

From vendor to consumer: Evaluating the microbial quality of generic bottled water in Rizal Province, Philippines

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ABSTRACT

Introduction: In Rizal Province, Philippines, generic bottled water is widely distributed through street vendors and small retail outlets and is often presumed by consumers to be safe. However, potential microbial contamination poses public health risks, particularly in areas lacking stringent quality controls. Therefore, this study assessed the microbial and physicochemical quality of these bottled waters and their compliance with national and international safety standards.

Materials and Methods: Seventy-five bottled water samples representing 15 brands were collected from five locations in Rizal Province between May and November 2025. Samples were analyzed for *Escherichia coli* (*E. coli*), total coliforms (TC), heterotrophic plate count (HPC) bacteria, and yeast and mold (YM) using Neogen Petrifilm™. Biochemical and phenotypic identification of *E. coli* was performed using the VITEK 2 system. Antimicrobial susceptibility testing was conducted on confirmed *E. coli* isolates. Physicochemical parameters (pH, turbidity, and chloride) were measured and analyzed for correlation with microbial concentrations.

Results: A total of 56% of samples exceeded at least one microbial safety threshold. *E. coli* was detected in 2.7% of samples, TC in 16%, HPC in 33%, and YM in 27%. All *E. coli* isolates were fully susceptible to all 30 antibiotics tested. Brand identity significantly influenced microbial contamination ($p < 0.01$). Positive correlations were found between *E. coli* and TC ($\rho = 0.447$), TC and HPC ($\rho = 0.487$), and HPC and YM ($\rho = 0.422$). pH had no significant correlation with microbial load.

Conclusions: A considerable proportion of bottled water sold in Rizal failed to meet microbial safety standards. Although all *E. coli* isolates were antibiotic-sensitive, their presence indicates inadequate treatment or post-treatment contamination. Enhanced regulation, routine monitoring, and public education are

recommended to ensure microbial safety and consumer protection in informal bottled water markets.

INTRODUCTION

Rizal is a densely populated province in the Philippines that functions as a suburban commuter zone for Metro Manila, with an estimated population of 3.42 million and a population density of approximately 2,866 per square kilometer as of the year 2024 (Philippine Statistics Authority 2024). The high volume of daily commuters and pedestrian activity has contributed to the widespread distribution of bottled drinking water, primarily through street vendors and small-scale retail establishments. This widespread availability caters to the demand for potable water, presumed to be safe for immediate consumption (Estabillo *et al.* 2025; Puspita *et al.* 2023; Tabar *et al.* 2023). Consumers expect that generic or unbranded bottled water, often marketed as purified or filtered, complies with microbiological safety standards. Recent investigations, however, indicate that the microbial quality of some bottled water may fail to meet national and international drinking water guidelines (DOH 2017; Mills *et al.* 2018; Traoré *et al.* 2023). Contamination can occur at various stages of production. For example, during treatment, bottling, or sealing steps, harmful microorganisms such as fecal bacteria and other heterotrophic microbes may be introduced (Bedada *et al.* 2018; Estabillo *et al.* 2025; Momtaz *et al.* 2013). Ingesting contaminated water typically results in gastrointestinal illness, and vulnerable populations (e.g., immunocompromised individuals and older adults) may experience more severe health effects (WHO 2022). Moreover, the presence of antibiotic-resistant bacteria in contaminated water could further complicate treatment options if infections occur (Aguilar-Salazar *et al.* 2023; Carreon *et al.* 2025). Despite these concerns, data on the microbial quality of bottled water sold by street vendors and small stores in Rizal Province remain sparse. This gap highlights the need to systematically evaluate these products against established water quality standards. Therefore, the present study assessed bottled drinking water from these sources

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by measuring key microbial indicators. Specifically, the analysis focuses on the presence and levels of *E. coli*, total coliforms (TC), heterotrophic plate count (HPC) bacteria, and yeasts/molds (YM) to determine compliance with both local and international safety guidelines (DOH 2017; BIS 2018).

