. In brief, this combination of moderate turbidity, near-neutral pH, low dissolved oxygen, and elevated conductivity reflects a polluted aquatic environment under significant anthropogenic influence, likely driven by sewage discharges and urban runoff. These conditions are conducive to the short-term survival of *E. coli*, especially in cases of recent contamination. However, long-term persistence may be inhibited by salinity stress unless bacteria are protected by environmental buffers such as sediments, particulate matter, or biofilm matrices (Adesakin et al., 2020; Shah et al., 2023; Sila, 2019).

Table 1. Mean values of measured water quality parameters

Site	NTU	pH at 25°C	DO (mg/L)	Conductivity (µS/cm)
1	13.1	7.19	1.13	29475
2	2.6	7.52	1.73	41300
3	15.8	7.27	1.11	7863
4	13.8	7.31	2.08	4530
5	8.8	7.45	1.24	29375
Average	10.8	7.35	1.46	22509

Note: DO, dissolved oxygen; µS/cm, microsiemens per centimeter; mg/L, milligrams per deciliter; NTU, nephelometric turbidity units;

3.2. Coliform and *Escherichia coli* concentrations

Water samples collected from five shoreline sites in Manila Bay revealed alarmingly high levels of microbial contamination, with mean concentrations of *E. coli* and total coliforms at 17.3×10^3 CFU/100 mL and 5.9×10^4 CFU/100 mL, respectively (Table 2). These values far exceed the national regulatory limits set by the Department of Environment and Natural Resources (DENR) under the Water Quality Guidelines (DENR, 2015, 2016). For Class SC marine waters intended for recreational activities such as boating and fishing, the maximum allowable *E. coli* concentration is 100 CFU/100 mL, while for Class SD waters designated for navigation and non-contact purposes, the threshold is 400 CFU/100 mL. The measured concentrations surpass these standards by more than two orders of magnitude, indicating a severe and persistent state of microbial pollution. Moreover, even when compared against the effluent standards for treated wastewater discharges into these water classes, set at 10×10^3 CFU/100 mL for Class SC and 15×10^3 CFU/100 mL for Class SD, the ambient *E. coli* levels remain elevated. This suggests that the shoreline waters are more contaminated than effluents that would be considered legally compliant under existing discharge regulations (DENR, 2015, 2016). Such contamination implies the presence of continuous and significant fecal pollution sources, potentially originating from direct sewage outfalls, stormwater runoff, failing sanitation infrastructure, or leaching from informal settlements (S. Suzuki et al., 2013). The magnitude of microbial load observed in this

study renders Manila Bay's nearshore waters legally non-compliant and unsuitable for their designated uses, including recreation, navigation, and ecosystem support.

Table 2. Average concentration of *Escherichia coli*, coliforms, and non-coliform according to site of collection

Site	<i>E. coli</i> CFU/100 mL Mean $\pm$ SD	Coliform CFU/100 mL Mean $\pm$ SD	Non-coliform CFU/100 mL Mean $\pm$ SD
1	$(10.5 \pm 5.9) \times 10^3$	$(4.7 \pm 3.1) \times 10^4$	$(2.4 \pm 1.9) \times 10^4$
2	$(9.0 \pm 4.4) \times 10^3$	$(4.5 \pm 1.0) \times 10^4$	$(2.9 \pm 1.4) \times 10^4$
3	$(31.3 \pm 2.1) \times 10^3$	$(8.8 \pm 1.8) \times 10^4$	$(6.6 \pm 1.8) \times 10^4$
4	$(18.8 \pm 5.4) \times 10^3$	$(6.9 \pm 1.0) \times 10^4$	$(6.2 \pm 2.9) \times 10^4$
5	$(17.0 \pm 9.8) \times 10^3$	$(4.4 \pm 2.4) \times 10^4$	$(5.5 \pm 0.6) \times 10^4$
Average	$(17.3 \pm 9.8) \times 10^3$	$(5.9 \pm 2.6) \times 10^4$	$(4.7 \pm 2.5) \times 10^4$

