Article

Insufficient Chlorination as the Critical Determinant of Microbiological Non-Compliance in Rural Water Systems: First Integrated Assessment in Azuero, Panama
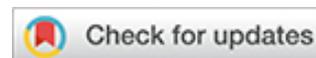
Astrid Moreno (iD) * [1], Michelle Aguirre (iD) [1], Alexis De La Cruz L (iD) [1]

[1] University of Panama, Azuero Regional University Center, Faculty of Natural, Exact and Technological Sciences, Panama.
michelle.aguirre@up.ac.pa
alexis.delacruz@up.ac.pa
Correspondence author: astrid-y.moreno-g@up.ac.pa

Check for updates

ABSTRACT

Access to safe drinking water in rural communities depends heavily on day-to-day system operation and sustained disinfection, both of which are often fragile. Although global reports describe widespread microbiological contamination of rural water supplies, there remains a lack of integrated studies that pinpoint the specific drivers of failure in regions such as Azuero, Panama. In this cross-sectional study, we evaluated rural water systems in the municipalities of Chitré, La Villa de Los Santos, and Santa Ana, using a combination of microbiological and physicochemical indicators. Total coliforms, *Escherichia coli,* and nematodes were assessed by membrane filtration and microscopic examination, while pH and free residual chlorine were measured in situ.

Nematodes were not detected in any sample. In contrast, total coliforms were detected at all sampling points, with concentrations ranging from 1 to 4,556 CFU/100 mL, clearly above the regulatory limit (<1 CFU/100 mL). *E. coli* was found at three sites (47–110 CFU/100 mL). Although pH values complied with national standards, free residual chlorine was frequently absent or below the recommended range. No significant differences in bacterial loads were observed between tanks and wells ($p > 0.05$), but a weak negative correlation between residual chlorine and total coliforms ($r = –0.25$) suggests that inconsistent chlorination is the main factor associated with microbiological non-compliance.

This work provides the first integrated assessment of rural water systems in the Azuero region, jointly analysing microbiological, physicochemical, and operational information. The results show that inadequate chlorination—rather than infrastructure type—undermines water quality, and they point to an urgent need to reinforce community-level disinfection practices, preventive maintenance, and continuous monitoring in rural Panama.

Keywords: Drinking water quality, Rural aqueducts, Total coliform bacteria, *Escherichia coli* contamination, Chlorination failure, Free residual chlorine, Microbiological water monitoring, Community water systems, Waterborne pathogens, Panama

INTRODUCTION

Access to microbiologically safe drinking water is still a major public health challenge, particularly in rural areas where supply systems often operate with limited resources and technical support. The World Health Organization (WHO) estimates that about 2 billion people worldwide rely on drinking-water sources contaminated with fecal matter, which contributes to the burden of diarrhoeal disease, dysentery, and cholera[1]. *Escherichia coli* is widely used as the primary indicator of recent fecal contamination, and its detection in drinking water suggests the possible presence of enteric pathogens[2]. The WHO Guidelines for Drinking-Water Quality therefore specify that both coliform bacteria and *E. coli* must be absent in 100 mL samples intended for human consumption[1].

In many low- and middle-income countries, rural water systems frequently fail to meet these microbiological criteria. Recent global assessments indicate that up to 70% of such systems show some degree of contamination[5,17], mainly due to weaknesses in disinfection, lack of routine maintenance, and the vulnerability of small-scale infrastructure[6,7]. The United States Environmental Protection Agency (EPA) likewise emphasises the role of free residual chlorine as the principal barrier against microbial contamination in decentralized supplies, recommending that residual concentrations be maintained within a narrow range to ensure effective inactivation of bacteria[3,15].

Panama has adopted national regulations such as DGNTI-COPANIT 21-2019 to govern drinking-water quality, but applying these standards in community-managed rural systems remains difficult in practice. Studies carried out in Azuero have reported the presence of coliforms and fluctuations in residual chlorine in local aqueducts, where populations are supplied mainly through wells and storage tanks that do not always receive systematic technical supervision[8]. Similar situations have been described elsewhere in Latin America, where microbiological contamination is often linked to irregular chlorination, leaky or precarious connections, and the absence of regular cleaning of storage infrastructure[9,18].