MATERIALS AND METHODS

Sample Collection

From May through November 2025, seventy-five generic bottled drinking water samples were obtained using convenience sampling from street vendors and small-scale stores in five study sites covering all four congressional districts of Rizal Province and Antipolo City (Figure 1). The bottles, with volumes between 250 mL and 1 L, were contained in standard polyethylene terephthalate (PET) packaging and represented fifteen unbranded or generic product lines. Each location contributed three distinct brands, and five units were purchased for every brand, yielding fifteen samples per site and an overall collection of seventy-five bottles. Immediately after purchase, the samples were kept in insulated containers with ice to maintain a low temperature and were delivered to the laboratory, where all analyses were initiated within four hours of collection.

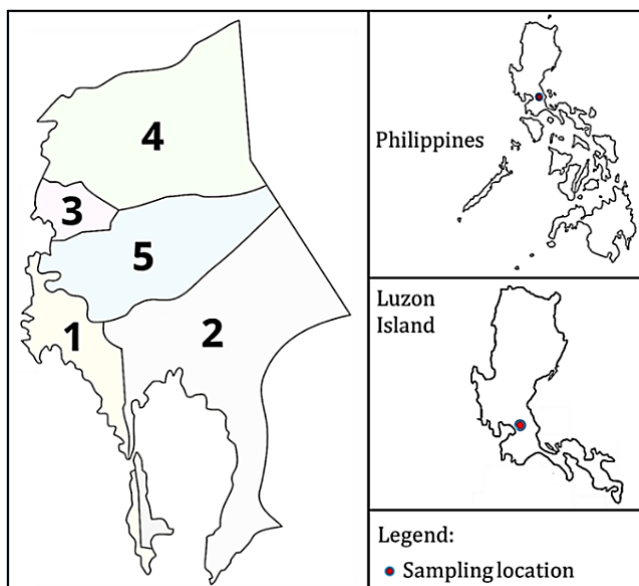


Figure 1: Map of sampling areas in Rizal Province. 1, 1st District of Rizal; 2, 2nd District of Rizal; 3, 3rd District of Rizal; 4, 4th District of Rizal; and 5, Component City of Antipolo.

Sample Preparation

All bottled water samples were processed using aseptic techniques to prevent contamination. The external surfaces of the bottles, including the cap and neck, were decontaminated with sterile gauze saturated with 70% isopropyl alcohol and allowed to air dry. The bottle necks were briefly flamed with an alcohol burner and opened aseptically without contact with the inner surface. Water samples were subsequently subjected to microbiological analysis. Primary isolation and enumeration were conducted using Neogen Petrifilm™ Aqua Heterotrophic Count (AQHC), *Escherichia coli*/Coliform (EC), and Yeast and Mold (YM) plates in accordance with the manufacturer's protocols.

Enumeration of Total Coliform (TC) and *Escherichia coli*

Total coliforms and *E. coli* were enumerated on the Neogen Petrifilm™ EC Plates. A 1-mL volume of each sample was inoculated directly onto the EC plate and incubated at 37.0 ± 0.1 °C for 48 ± 1 hours in stacks not exceeding five plates. After incubation, colonies were enumerated based on color and gas production using a standard colony counter. Colonies producing gas were differentiated by color for identification purposes: gas-

forming red colonies were classified as presumptive coliforms, whereas gas-forming blue colonies (due to β -glucuronidase activity) were identified as presumptive *E. coli*. The overall coliform concentration was determined by adding together all red and blue gas-producing colonies. Specifically, total coliform concentration was determined using the formula $CFU/mL = \text{number of colonies} \div \text{volume plated (mL)}$. The countable range for total coliforms was up to 150 colonies per plate.

Enumeration of Heterotrophic Plate Count (HPC) Bacteria

Heterotrophic bacteria were enumerated on the Neogen Petrifilm™ Aqua Heterotrophic Count (AQHC) plates. For each water sample, two parallel preparations were made: one consisting of the original sample and another subjected to a tenfold dilution in sterile 0.9% sodium chloride solution. A volume of 1 mL from each preparation was aseptically dispensed onto individual Petrifilm™ AQHC plates. The inoculated media were incubated at 37.0 ± 0.1 °C for 48 ± 1 h, with plates arranged in stacks of no more than five to ensure uniform incubation conditions. Following incubation, bacterial growth was quantified by enumerating colony-forming units using a standard colony counter. All red-colored colonies were included in the count, irrespective of their diameter or color intensity. When growth on the undiluted plate exceeded the countable limit of 300 colonies per plate, results from the tenfold diluted preparation were used, and values were adjusted by the dilution factor. Specifically, HPC concentration was also determined using the formula $CFU/mL = (\text{number of colonies} \times \text{dilution factor}) \div \text{volume plated (mL)}$. In the diluted preparation, 111 μ L of sample was added to 1000 μ L of diluent, yielding an approximate tenfold dilution, so colony counts are multiplied by 10.