Note: CFU, colony forming units; SD, standard deviation

3.3. The antimicrobial susceptibility profile of *Escherichia coli*

Antimicrobial susceptibility testing (AST) was performed on 40 *E. coli* isolates. The highest resistance was observed against AMP (60%), followed by GEN (25%), SXT (22.5%), and CIP (12.5%). Resistance to SAM, CXM, CXM-AX, FOX, and CFM was lower, each at 5% (Figure 2). All isolates were fully susceptible to 20 other antibiotics spanning seven classes, including penicillins (AMC, TIC-CLA, TZP), cephalosporins (CTX, CAZ, CRO, CZA, C/T, FEP), monobactams (ATM), carbapenems (DOR, ETP, IMP, MEM, IMIREL, MEM-VAB), polymyxins (CST), aminoglycosides (AMK, TOB), and quinolones (LEV, NOR). Thus, they were not included in Figure 2. The observed resistance profile is generally consistent with the Department of Health's 2023 Antimicrobial Resistance Surveillance Program (ARSP), which reported high resistance of *E. coli* to AMP (78%), and SXT (56%) (ARSP, 2024). AMP, a widely used first-line β -lactam, showed the highest resistance in this study, posing a concern for both human and veterinary treatment. This higher resistance rate may lead to an increase in reliance on more expensive drugs like carbapenems or CST. Moreover, moderate resistance to SXT and GEN, frequently prescribed for urinary tract and community-acquired infections also indicates that standard therapies may be increasingly ineffective, due to increasing misuse and overuse of

such drugs. (Collignon & McEwen, 2019; Rousham et al., 2018). Strengthened antibiotic stewardship, environmental controls, and regulatory oversight are essential to slow the spread of resistance and preserve treatment efficacy (Ancheta, 2021; Chen et al., 2023; Essert et al., 2023; Y. Suzuki et al., 2020).