Against this backdrop, the present study is novel in two ways. First, it represents the first integrated assessment of rural water systems in the Azuero region, simultaneously examining microbiological, physicochemical, and operational variables. Second, it explicitly tests the hypothesis that the key determinant of regulatory failure is not the type of infrastructure (tanks versus wells), but inadequate operational management, particularly insufficient free residual chlorine. By combining field measurements with a detailed comparison with international standards, this work generates robust, actionable evidence to inform public health policy and community water-safety interventions.

MATERIAL AND METHODS

Study Design

A descriptive, cross-sectional study was conducted to evaluate physicochemical and microbiological water quality in rural supply systems located in the Azuero region of Panama. Sampling was performed during a single field campaign and included community-managed storage tanks and wells from the localities of La

Arena, Chitré, Monagrillo, Santa Ana, and Los Santos. The study focused on detecting total coliforms, *Escherichia coli*, and nematodes, and on measuring pH and free residual chlorine.

Sampling Sites

Five representative rural communities were selected based on population relevance, accessibility, and their dependence on decentralized water systems. Coordinates were recorded following GPS verification.

Sequence	Sampling Site	Coordinates
1	La Arena	7°58′16″N, 80°27′51″W
2	Chitré	7°58′00″N, 80°26′00″W
3	Monagrillo	7°59′36″N, 80°26′40″W
4	Santa Ana	7°40′00″N, 80°25′00″W
5	Los Santos	7°36′00″N, 80°27′00″W

Table 1. Sampling points in the Azuero region.

Sample Collection

Two volumes were collected at each sampling point using sterile sampling bottles:

- 300 mL for nematode detection.
- 100 mL for bacteriological analysis of total coliforms and *E. coli*.

Samples were collected following aseptic procedures, ensuring no contact between bottle, hands, or external surfaces. Each bottle was labeled with the sample code, date, site, and time of collection.

During sampling, pH and free residual chlorine were measured *in situ* using a DPD colorimetric kit (Hach® or equivalent), following manufacturer instructions and standards for drinking water field monitoring.

Samples were transported to the Environmental Analysis Laboratory of Chitré in insulated coolers at 4 °C and processed within 6 hours of collection.

Microbiological Analysis

Detection of Nematodes

A 300 mL water sample was filtered through a 0.45 µm cellulose membrane using a sterile filtration system. The membrane was then placed into a sterile container with distilled water and examined under optical microscopy (10× and 40× objectives) to determine the presence or absence of nematodes.

Total Coliforms and *Escherichia coli*

A 100 mL sample was filtered through a 0.45 µm sterile membrane, which was transferred onto a chromogenic medium (Colilert®, m-ColiBlue24®, or equivalent). Plates were incubated at 37 °C for 24 hours.

Colonies of total coliforms and *E. coli* were counted and expressed as CFU/100 mL, following color-based differentiation outlined in the chromogenic protocol.

Physicochemical Analysis

pH and free residual chlorine were measured in the field immediately after sample collection.

Results were compared against the national drinking water standard DGNTI-COPANIT 21-2019, which establishes:

- pH: 6.5–8.5
- Free residual chlorine: 0.3–0.8 mg/L

Statistical Analysis

Data processing was performed using standard statistical procedures:

- Shapiro–Wilk test was used to assess data normality.
- Student's t-test compared microbiological values between tanks and wells.
- Pearson correlation coefficient (r) evaluated the association between free residual chlorine and total coliform levels.

A significance threshold of $p < 0.05$ was applied.

Ethical Considerations

No human subjects or laboratory animals were involved in this study.

Water samples were collected from public rural water systems following environmental monitoring practices and did not require institutional ethics approval.