Enumeration of Yeast and Mold (YM)

Fungal populations were enumerated on the Neogen Petrifilm™ Yeast and Mold (YM) count plates. For each water sample, two parallel preparations were also made: one consisting of the original sample and another subjected to a tenfold dilution in sterile 0.9% sodium chloride solution. A volume of 1 mL from each preparation was aseptically dispensed onto individual 3M Petrifilm™ YM plates. The inoculated plates were incubated at 25.0 ± 0.1 °C for a maximum duration of five days (120 ± 2 h), with plates arranged in stacks of no more than five to ensure uniform incubation conditions. Following incubation, fungal growth was assessed based on macroscopic colony characteristics. Yeasts were distinguished as discrete colonies that were typically small, circular, uniformly colored, and sharply demarcated, frequently exhibiting a convex profile and lacking a dark central region. In contrast, molds were identified as comparatively larger colonies with flattened growth, poorly defined or spreading margins, heterogeneous pigmentation (including but not limited to beige, blue-green, tan, or orange hues), and commonly presenting a darker central area. Yeast and mold colonies were enumerated independently, and the combined count was expressed as total fungal colony-forming units (CFU) per milliliter. Specifically, fungal concentration was also determined using the formula $CFU/mL = (\text{number of colonies} \times \text{dilution factor}) \div \text{volume plated (mL)}$. In the diluted preparation, 111 μ L of sample was also added to 1000 μ L of diluent, yielding an approximate tenfold dilution, so colony counts are also multiplied by 10.

Isolation and Purification of Presumptive *Escherichia coli*

Presumptive *E. coli* isolates, characterized by blue colonies with gas formation on EC plates, were further processed for biochemical confirmation. Individual colonies were aseptically selected and purified through streak isolation on Eosin Methylene Blue (EMB) agar (HiMedia Laboratories, Mumbai, India). The inoculated plates were incubated at 37 ± 0.5 °C for 24 h to allow colony development. Following incubation, isolates exhibiting dark violet colonies with a distinctive metallic green sheen on EMB agar consistent with strong lactose fermentation were considered putative *E. coli*. These colonies were subsequently transferred onto

MacConkey (MAC) agar (HiMedia Laboratories, Mumbai, India) and incubated at 37.0 ± 0.5 °C for 18 h. On MAC agar, lactose-fermenting *E. coli* were identified by the formation of pink colonies, with surrounding zones of precipitated bile salts.

Biochemical Identification and Antimicrobial Susceptibility Testing

Presumptive *E. coli* colonies recovered on MacConkey agar were processed for confirmatory identification and antimicrobial susceptibility profiling. For each isolate, a standardized bacterial suspension was prepared in sterile 0.5% sodium chloride, and optical density was adjusted to a 0.55 McFarland equivalent using the VITEK DensiCHEK™ instrument (bioMérieux, Marcy-l'Étoile, France). Species-level identification was carried out using the automated VITEK 2 platform (bioMérieux, Durham, NC, USA) with the ID-N261 identification card. Antimicrobial susceptibility testing was initially performed using the VITEK 2 AST-N261 card operated under software version 9.03.3. This testing algorithm incorporated an integrated extended-spectrum β -lactamase (ESBL) screening module specific for *E. coli*. The primary susceptibility panel encompassed seventeen antimicrobial agents representing seven major therapeutic classes. These included β -lactam penicillins [ampicillin (AMP), amoxicillin/clavulanate (AMC), and piperacillin/tazobactam (TZP)]; cephalosporins [cefoxitin (FOX), cefepime (FEP), ceftazidime (CAZ), ceftriaxone (CRO), and cefuroxime or cefuroxime axetil (CXM/CXM-AX)]; carbapenems [meropenem (MEM), ertapenem (ETP), and imipenem]; aminoglycosides [gentamicin (GEN) and amikacin (AMK)]; the fluoroquinolone ciprofloxacin (CIP); the polymyxin colistin (CST); and the folate pathway inhibitor combination cotrimoxazole (SXT). Susceptibility categorizations such as susceptible, intermediate, or resistant were assigned based on minimum inhibitory concentration (MIC) values interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) performance standards (Bonnin *et al.* 2022; Thermo Fisher Scientific 2018). To broaden the resistance profile, additional MIC