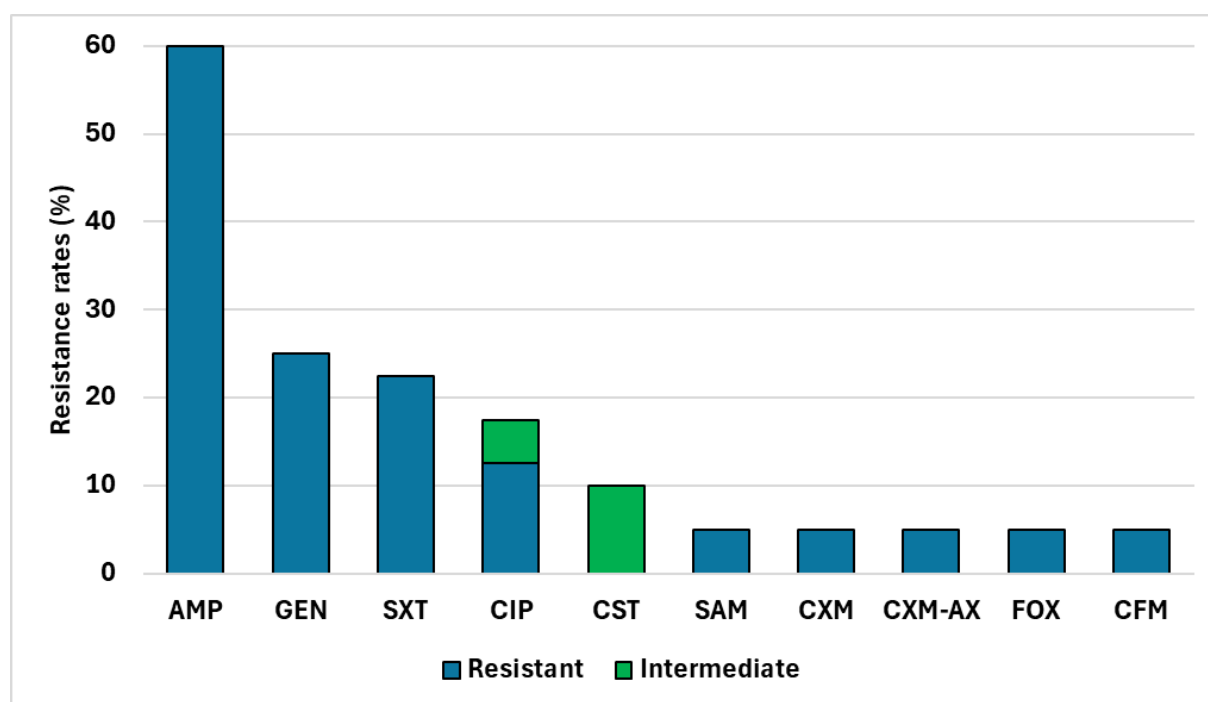


Figure 2. Antibiotic resistance profile of *E. coli* from water samples in order of resistance level. AMP, ampicillin; GEN, gentamicin; SXT, cotrimoxazole; CIP, ciprofloxacin; SAM, ampicillin/sulbactam; CXM, cefuroxime; CXM-AX, cefuroxime axetil; FOX, ceftiofur; and CFM cefixime. Note: The figure includes only antibiotics for which resistance was observed; fully susceptible antibiotics are not shown.

3.4. The frequency of antibiotic-resistant *Escherichia coli*

Of the 40 *E. coli* isolates tested, 12 (30.0%) were FS to all antibiotics, while 20 (50.0%) were AR, 8 (20.0%) were MAR, and 7 (17.5%) MDR. No ESBL producers were detected (Table 3). The presence of 30% FS isolates indicates that a portion of the bacterial population remains treatable with first-line antibiotics. However, the fact that 70% of isolates displayed some form of resistance raises concerns for standard therapy, especially in complicated or recurrent infections. Half of the isolates were resistant to at least one antibiotic (AR), pointing to a

widespread resistance problem that, if unchecked, could progress to more severe resistance profiles. The emergence of MAR strains (20%) further suggests narrowing treatment options, as these bacteria have enhanced survival under selective pressure and greater potential for transmission (Brepoels et al., 2024). Even more concerning is the 17.5% MDR rate, defined by resistance to at least one agent in three or more antibiotic classes, significantly limiting treatment choices and increasing the likelihood of therapy failure (Rafailidis & Kofteridis, 2021). MDR strains are often associated with healthcare-associated infections and can lead to longer hospital stays, increased costs, and elevated mortality. The distribution of FS, AR, MAR, and MDR isolates reflects a continuum of resistance development. Without proper antibiotic stewardship and surveillance, FS strains may evolve into MDR or even ESBL-producing forms under continued selective pressure (Fodor et al., 2020).

Table 3. Frequencies and percentage of antimicrobial resistance (AMR) profiles

Result	Frequency (Percent, %)				
	FS	AR	MAR	MDR	ESBL
Positive	12 (30.0)	20 (50.0)	8 (20.0)	7 (17.5)	0 (0.0)
Negative	28 (70.0)	20 (50.0)	32 (80.0)	33 (82.5)	40 (100.0)

Note: AR, antibiotic resistant; ESBL, extended spectrum beta lactamase producer; FS, fully susceptible; MAR, multiple antibiotic resistant; MDR, multidrug resistant

3.5. Spearman correlations of MICs and other measured parameters

Spearman correlation analysis was conducted to assess potential relationships between the minimum inhibitory concentrations (MICs) of seven antibiotics (AMP, GEN, SXT, CIP, SAM, CXM, and FOX) and key water quality parameters across 40 *E. coli* isolates (Table 4). The results showed no statistically significant correlations between the MICs of any antibiotic and turbidity (NTU), pH, dissolved oxygen (DO), or conductivity ($p > 0.01$). This suggests that resistance expression in *E. coli* is not directly influenced by these environmental parameters, and may instead be driven by factors such as the presence of antibiotic residues, resistance genes, or horizontal gene transfer (Carney et al., 2019; Leonard et al., 2018; Nappier et al., 2020). However, significant correlations were observed between *E. coli* concentrations and certain water