RESULTS

Sampling Locations

Five rural communities in the Azuero region were evaluated. Their coordinates and geographic positions are shown in Table 1, and their spatial distribution is illustrated in Figure 1.
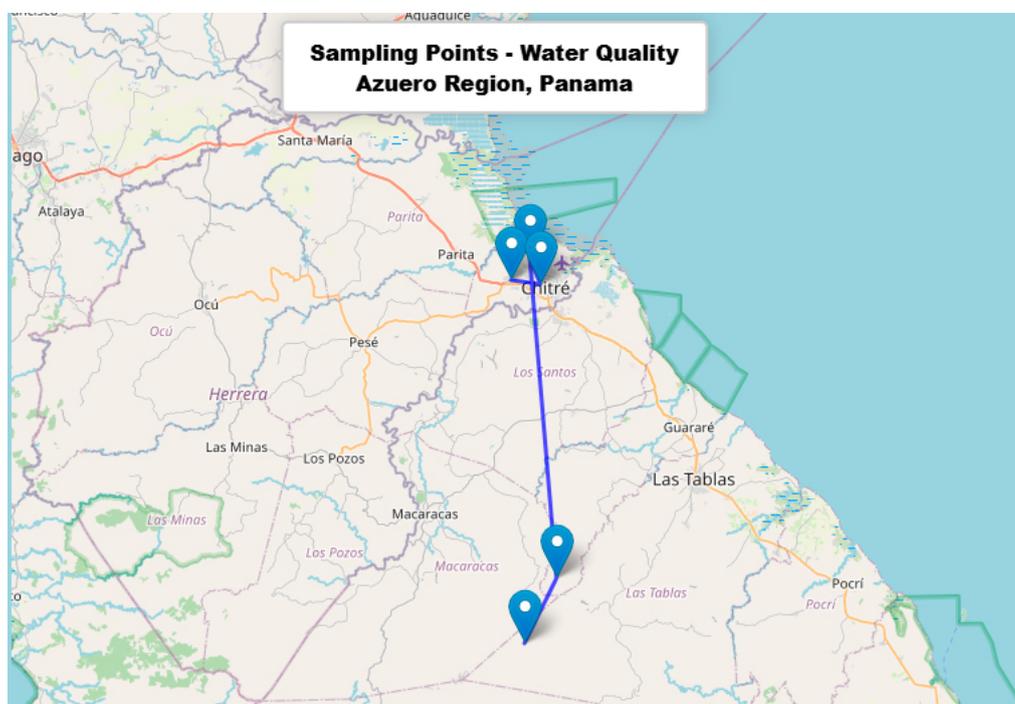
Figure 1. Geographic distribution of the five rural sampling sites evaluated in the Azuero region of Panama.

The map shows the locations of La Arena, Chitré, Monagrillo, Santa Ana, and Los Santos, where physicochemical and microbiological water quality parameters were assessed. All sites exhibited microbiological non-compliance, highlighting a widespread and systemic issue in rural water systems.

Physicochemical Parameters

Most physicochemical parameters complied with DGNTI-COPANIT 21-2019 requirements, except for free residual chlorine, which exhibited substantial variability and recurrent non-compliance.

In storage tanks, pH ranged from 6.8 to 7.8 (mean 7.13), while free residual chlorine ranged from 0 to 3.0 mg/L (mean 0.5066 mg/L).
In wells, pH was slightly more stable (6.8–7.2; mean 6.96), and free residual chlorine levels were consistently low (0–0.2 mg/L; mean 0.12 mg/L).

These results are detailed in Table 2.

Tanks

pH	6.8	7.8	7.13	6.8	0.3976	6.5–8.5
Free residual chlorine (mg/L)	0	3.0	0.5066	0.3	0.7573	0.3–0.8

Wells

Parameter	Min	Max	Mean	Median	SD	Permitted Range
pH	6.8	7.2	6.96	6.8	0.21908	6.5–8.5
Free residual chlorine (mg/L)	0	0.2	0.12	0.2	0.1095	0.3–0.8

Table 2. Physicochemical parameters in tanks and wells compared with DGNTI-COPANIT 21-2019 standards.

Microbiological Parameters

Microbiological analysis revealed universal contamination.
Total coliforms were detected in 100% of samples, exceeding the permitted limit (<1 CFU/100 mL) in all locations.

- Tanks: 1–4,556 CFU/100 mL
- Wells: 110–387 CFU/100 mL

Escherichia coli was detected at three sampling points:

- Minimum: 47 CFU/100 mL (well 2)
- Maximum: 110 CFU/100 mL (tank 9)
- Overall median: 88 CFU/100 mL

Mesophilic heterotrophs exceeded the DGNTI-COPANIT 77-2007 limit in one tank (581 CFU/mL).