determinations were conducted using Sensititre™ broth microdilution panels (Thermo Fisher Scientific, Dardilly, France). This expanded analysis evaluated thirteen further antimicrobial compounds spanning five antimicrobial classes or subclasses. Tested agents included β -lactam/ β -lactamase inhibitor combinations [ampicillin/sulbactam (SAM) and ticarcillin/clavulanic acid (TIC-CLA)]; extended-spectrum and novel cephalosporins [cefexime (CFM), cefotaxime (CTX), ceftazidime/avibactam (CZA), and ceftolozane/tazobactam (C/T)]; the monobactam aztreonam (ATM); carbapenems and carbapenem-inhibitor combinations [doripenem (DOR), imipenem/relebactam (IMI-REL), and meropenem/vaborbactam (MEM-V)]; the aminoglycoside tobramycin (TOB); and the fluoroquinolones levofloxacin (LEV) and norfloxacin (NOR). Interpretation of all MIC results generated from the expanded testing panel followed the breakpoint criteria established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2026).

Quality Standards Evaluation

Microbial indicators detected in the collected water samples were interpreted with reference to applicable international and national regulatory thresholds (Table 1). Enumerations of HPC bacteria, TC, and *E. coli* were evaluated in relation to the World Health Organization Guidelines for Drinking-Water Quality (GDWQ) and the Philippine National Standards for Drinking Water (PNSDW) issued by the Department of Health (DOH 2017; WHO 2022). In the absence of specified permissible limits for yeasts and molds in both GDWQ and PNSDW, fungal concentrations were instead evaluated using the Philippine Food and Drug Administration microbiological criteria for non-alcoholic beverages (FDA Philippines 2022). For each microbial indicator, sample results were classified as compliant or non-compliant based on whether measured values fell within or exceeded the corresponding reference limits.

Table 1: Reference criteria used for evaluating microbiological and physicochemical quality of drinking water and selected non-alcoholic beverages.

Indicator category	Standard value	Unit of measurement	Reference standard
Total coliform and <i>E. coli</i>	< 1	CFU·100 mL ⁻¹	WHO (2022); DOH (2017)
Total yeast and mold	< 1	CFU mL ⁻¹	FDA Philippines (2022)
HPC	< 500	CFU mL ⁻¹	WHO (2022); DOH (2017)
Chloride	≤ 250	ppm	WHO (2022); DOH (2017)
Turbidity	≤ 5	NTU	WHO (2022); DOH (2017)
pH (25 °C)	6.5–8.5	Dimensionless	WHO (2022); DOH (2017)

Notes: Microbial quantities are expressed as colony-forming units (CFU). Concentrations of dissolved ions are reported in parts per million (ppm), while turbidity is expressed in nephelometric turbidity units (NTU).

Physicochemical and Data Analysis

Following microbiological evaluation, bottled water samples representing all surveyed brands underwent physicochemical characterization. Analyses focused on turbidity, pH, and chloride concentration. Turbidity was quantified using a nephelometric technique in accordance with Standard Methods 2130 B. The pH was measured potentiometrically with a calibrated glass electrode following Standard Methods 4500-H⁺ B. Chloride ion concentration was determined by ion chromatography based on Standard Methods 4511B (Addisie 2022). Measured values were evaluated against the allowable limits specified in PNSDW (DOH 2017). Statistical analyses were performed using SPSS version 30.0 (SPSS Inc., Chicago, IL, USA) to examine associations between bottled water brands and compliance with microbiological drinking water standards, as well as relationships between physicochemical parameters and microbial indicators. Brand-related compliance was assessed using Fisher's exact test, while correlations between microbial counts and physicochemical variables were analyzed using Spearman's rank correlation coefficient. Statistical significance was defined as $p < 0.01$.