quality parameters. Specifically, *E. coli* counts were positively correlated with turbidity ($p < 0.01$), indicating that higher turbidity often resulting from suspended solids or organic matter, may serve as an indicator of fecal contamination and increased microbial load (Huey & Meyer, 2010). In contrast, *E. coli* concentrations were negatively correlated with conductivity ($p < 0.01$), suggesting that high ionic strength may inhibit bacterial survival (Agwu et al., 2017). These findings may imply that while water quality indicators like turbidity and conductivity may not predict antibiotic resistance levels, they are useful proxies for assessing microbial pollution and public health risks (Zhai et al., 2020). Correlating MICs with water quality parameters may help identify resistance "hotspots" and guide targeted interventions. While correlation does not imply causation, the observed associations may offer important insights into environmental influences on resistance (Farias et al., 2015).

Table 4. Spearman correlation coefficients and their respective p-values describing relationships between the MICs and other measured parameters.

Variable	<i>E. coli</i> (CFU/dL)		AMP		GEN		SXT	
	Spearman ρ	Prob> ρ	Spearman ρ	Prob> ρ	Spearman ρ	Prob> ρ	Spearman ρ	Prob> ρ
NTU	0.596	<.001	-0.066	0.687	0.245	0.127	-0.278	0.083
pH	-0.300	0.060	0.070	0.67	0.010	0.951	0.350	0.027
DO	-0.159	0.326	0.173	0.287	-0.274	0.087	0.084	0.608
Cond	-0.464	0.003	-0.022	0.892	-0.334	0.035	-0.100	0.540
Variable	CIP		SAM		CXM		FOX	
	Spearman ρ	Prob> ρ	Spearman ρ	Prob> ρ	Spearman ρ	Prob> ρ	Spearman ρ	Prob> ρ
NTU	0.249	0.121	-0.341	0.031	-0.132	0.415	-0.341	0.031
pH	0.017	0.917	0.026	0.105	0.117	0.117	0.105	0.105
DO	-0.199	0.217	0.149	0.357	0.144	0.375	0.149	0.357
Cond	-0.365	0.021	0.302	0.058	0.001	0.994	0.302	0.058

Note: AMP, ampicillin; GEN, gentamicin; SXT, cotrimoxazole; CIP, ciprofloxacin; SAM, ampicillin/sulbactam; CXM, cefuroxime; and FOX, ceftiofur. Statistically significant relationships ($p < 0.01$) are bolded.

3.6. Limitations of the Study

Despite yielding valuable insights, this study has several limitations. First, the temporal scope was restricted to two sampling periods within a single dry season month, which may not capture seasonal variations in water quality and microbial resistance patterns (Jiang et al., 2021; Liang et al., 2020). Second, the geographic focus was limited to five shoreline sites within Metro Manila,

potentially overlooking broader spatial heterogeneity across Manila Bay (Wang et al., 2020). Third, while correlations between physicochemical parameters and *E. coli* concentrations were explored, the absence of significant associations with MICs suggests that other unmeasured environmental factors, such as antibiotic residues or genetic determinants, may play a more direct role in resistance dynamics (Fodor et al., 2020). Additionally, the study relied solely on phenotypic assays for resistance profiling, without conducting molecular characterization of resistance genes or mobile genetic elements, which limits understanding of the mechanisms and potential for horizontal gene transfer (Maal-Bared et al., 2013). Finally, the sample size, particularly for antimicrobial susceptibility testing, was relatively small ($n = 40$), which may reduce statistical power and the generalizability of findings.