A consolidated summary is provided in Table 3.

Parameter	Tanks	Wells	Interpretation
Total coliforms (range)	1–4,556	110–387	All samples exceeded the limit
Total coliforms (mean)	393.27	125.6	Higher variability in tanks
Geometric mean	~32.84	~32.84	Similar central tendency
***E. coli* detected**	110 (Tank 9)	47 (Well 2)	Recent fecal contamination
Mesophilic heterotrophs	581 (Tank 15)	<100	One tank exceeded the limit
Nematodes	Not detected	Not detected	No helminth contamination

Table 3. Summary of microbiological results in rural water systems (Azuero region).

Comparison with Regulatory Standards

All water sources exceeded DGNTI-COPANIT 21-2019 microbiological limits.
Regulatory thresholds are shown in Table 4.

Parameter	Unit	Permitted Limit
Total coliforms	CFU/100 mL	<1
E. coli	CFU/100 mL	<1
Mesophilic heterotrophs	CFU/mL	<100

Table 4. Microbiological regulatory limits (DGNTI-COPANIT 21-2019; DGNTI-COPANIT 77-2007).

Nematode Analysis

Microscopic examination revealed no presence of nematodes in any of the 300 mL samples.

Statistical Analysis

Normality was confirmed using the Shapiro–Wilk test for total coliforms and *E. coli*.

A Student's t-test indicated no significant differences between tanks and wells (t = 0.50465; p = 0.61993).

Pearson's correlation demonstrated a weak negative association between free residual chlorine and total coliform levels (r = –0.2505).
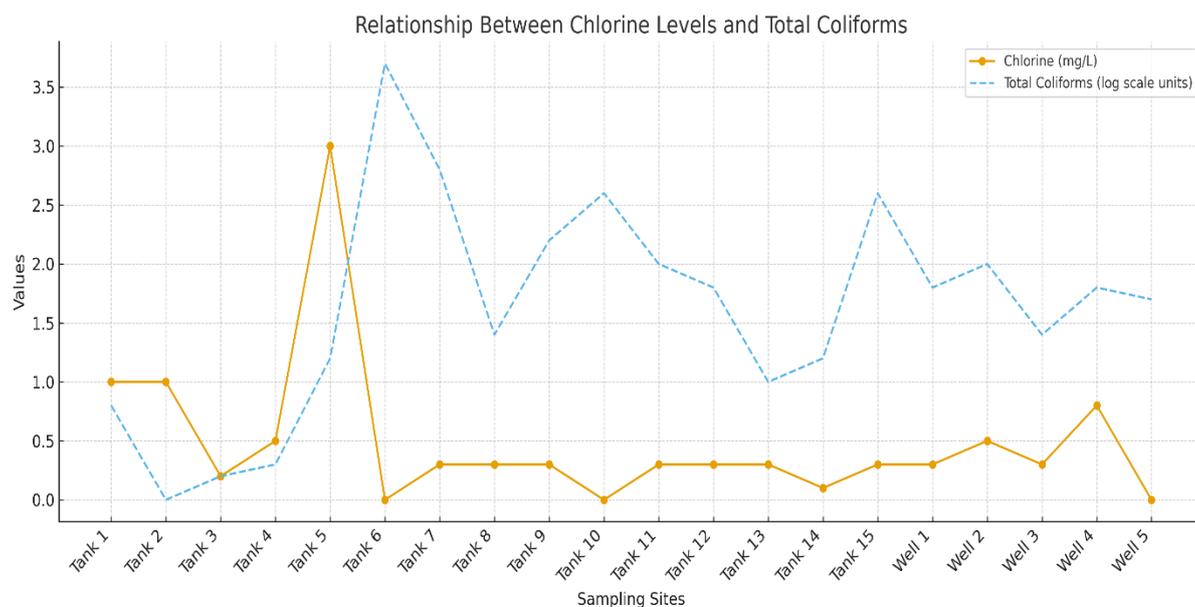
This relationship is visually represented in Figure 2.

Figure 2. Scatterplot showing the relationship between free residual chlorine and total coliform concentrations.