RESULTS

Microbial Quality and pH of Bottled Water Samples

Table 2 summarizes the average concentration of *E. coli*, TC, HPC, and YM detected in each bottled water brand. Brand B exhibited the highest average *E. coli* (0.4 CFU mL⁻¹), TC (5.0 CFU mL⁻¹), and HPC (892 CFU mL⁻¹), while the highest average YM count was observed in Brand G (6.6 CFU mL⁻¹). Measured pH values across all samples varied between 6.02 and 7.48; however, three brands (F, H, and N) recorded pH levels outside the acceptable range of 6.5–8.5 established by the PNSDW. Chloride concentrations in every sample were consistently below 0.5 ppm, remaining far under the regulatory limit of 250 ppm. In addition, turbidity measurements for all samples were below the analytical detection threshold of 0.5 nephelometric turbidity units (NTU), which is well within the allowable maximum of 5.0 NTU. Owing to these consistently low values, chloride and turbidity data were excluded from Table 2.

Table 2: Mean (\pm SD) microbial concentrations and pH of bottled water samples (n = 5 replicates per sample).

Brand code	<i>E. coli</i> (CFU mL ⁻¹)	TC (CFU mL ⁻¹)	HPC (CFU mL ⁻¹)	YM (CFU mL ⁻¹)	pH (25 °C)
A	ND	ND	ND	4.0 \pm 5.8	6.72
B	0.4 \pm 0.5	5.0 \pm 1.6	892 \pm 219	0.4 \pm 0.5	6.73
C	ND	0.8 \pm 0.8	679 \pm 110	0.2 \pm 0.4	6.65
D	ND	ND	ND	ND	7.39
E	ND	ND	ND	ND	6.95
F	ND	ND	764 \pm 146	3.2 \pm 2.6	6.42
G	ND	ND	852 \pm 191	6.6 \pm 4.8	7.48
H	ND	ND	ND	ND	6.22
I	ND	ND	112 \pm 245	0.4 \pm 0.9	6.64
J	ND	ND	120 \pm 268	0.8 \pm 1.3	6.62
K	ND	ND	8.8 \pm 8.2	ND	7.08
L	ND	ND	ND	ND	6.58
M	ND	ND	ND	ND	6.75
N	ND	ND	255 \pm 313	1.0 \pm 1.4	6.02
O	ND	0.6 \pm 0.9	250 \pm 103	1.6 \pm 2.6	6.97
MAL	<1	<1	<500	<1	6.5–8.5

Notes: Values are expressed as mean \pm standard deviation. CFU, colony-forming units; mL, milliliter; HPC, heterotrophic plate count bacteria; MAL, maximum allowable level; ND, not detected.

Incidence of Samples Exceeding Microbial Standards

Table 3 summarizes the occurrence of bottled water samples, grouped by brand, that exceeded acceptable microbiological standards. The results show a statistically significant relationship between the bottled water brand and noncompliance with established limits for TC, HPC, and YM ($p < 0.01$). Notably, none of the product labels disclosed the specific water treatment processes applied. Among the brands analyzed, two samples

belonging to Brand B were noncompliant with at least three microbiological parameters. Conversely, samples obtained from five brands (D, E, H, K, and M) consistently satisfied all required microbial quality thresholds. Additionally, Figure 2 illustrates the percentage of samples failing microbiological standards across different sampling locations.

Table 3: Incidence of bottled water samples not meeting the microbial quality standard (n = 5 replicates per brand code/sample).

Brand code	<i>E. coli</i> (CFU mL ⁻¹)	TC (CFU mL ⁻¹)	HPC (CFU mL ⁻¹)	YM (CFU mL ⁻¹)
A	0	0	0	3
B	2	5	5	2
C	0	3	5	1
D	0	0	0	0
E	0	0	0	0
F	0	0	5	4
G	0	0	5	4
H	0	0	0	0
I	0	0	2	1
J	0	0	1	2
K	0	0	0	0
L	0	2	0	0
M	0	0	0	0
N	0	0	2	2
O	0	2	0	1
Total n = 75 (%)	2 (2.67)	12 (16)	25 (33)	20 (27)
p-value	0.054	<0.01	<0.01	<0.01

Notes: Heterotrophic bacteria quantified via heterotrophic plate count method; total coliforms indicate general sanitary quality; yeasts and molds represent fungal contamination.