4. Conclusion

This study provides compelling evidence of severe microbial contamination and emerging antimicrobial resistance (AMR) in the nearshore waters of Manila Bay. *E. coli* concentrations far exceeded national regulatory thresholds, confirming widespread and persistent fecal pollution potentially driven by untreated sewage, urban runoff, and inadequate wastewater infrastructure. Physicochemical conditions characterized by moderate turbidity, low dissolved oxygen, and elevated conductivity create a conducive environment for the short-term survival of *E. coli*. Moreover, antimicrobial susceptibility testing revealed that 70% of *E. coli* isolates exhibited resistance to at least one antibiotic, with 17.5% classified as MDR. While no ESBL producers were detected, the detection of MAR and MDR strains underscores a troubling trajectory that threatens both environmental and public health. Notably, the lack of significant correlations between resistance levels and water quality parameters suggests that resistance is likely driven by complex factors beyond basic physicochemical conditions, such as antibiotic residues and horizontal gene transfer. These findings highlight the urgent need for integrated interventions including enhanced wastewater treatment, continuous surveillance, long-term investment in

pollution control, and responsible antibiotic stewardship to curb microbial pollution and mitigate the spread of AMR in Manila Bay and similar urban coastal environments.

Acknowledgements. The authors are grateful to the Department of Medical Technology, Far Eastern University, for allowing the use of research facilities and the technical assistance of staff for this work.

Declarations Conflict of interest: The authors have no relevant financial or non-financial interests to disclose

References

- Addisie, M. B., 2022, Evaluating drinking water quality using water quality parameters and esthetic attributes. *Air, Soil and Water Research*. *Air, Soil and Water Research*, 15: 1–8. <https://doi.org/10.1177/11786221221075005>
- Adesakin, T. A., Oyewale, A. T., Bayero, U., Mohammed, A. N., Aduwo, I. A., Ahmed, P. Z., Abubakar, N. D., & Barje, I. B., 2020. Assessment of bacteriological quality and physico-chemical parameters of domestic water sources in Samaru community, Zaria, Northwest Nigeria. *Heliyon*, 6(8), e04773. <https://doi.org/10.1016/j.heliyon.2020.e04773>
- Agwu, O. A., Oluwagunke, T., & Ebohon, J., 2017, Survival and growth rate of coastal water *Escherichia coli* isolates in different salt concentrations. *Ife Journal of Science*, 19(1): 41. <https://doi.org/10.4314/ijis.v19i1.6>
- Ancheta, A. A., 2021, Sustaining Estuarial Creeks: Water Corridors in Mitigating Flooding in Manila, Philippines. *IOP Conference Series: Earth and Environmental Science*, 799: 012004. <https://doi.org/10.1088/1755-1315/799/1/012004>
- ARSP, 2024, Antimicrobial Resistance Surveillance Program Annual Report - 2023. In Department of Health - Research Institute for Tropical Medicine.
- Azuma, T., Uchiyama, T., Zhang, D., Usui, M., & Hayashi, T., 2022, Distribution and characteristics of carbapenem-resistant and extended-spectrum β -lactamase (ESBL) producing *Escherichia coli* in hospital effluents, sewage treatment plants, and river water in an urban area of Japan. *Science of the Total Environment*, 839: 156232. <https://doi.org/10.1016/j.scitotenv.2022.156232>
- Bonnin, R. A., Bernabeu, S., Emeraud, C., Creton, E., Vanparis, O., Naas, T., Jousset, A. B., & Dortet, L., 2022, Susceptibility of OXA-48-producing Enterobacterales to imipenem/relebactam, meropenem/vaborbactam and ceftazidime/avibactam. *International Journal of Antimicrobial Agents*, 60(4): 106660. <https://doi.org/10.1016/j.ijantimicag.2022.106660>
- Brepoels, P., De Wit, G., Lories, B., Belpaire, T. E. R., & Steenackers, H. P., 2024, Selective pressures for public antibiotic resistance. *Critical Reviews in Microbiology*, 51(3): 417–426. <https://doi.org/https://doi.org/10.1080/1040841X.2024.2367666>
- Cardos, H. B., Dalmacio, L. M. M., & Balolong, M. P., 2023, Bacterial composition and antibiotic resistome profile of water in the Manila Bay South Harbor. *SciEnggJ*, 16(2): 254–267.
- Carney, R. L., Labbate, M., Siboni, N., Tagg, K. A., Mitrovic, S. M., & Seymour, J. R., 2019,