DISCUSSION

The findings of this study show that all analysed samples contained total coliforms and that *E. coli* was detected at several sites, indicating clear non-compliance with WHO recommendations, which require the complete absence of these indicators in water intended for human consumption[1,2]. This pattern mirrors reports from other rural settings around the world, where microbial contamination of small systems is common and is frequently linked to shortcomings in disinfection and day-to-day operation[5,17].

In our study, pH values remained within acceptable limits, suggesting that the main problem is not related to major physicochemical imbalances but to the instability of free residual chlorine. Residual chlorine was often absent or below the recommended range, particularly in wells, which is consistent with the EPA's view of chlorine as the primary defence against microbial contamination in decentralized systems[3,15]. Studies from Africa, Asia, and Latin America have similarly documented that decreases in residual chlorine are associated with higher levels of coliforms and *E. coli* in rural supplies[6,7,18].

The presence of *E. coli* at three sampling points is particularly relevant from a public health perspective, as it reflects recent fecal contamination and indicates an increased risk of waterborne disease. Global estimates attribute millions of cases of gastrointestinal illness each year to untreated or poorly disinfected rural water systems[10,17]. The absence of significant differences between tanks and wells in bacterial load suggests that infrastructure type is not the main driver of risk. Instead, what appears to matter most is how systems are operated—whether chlorination is consistent, whether tanks are cleaned, and whether distribution networks are protected from intrusions[6,7]. This interpretation is consistent with previous observations in Panama and

other Latin American countries, where community management often lacks clear, standardised protocols for chlorine handling and infrastructure maintenance[8,18].

A key contribution of this study is to demonstrate that inadequate chlorination is the critical factor associated with microbiological non-compliance in the evaluated systems. International assessments have shown that even when pH and turbidity values meet guideline levels, microbiological quality may still fail if residual chlorine is not maintained within recommended ranges[1,3,5]. The negative correlation between residual chlorine and total coliforms observed here, although modest, aligns with studies describing highly variable conditions in rural systems, where sediment accumulation, biofilm development, infiltration, and lack of cleaning can all reduce the effectiveness of chlorine[6,7,18].

Finally, the absence of nematodes in the samples analysed is consistent with the low prevalence typically reported for deep or semi-protected supply systems. Nevertheless, targeted surveillance of these organisms remains relevant in areas where water sources may be exposed to surface runoff or other environmental inputs[1,7].

Implications for policy and practice

The results of this study have clear implications for the management of rural water services in Panama and similar contexts. Demonstrating that tanks and wells show comparable microbial loads challenges the common assumption that the source itself is the main determinant of risk. Policies focused solely on constructing new wells or storage structures are therefore unlikely to solve the problem if operational practices do not improve at the same time. Given limited resources, priority should be placed on strengthening community operators' skills and ensuring a reliable supply of disinfectants.

Identifying insufficient chlorination as the central weakness enables targeted, measurable interventions. Rather than generic calls to "improve water quality", the evidence supports three concrete lines of action: (i) standardised training programmes based on WHO Water Safety Plans, emphasising correct dosing, verification of residual chlorine and corrective actions; (ii) simplified community monitoring, using affordable DPD kits so that users can check residual chlorine daily; and (iii) mandatory preventive maintenance, including regular cleaning and disinfection of storage tanks to limit sediment and biofilm accumulation that consume residual chlorine and shelter bacteria.

CONCLUSIONS

In summary, this study shows that the microbiological quality of water in the evaluated rural systems in Azuero is inadequate, with universal detection of total coliforms and sporadic occurrence of *Escherichia coli*. Basic physicochemical parameters such as pH met national standards, but residual chlorine was frequently absent or below the recommended range. Taken together, the findings indicate that the main problem is not the type of infrastructure but inconsistent chlorination and insufficient operational management of community systems. Strengthening disinfection protocols, standardising chlorination procedures, ensuring periodic cleaning of tanks and wells, and establishing continuous monitoring programmes are essential steps to secure safe water,

reduce the burden of waterborne disease, and improve local stewardship of rural water supplies in the Azuero region.

Institutional Review Board Statement

Not applicable. This study did not involve human participants or laboratory animals. All water samples were collected from rural community water systems following standard environmental monitoring practices.

Informed Consent Statement

Not applicable. No human subjects were involved in the study.