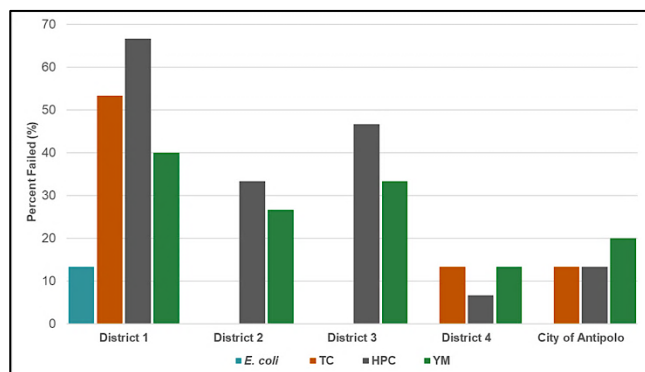


Figure 2: Percentage of bottled water samples exceeding microbial safety limits across sampling areas in Rizal Province, based on PNSDW and FDA criteria.

Relationships Between Microbial Concentration and pH

The relationships among the concentrations of *E. coli*, total coliforms (TC), heterotrophic plate count (HPC) bacteria, yeast and molds (YM), and pH are shown in Table 4. Correlation analysis with turbidity and chloride levels was not performed because both variables were below detection limit and exhibited no variability across samples. Spearman correlation analysis showed significant associations only among selected microbial indicators, specifically between *E. coli* and TC ($\rho = 0.447$, $p < 0.01$), TC and HPC ($\rho = 0.487$, $p < 0.01$), and HPC and YM ($\rho = 0.422$, $p < 0.01$).

Table 4: Spearman's rank correlation coefficients (ρ) and corresponding probability values illustrating the associations among microbiological indicators and pH.

Variables	<i>E. coli</i> (CFU mL ⁻¹)		TC (CFU mL ⁻¹)		YM (CFU mL ⁻¹)		HPC bacteria (CFU mL ⁻¹)	
	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
TC	0.447	<0.01	-	-	-	-	-	-
YM	0.039	0.742	0.001	0.991	-	-	-	-
HPC	0.275	0.017	0.487	<0.01	0.422	<0.01	-	-
pH	0.029	0.807	0.036	0.762	0.026	0.826	-0.006	0.958

Notes: Microbial concentrations are expressed as colony-forming units per millilitre (CFU mL⁻¹). HPC refers to heterotrophic plate count bacteria. ρ denotes Spearman's correlation coefficient. Statistically significant correlations are bolded ($p < 0.01$).

The Antibiotic Susceptibility Profile of Isolated *Escherichia coli*

Among the 75 bottled water samples examined, presumptive *E. coli* was detected in two samples, corresponding to a positivity rate of 2.7%. Presumptive identification was based on the formation of characteristic blue colonies accompanied by gas production on EC Petrifilm. Isolates recovered from the positive samples were subsequently subjected to automated phenotypic confirmation and

antimicrobial susceptibility testing using the VITEK[®] 2 system. The isolates obtained from samples B2 and B5 were definitively identified as *E. coli*. Antimicrobial susceptibility profiling demonstrated that all confirmed isolates were susceptible to the complete panel of antibiotics evaluated in this study, as summarized in Table 5.

Table 5: Minimum inhibitory concentrations and susceptibility classifications of *Escherichia coli* isolates against selected antimicrobial agents.

Code	AMP AMC	TZP	CXM CXM-AX	FOX	CAZ CRO	FEP	ETP	IMP MEM	AMK	GEN	CIP	SXT
B2	4 (S)	2 (S)	4 (S)	4 (S)	1 (S)	1 (S)	0.5 (S)	0.25 (S)	2 (S)	1 (S)	0.25 (S)	2 (S)
B5	2 (S)	4 (S)	2 (S)	4 (S)	1 (S)	1 (S)	0.5 (S)	0.25 (S)	2 (S)	1 (S)	0.25 (S)	2 (S)

Notes: MIC values are expressed in $\mu\text{g/mL}$, with susceptibility interpretations provided in parentheses based on established clinical breakpoints from CLSI M100 35th Edition (2025). Abbreviations: AMP, ampicillin; AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam; CXM, cefuroxime, CXM-AX, cefuroxime axetil; FOX, ceftiofur; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ETP, ertapenem; IMP, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; S, susceptible.