- Urban beaches are environmental hotspots for antibiotic resistance following rainfall. *Water Research*, 167, 115081. <https://doi.org/10.1016/j.watres.2019.115081>
- Carreon, P. B., Bombeo, H. P., Castillo, T. J., Closa, J. M., Damaso, J. M., Gellecanao, F., & Palmares, A. J., 2025, Antimicrobial susceptibility profile of gram - negative bacteria isolated from selected hot springs in Laguna , Philippines. *Journal of Applied and Natural Science*, 17(4), 1550–1560. <https://doi.org/10.31018/jans.v17i4.6860>
- Chen, J., Hsu, B., Ko, W., & Wang, J., 2023, Ecotoxicology and Environmental Safety Comparison of antibiotic-resistant *Escherichia coli* and extra-intestinal pathogenic *E. coli* from main river basins under different levels of the sewer system development. *Ecotoxicology and Environmental Safety*, 263(138): 115372. <https://doi.org/10.1016/j.ecoenv.2023.115372>
- CLSI, 2021, M100 Performance Standards for Antimicrobial Susceptibility Testing (31st ed.). Clinical Laboratory Standards Institute.
- Collignon, P. J., & McEwen, S. A., 2019, One health-its importance in helping to better control antimicrobial resistance, *Tropical Medicine and Infectious Disease*, 4: 11. <https://doi.org/10.3390/tropicalmed4010022>
- Dela Peña, L. B. R. O., Nacario, M. A. G., Bolo, N. R., & Rivera, W. L., 2022, Multiple antibiotic resistance in *Escherichia coli* isolates from fecal and water sources in Laguna Lake, Philippines. *Water*, 14: 1517. <https://doi.org/10.3390/w14091517>
- DENR, 2015, DENR MC 2015-006 Guidelines for recreational waters monitoring program. Department of Environment and Natural Resources, Republic of the Philippines.
- DENR, 2016, DENR AO 2016-08. Water quality guidelines and general effluent standards of 2016. Department of Environment and Natural Resources, Republic of the Philippines.
- Enova, A. E. R., Singh, R. R., Aga, D. S., Mariano, S. M. F., Villanoy, C. L., & Jaraula, C. M. B., 2025, Modeling pharmaceutical contaminant transport in an urban coastal estuarine system: The case of Manila Bay, Philippines. *ACS ES and T Water*, 5(1): 156–157. <https://doi.org/10.1021/acsestwater.4c00736>
- Essert, S. M., Zacharias, N., Precht, T., Pankratz, D., Funken, K., Mutters, N. T., Kistemann, T., & Schreiber, C., 2023, Persistence of MRSA and ESBL-producing *E. coli* and *K. oxytoca* in river water. *Hygiene and Environmental Health Advances*, 7: 100072. <https://doi.org/10.1016/j.heha.2023.100072>
- Estabillo, C. F., Hingpit, M., John, O., Lacambra, R., Cuyugan, C., Gellecanao, F., & Palmares, A. J., 2025, Assessment of microbial quality of bottled water from street vendors and sundry stores in Metro Manila , Philippines. *Journal of Applied and Natural Science*, 17(4), 1591–1599. <https://doi.org/10.31018/jans.v17i4.6861>
- Farias, P., Santo, C. E., Branco, R., Francisco, R., Santos, S., Hansen, L., Sorensen, S., & Morais, P. V., 2015, Natural hot spots for gain of multiple resistances: Arsenic and antibiotic resistances in heterotrophic, aerobic bacteria from marine hydrothermal vent fields. *Applied and Environmental Microbiology*, 81(7): 2534–2543. <https://doi.org/10.1128/AEM.03240-14>
- Fodor, A., Abate, B. A., Deák, P., Fodor, L., Gyenge, E., Klein, M. G., Koncz, Z., Muvevi, J., Ötvös, L., Székely, G., Vozik, D., & Makrai, L., 2020, Multidrug resistance (MDR) and collateral sensitivity in bacteria, with special attention to genetic and evolutionary aspects and to the perspectives of antimicrobial peptides—a review. *Pathogens*, 9(7): 1–55. <https://doi.org/10.3390/pathogens9070522>
- Huey, G. M., & Meyer, M. L., 2010, Turbidity as an indicator of water quality in diverse watersheds of the upper Pecos River Basin. *Water (Switzerland)*, 2(2): 273–284. <https://doi.org/10.3390/w2020273>
- Jacinto, G. S., Sotto, L. P. A., Senal, M. I. S., Diego-McGlone, M. L. S., Escobar, M. T. L., Amano, A., & Miller, T. W., 2011, Hypoxia in Manila Bay, Philippines during the northeast monsoon. *Marine Pollution Bulletin*, 63: 243–248.