Data Availability Statement

All data generated in this study were obtained directly from field sampling and laboratory analyses conducted by the research team.
No external datasets were used. Additional details are available upon reasonable request from the corresponding author.

Data and AI Disclosure

All figures and tables were created by the authors using data obtained through field sampling and laboratory analyses described in this manuscript.
Figures 1–3 were generated, edited, or redesigned using author-supervised AI-assisted workflows (OpenAI),

strictly for visualization and language editing purposes. No AI system generated scientific data, processed raw measurements, or produced original scientific interpretations.

Generative artificial intelligence was used only for language polishing, grammatical correction, and formatting standardization, always under complete human supervision. The authors independently verified all scientific results, interpretations, and conclusions in accordance with BioNatura Journal's policy on AI-assisted content (https://bionaturajournal.com/artificial-intelligence--ai-.html).

REFERENCES

1. World Health Organization. *Guidelines for Drinking-Water Quality*. 4th ed. Geneva: WHO; 2022.

2. World Health Organization. *Escherichia coli – Fact Sheet*. Geneva: WHO; 2017. Disponible en: https://www.who.int/es/news-room/fact-sheets/detail/e-coli

3. United States Environmental Protection Agency (USEPA). *National Primary Drinking Water Regulations*. Washington, DC: USEPA; 2023.

4. Serrano-Suárez A, Dellundé J, Salvadó H, Cervero-Aragó S, Méndez J, Canals O, et al. Microbial and physicochemical parameters associated with *Legionella* contamination in hot-water recirculation systems. *Environ Sci Pollut Res Int.* 2013;20(8):5534–44.

5. Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Global assessment of rural drinking water contamination. *Nature Water.* 2021;1:1–12.

6. Shields KF, Bain R, Cronk R, Wright J, Bartram J. Assessing rural water supply reliability and health impacts. *Water Res.* 2020;185:116283.

7. Anthonj C, Setty K, Ezbakhe F, Manga M, Hoeser C. Pathways of microbial contamination in rural drinking water systems: a systematic assessment. *Sci Total Environ.* 2022;807:150928.

8. Cruz H, Montañez D. Diagnóstico de la calidad bacteriológica del agua potable en comunidades rurales de Azuero. *Centros.* 2024;13(1):24–37.

9. Sánchez H, Vargas M, Méndez J. Calidad bacteriológica del agua para consumo humano en zonas de alta marginación de Chiapas. *Salud Pública Méx.* 2000;42(5):10–6.

10. Banco Mundial. Las enfermedades transmitidas por alimentos cuestan US$110 000 millones anuales. Washington, DC: The World Bank; 2018.

11. Organización Panamericana de la Salud (OPS). *Día Mundial de la Higiene de las Manos 2023*. Washington, DC: OPS; 2023.

12. Rosales Borjas DM, Arévalo Rosales MA. *Pseudomonas aeruginosa: un problema hospitalario*. Repositorio Latinoamericano; 2017. Disponible en: http://repositorioslatinoamericanos.uchile.cl/handle/2250/210997

13. World Health Organization. *Microbial Aspects of Drinking Water*. Geneva: WHO; 2020.

14. World Health Organization. *Water Safety Plans: A Field Guide to Improving Drinking-Water Safety*. Geneva: WHO; 2017.

15. United States Environmental Protection Agency (USEPA). *Ground Water Rule*. Washington, DC: USEPA; 2022.

16. Sobsey MD, Stauber CE, Casanova LM, Brown JM, Elliott MA. Point-of-use household drinking water filtration: a practical, effective solution for improving drinking water quality. *J Water Health.* 2008;6(1):1–7.

17. Bain R, Johnston R, Mitis F, Chatterley C, Slaymaker T. Drinking-water quality in low- and middle-income countries: 30 years of evidence. *Int J Hyg Environ Health.* 2020;224:113439.

18. Flores J, Galvis A, Pérez S, Martínez L. Microbiological quality of rural drinking water and factors affecting contamination in community-managed systems in Latin America. *Water Sci Technol.* 2022;86(1):249–60.

Additional Information

Correspondence should be addressed to: *astrid-y.moreno-g@up.ac.pa*

Governance

For editorial governance and co-publisher responsibilities, see the **BIPC Governance Framework (PDF)** at:

https://clinicalbiotec.com/bipc