DISCUSSION

The World Health Organization (2022) recommends that the microbial safety of drinking water be assessed by monitoring specific indicator microorganisms that are commonly found in the gastrointestinal systems of humans and warm-blooded animals. These microorganisms are typically present in large numbers and can reach water supplies or distribution networks as a result of fecal pollution. For practical monitoring purposes, such indicators must also be readily detectable using simple laboratory techniques. Therefore, both the GDWQ and the PNSDW assess microbiological safety using representative bacterial indicators, including HPC bacteria, TC, and *E. coli*.

About HPC determination, it measures the number of heterotrophic bacteria that can grow on nonselective general-isolation culture media. These bacteria are typically not fastidious and can grow quickly; many also survive for long periods in treated water (WHO 2022). HPC is commonly used as a general indicator of treatment effectiveness (e.g., filtration or distillation). In our study, every sample from Brands B, C, F, and G had HPC counts above the allowed limit of 500 CFU/mL (DOH 2017; WHO 2022) (Table 3). A significant association was observed between the elevated HPC levels and the specific brands examined ($p < 0.01$). Despite refrigeration or ice storage of the bottled water prior to analysis, prior exposure to elevated temperatures or sunlight during handling and transport may have promoted microbial growth. It is important to note that a high HPC level alone does not necessarily indicate fecal contamination or an imminent health risk. However, elevated HPC counts can signal the presence of opportunistic bacteria (such as *Acinetobacter*, *Aeromonas*, or *Pseudomonas*), which could pose infection risks to immunocompromised individuals (Herath *et al.*, 2014).

Regarding yeast and mold contamination, neither the WHO water-quality guidelines nor the Philippine drinking water standard specifies limits (DOH 2017). In our study, the FDA Philippines standards for non-alcoholic beverages were used. Under these criteria, a minimum of one specimen from each of the nine

examined brands (A, B, C, F, G, I, J, N, and O) exceeded the maximum allowable yeast and mold count (Table 3). While testing for YM is not generally required, their presence may reflect lapses in hygiene during treatment, bottling, or storage, potentially compromising product quality and reducing shelf life. Certain fungal contaminants in water can also produce mycotoxins that may cause allergic reactions or infections in susceptible individuals (Ameen *et al.*, 2018; Svagzdiene *et al.*, 2010). Thus, yeast and mold contamination in bottled water is a potential public health concern (Babič *et al.*, 2017). In this study, failure to meet the YM count standard was significantly associated with the specific brands mentioned ($p < 0.01$).

Regarding numerical limits for total coliforms (TC), all five samples from Brand B had TC levels above the standard limit. In addition, two to three samples from each of Brands C, L, and O exceeded the coliform standard. Because coliform bacteria are naturally present in the environment, their detection in water does not automatically imply fecal contamination or a health risk for most consumers (Burlakoti *et al.* 2020; Palmares *et al.* 2024). However, coliforms can cause opportunistic infections in vulnerable populations such as children, the elderly, and immunocompromised individuals. Since proper disinfection normally kills coliforms, finding them in bottled water suggests inadequate disinfection, poor storage conditions, and possible post-treatment contamination (for example, by equipment or handlers in the bottling facility) (Curutiu *et al.* 2020; Hamad *et al.* 2022). In our results, failing to meet the coliform standard indicated a significant association with the particular brands mentioned ($p < 0.01$) (Table 3).

Regarding the numerical limits for *E. coli*, two out of five samples from Brand B were positive (Table 3). Detection of *E. coli* in the analyzed bottled water samples raises significant public health concerns, suggesting recent contamination with fecal matter and the possible co-occurrence of harmful microbial agents. *E. coli* serves as a key indicator organism in water quality monitoring because its presence reflects a failure in water treatment, bottling hygiene, or post-treatment handling processes (Keleb *et al.* 2022).

In contrast to the broader coliform population, *E. coli* is predominantly confined to the intestines of warm-blooded animals, which enhances its reliability as a specific marker for detecting fecal pollution (Carreon *et al.* 2025; Palmares *et al.* 2025). The detection of *E. coli* in bottled water not only violates international and national microbial safety standards, which mandate zero tolerance for this organism in 100 mL of water, but also raises the risk of waterborne infections, particularly in vulnerable populations (e.g., elderly, immunocompromised individuals, young children). Its presence underscores the need for stricter quality control, regular monitoring, and enforcement of sanitary practices in the production and distribution of bottled water (Gautam 2021).