- <https://doi.org/10.1016/j.marpolbul.2011.02.026>
- Jiang, X., Liu, L., Chen, J., Fan, X., Xie, S., Huang, J., & Yu, G., 2021, Antibiotic resistance genes and mobile genetic elements in a rural river in Southeast China: Occurrence, seasonal variation and association with the antibiotics. *Science of the Total Environment*, 778: 146131. <https://doi.org/10.1016/j.scitotenv.2021.146131>
- Larsson, D. G. J., & Flach, C. F., 2022, Antibiotic resistance in the environment. *Nature Reviews Microbiology*, 20(5): 257–269. <https://doi.org/10.1038/s41579-021-00649-x>
- Leonard, A. F. C., Zhang, L., Balfour, A. J., Garside, R., Hawkey, P. M., Murray, A. K., Ukoumunne, O. C., & Gaze, W. H., 2018, Exposure to and colonisation by antibiotic-resistant *E. coli* in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). *Environment International*, 114: 326–333. <https://doi.org/10.1016/j.envint.2017.11.003>
- Liang, X., Guan, F., Chen, B., Luo, P., Guo, C., Wu, G., Ye, Y., Zhou, Q., & Fang, H., 2020, Spatial and seasonal variations of antibiotic resistance genes and antibiotics in the surface waters of Poyang Lake in China. *Ecotoxicology and Environmental Safety*, 196: 110543. <https://doi.org/10.1016/j.ecoenv.2020.110543>
- Maal-Bared, R., Bartlett, K. H., Bowie, W. R., & Hall, E. R., 2013, Phenotypic antibiotic resistance of *Escherichia coli* and *E. coli* O157 isolated from water, sediment and biofilms in an agricultural watershed in British Columbia. *Science of the Total Environment*, 443: 315–323. <https://doi.org/10.1016/j.scitotenv.2012.10.106>
- Nappier, S. P., Liguori, K., Ichida, A. M., Stewart, J. R., & Jones, K. R., 2020, Antibiotic resistance in recreational waters: State of the science. *International Journal of Environmental Research and Public Health*, 17(21): 1–28. <https://doi.org/10.3390/ijerph17218034>
- Osorio, E. D., Tanchuling, M. A. N., & Diola, M. B. L. D., 2021, Microplastics occurrence in surface waters and sediments in five river mouths of Manila Bay. *Frontiers in Environmental Science*, 9: 719274. <https://doi.org/10.3389/fenvs.2021.719274>
- Palmares, A. J., Rongalirios, J. J., Santos, M., Siggaoat, M. J., Sugang, P. L., Tipa, N. N., Torres, J. C. A., Vasquez, C. A., Viernes, A. M., & Gellecanao, F., 2024, The antibiotic resistance profile of *Escherichia coli* from selected estuaries in the City of Manila, Philippines. *Asian Journal of Water, Environment and Pollution*, 21(6): 15–22. <https://doi.org/10.3233/AJW240068>
- Palmares, A. J., Tacsagon, K. J., Taopo, F. C., Taroma, X., Toledo, S., Torres, I. S., Ventura, J. R., Villanueva, A. M., Yamat, L. G., & Gellecanao, F., 2025, Antibiotic resistance profile of *Escherichia coli* from Marikina River in the Philippines: Environmental and public health implications. *Journal of Applied and Natural Science*, 17(2).
- Rafailidis, P. I., & Kofteridis, D., 2021, Proposed amendments regarding the definitions of multidrug-resistant and extensively drug-resistant bacteria. *Expert Review of Anti-Infective Therapy*. <https://doi.org/10.1080/14787210.2021.1945922>
- Rousham, E. K., Unicomb, L., & Islam, M. A., 2018, Human, animal and environmental contributors to antibiotic resistance in low-resource settings: Integrating behavioural, epidemiological and one health approaches. *Proceedings of the Royal Society B: Biological Sciences*, 285: 20180332. <https://doi.org/10.1098/rspb.2018.0332>
- Shah, A., Arjunan, A., Baroutaji, A., & Zakharova, J., 2023, A review of physicochemical and biological contaminants in drinking water and their impacts on human health. *Water Science and Engineering*, 16(4): 333–344. <https://doi.org/10.1016/j.wse.2023.04.003>
- Sila, O. N., 2019, Physico-chemical and bacteriological quality of water sources in rural settings, a case study of Kenya, Africa. *Scientific African*, 2: e00018. <https://doi.org/10.1016/j.sciaf.2018.e00018>
- Smith, R. P., Paiba, G. A., & Ellis-Iversen, J., 2008, Turbidity as an indicator of *Escherichia coli* presence in water troughs on cattle farms. *Journal of Dairy Science*, 91(5): 2082–2085.

<https://doi.org/10.3168/jds.2007-0597>

- Suzuki, S., Ogo, M., Miller, T. W., Shimizu, A., Takada, H., & Siringan, M. A. T., 2013, Who possesses drug resistance genes in the aquatic environment?: Sulfamethoxazole (SMX) resistance genes among the bacterial community in water environment of Metro-Manila, Philippines. *Frontiers in Microbiology*, 4: 102. <https://doi.org/10.3389/fmicb.2013.00102>
- Suzuki, Y., Nazareno, J., Nakano, R., Mondoy, M., Nakano, A., & Bugayong, P., 2020, Environmental Presence and Genetic Characteristics of Carbapenemase-Producing Enterobacteriaceae from Hospital Sewage and River Water in the Philippines. *Applied and Environmental Microbiology*, 86(2): e01906-19.
- Sy, K. H., & Ong, A. K., 2023, Fecal Coliform Reduction Management in Manila Bay Bathing Beaches: A Case Study in the Philippines. *Proceedings of the First Australian International Conference on Industrial Engineering and Operations Management*, Sydney, Australia, December 20-21, 2022, 1161–1166. <https://doi.org/10.46254/au01.20220250>
- Vallejo, B. M., Aloy, A. B., Ocampo, M., Conejar-Espedido, J., & Manubag, L. M., 2019, Manila bay ecology and associated invasive species. In *Impacts of Invasive Species on Coastal Environments*, 29: 45–169. Coastal Research Library. https://doi.org/10.1007/978-3-319-91382-7_5
- Wang, Z., Han, M., Li, E., Liu, X., Wei, H., Yang, C., Lu, S., & Ning, K., 2020, Distribution of antibiotic resistance genes in an agriculturally disturbed lake in China: Their links with microbial communities, antibiotics, and water quality. *Journal of Hazardous Materials*, 393, 122426. <https://doi.org/10.1016/j.jhazmat.2020.122426>
- Yoneda, I., Rozanah, U. N., Nishiyama, M., Mith, H., & Watanabe, T., 2022, Detection and genetic analysis of *Escherichia coli* from Tonle Sap Lake and its tributaries in Cambodia: Spatial distribution, seasonal variation, pathogenicity, and antimicrobial resistance. *Environmental Pollution*, 315, 120406. <https://doi.org/10.1016/j.envpol.2022.120406>
- Zhai, R., Fu, B., Shi, X., Sun, C., Liu, Z., Wang, S., Shen, Z., Walsh, T. R., Cai, C., Wang, Y., & Wu, C., 2020, Contaminated in-house environment contributes to the persistence and transmission of NDM-producing bacteria in a Chinese poultry farm. *Environment International*, 139: 105715. <https://doi.org/10.1016/j.envint.2020.105715>