Regarding the assessment of the physicochemical parameters, none of the bottled water samples exhibited any visible color. Measured turbidity values were consistently lower than the instrument's detection limit of 0.5 NTU. Under the GDWQ standards, both color and turbidity are considered indicators that may signal the presence of organic matter or microbial proliferation. Moreover, from a consumer perspective, deviations from these parameters are interpreted as a potential safety concern or evidence of a substandard water source and insufficient treatment process (De Queiroz *et al.* 2013; Gharibi *et al.* 2018). Although international guidelines provide recommended values for color and turbidity, these parameters are generally considered aesthetic and operational indicators rather than health-based limits (WHO 2022). In contrast, pH analysis revealed that bottled water from Brands F, H, and N fell below the WHO's recommended lower limit of 6.5. According to the GDWQ, acidic water can corrode pipes, potentially leading to secondary contamination from metals or microbes in the distribution system. Notably, Brands F and N, which had low pH values, also failed some of the microbial standards. Despite this, there is no direct health threshold for slightly acidic bottled water; the main concern is its effect on plumbing and tooth enamel (Schmidt and Huang 2022). Regarding chloride, all samples had levels below 0.5 ppm, far under the maximum 250 ppm. Therefore, the data were not presented in the results. High chloride can accelerate pipe corrosion, but like color and turbidity, it is also more of an infrastructural concern than a direct health hazard (Gharibi *et al.* 2018).

Regarding the distribution of microbiological non-compliance among bottled water samples, it varied across the sampling locations in Rizal Province (Figure 2). District 1 showed the highest percentage of samples failing microbiological standards, particularly for TC (53%), HPC (67%), and YM (40%), and was the only district where *E. coli* was detected (13%). In contrast, no *E. coli* contamination was observed in Districts 2–4 and Antipolo City (Figure 2). Nevertheless, elevated HPC and YM levels were still recorded in several districts, notably District 3 (47% and 33%) and District 2 (33% and 27%). The higher proportion of non-compliant samples in District 1 suggests that microbiological contamination is not uniformly distributed across Rizal Province. This spatial variability may reflect differences in water sources used by bottling facilities, sanitation practices during processing, storage conditions during distribution, or hygiene practices at retail points such as street vendors and sari-sari stores (Adesakin *et al.* 2022).

Overall, more than half of the analyzed samples (56%, 42/75) exhibited microbial indicators exceeding the permissible limits established by the PNSDW and GDWQ for HPC, TC, and *E. coli*, and by the FDA Philippines for YM (Table 3). Specifically, the proportion of samples exceeding acceptable limits was 2.7% for *E. coli*, 16% for total coliforms, 33% for heterotrophic plate counts, and 27% for yeast and molds, highlighting significant deviations from regulatory standards (Table 3). Statistical analysis revealed that higher *E. coli* levels were positively correlated with elevated total coliform counts, while increased HPC levels were significantly associated with both total coliform and yeast/mold

counts ($p < 0.01$). No statistically significant correlation was observed between the microbial concentrations and pH levels of the bottled water samples ($p > 0.05$). Additionally, two coliform isolates identified as *E. coli* were found to be fully susceptible to all 30 antibiotics included in the test panel. This may indicate limited exposure of the source water or contamination pathways to antibiotic-selective pressures, reflecting low levels of antimicrobial contamination or minimal anthropogenic impact (Carreon *et al.*, 2025). Nevertheless, the small number of isolates limits definitive conclusions, and continued monitoring of antimicrobial resistance in waterborne *E. coli* remains important for public health surveillance.

CONCLUSION

These results indicate that some unbranded or generic bottled waters sold by street vendors and “sari-sari stores” may not receive adequate treatment or quality assurance before sale, leading to contaminated products. Regulatory authorities should ensure that all bottled water producers and distributors are properly registered, licensed, and fully compliant with safety standards throughout production, storage, and delivery. Special attention should be given to preventing post-packaging contamination, for example, by improving hygiene in vending environments. Regular inspection of water supply and bottling systems (pipes, tanks, and taps) is also advisable. Finally, conducting local surveys on public perceptions of bottled water quality and consumption habits could help inform education campaigns, guiding consumers to make safer choices when purchasing bottled water.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

AJ Palmares conceived and supervised the study. All authors contributed to methodology design, sample collection, experimentation, data analysis, and manuscript preparation.

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