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# DETECTION OF ANTIBIOTIC-RESISTANT BACTERIA IN DRINKING WATER SOURCES WITHIN THE COLOMBO DISTRICT OF SRI LANKA

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The rise of antibiotic-resistant bacteria in water distribution systems poses a significant risk to public health, particularly in regions such as Sri Lanka, where much of the infrastructure was built decades ago and may not meet present-day standards for bacterial control. This study aimed to detect the presence of antibiotic-resistant bacteria in drinking water sources within the Colombo District of Sri Lanka, focusing on two household sampling sites to which water is supplied by the National Water Supply and Drainage Board (NWSDB). Five biological replicates were analysed using various culturing methods: direct spread plating, direct quadrant streaking, and nutrient broth enrichment spread plating. This was followed by colony morphology observation, Gram staining, biochemical tests: catalase test, oxidase test, methyl-red, and Antibiotic susceptibility testing (ABST) via Kirby-Bauer disc diffusion method. The antibiotics tested were Gentamycin (10µg), Vancomycin (30µg), Ciprofloxacin (5µg), and Ampicillin (10µg). The results were analysed using Cowan and Steel's biochemical guidelines, WHO-listed waterborne pathogens, and CLSI guidelines. No fully resistant bacterial species were observed across all samples. However, potential intermediate resistance to Ciprofloxacin (5µg) by provisional *Campylobacter* spp. or *Pseudomonas* spp. was observed across an isolate from each sampling site, validating the importance of performing routine ABST to monitor bacterial resistance trends. Statistical analysis (ANOVA) confirmed no significant variation in antibiotic response across the samples. Future work should utilise molecular methods such as PCR for bacterial species confirmation and sample collection from multiple districts to assess bacterial resistance trends.

*Keywords*: antibiotic resistance, bacterial identification, culturing water samples, Kirby-Bauer disc diffusion, water distribution systems, waterborne pathogens, water quality monitoring.

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## INTRODUCTION

Antibiotic resistance has become a growing public health concern globally, where many bacterial species, such as methicillin-resistant *Staphylococcus aureus* (MRSA), certain strains of *Klebsiella pneumoniae* and *Escherichia coli,* are no longer responding to prescribed antibiotics. This is largely due to overexposure of bacteria to antibiotics via patients taking them without a prescription and improper disposal (WHO, 2019; Kamal et al., 2022; Cantón and Morosini, 2011)

These antibiotics and resistant bacteria can make their way into household drinking water systems due to the inability of current water filtration systems to remove them from water sources. They proliferate within the water distribution systems via the formation of biofilms, which compounds the issue, and this creates a vicious cycle in an area with rising antibiotic resistance (Duarte et al., 2022).

In Sri Lanka, water distribution systems have poor maintenance records, and infrastructure as old as 50 years is still in use, leading to excess amounts of biofilms forming within the pipelines of the water distribution systems, which further exacerbates the situation (Munasinghe et al., 2017; Asian Development Bank, 2012).

A study conducted by Wijesekara, Weerasinghe, and Seneviratne (2021) across 4,508 households in the Western Province revealed widespread bacterial contamination in drinking tap water. Their findings, which reported elevated total coliform and thermotolerant coliform counts, highlight the significant health risks posed by bacterial exposure in household water supplies.

When such contaminated water comes into contact with antibiotics, biofilm-associated bacteria can acquire resistance and subsequently contaminate wider water systems. These bacteria may then reach households and ultimately enter the bodies of people consuming the water. This demonstrates a clear public health concern for the Sri Lankan population, yet research on the presence of antibiotic-resistant bacteria in household drinking water remains limited.

**Null hypothesis:** In Colombo District, tap water is contaminated with at least one antibiotic-resistant bacterium.

This research aims to validate a suitable methodology to culture and test drinking water samples for antibiotic resistance using biochemical tests and disc diffusion-based antibiotic discs.

## METHODOLOGY

Two random household drinking water sampling sites (sampling site 01: 6°54'03.3"N 79°53'42.9"E, sampling site 02: 6°55'59.2"N 79°53'35.8"E) were selected from the Colombo District. Weekly, a biological replicate consisting of three technical replicates (each at 300mL) was obtained consecutively from each location for 5 weeks during the time period December 2024- January 2025. Samples were collected in sterile glass jars and stored at room temperature for 24 hours before culturing, simulating typical household water storage conditions.

Three culturing methods were utilised throughout the research.

Method 01- Direct spread plating of 1 mL onto Nutrient Agar at 37°C for 48 hours.

Method 02- Drop-inoculation and quadrant streaking on Nutrient Agar (and MacConkey agar) at 37℃ for 48 hours.

Method 03- Enrichment of water sample with Nutrient Broth (at 3:1) at 37℃ for 24 hours, followed by drop-inoculation and quadrant streaking on Nutrient Agar (and MacConkey agar) at 37℃ for 24 hours.

Colonies were characterised by their morphology (macroscopic and microscopic observations). An identified subset of isolates underwent the Catalase test, Oxidase test, and Methyl Red Test. The manual by Cowan and Steel (1993) was used for the profiling of the bacteria using biochemical test results. Antibiotic susceptibility testing (ABST) was done via the Kirby-Bauer disc diffusion method using Gentamycin 10µg, Vancomycin 30µg, Ciprofloxacin 5µg, and Ampicillin 10µg to cover gram-positive and gram-negative bacterial species.

An ANOVA test was performed via R Studio (v 2024.09.1) using data from the ABST across all five biological replicates based on their clear zone diameter.

## RESULTS AND DISCUSSION

Method 03 proved to be most effective at obtaining optimum colony growth. A total of 16 isolates were cultured. Biochemical profiling narrowed potential genera to *Acinetobacter, Campylobacter, Pseudomonas, Micrococcus, Helicobacter* and *Mycobacterium,* with species-level identification beyond the scope of this study. There was no growth seen on the MacConkey agar plates.

According to the colony-forming unit (CFU) testing, sampling site 01 showed a higher colony count (with an average of $28.38 \times 10^6$ $CFU$/mL) compared to sampling site 02 (with colonies too few to count)

No isolates met CLSI-defined resistance criteria. However, two isolates show concern of intermediate resistance (as seen in Figure 01): An isolate from sampling site 01 (provisionally *Acinetobacter* spp., *Campylobacter* spp., or *Pseudomonas* spp.) and sampling site 02 (provisionally *Campylobacter* spp. or *Pseudomonas* spp.), if confirmed as either *Campylobacter* spp. or *Pseudomonas* spp. these would be interpreted as showing intermediate resistance to Ciprofloxacin 5µg.

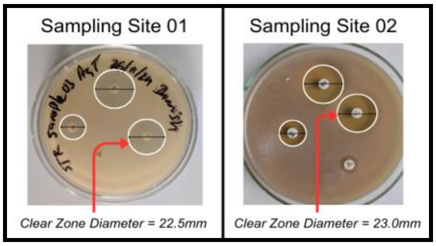


Figure 26 Images of Mueller-Hinton Agar Culture plates for ABST of isolates from sampling site 01 (left image) and sampling site 02 (right image) showing potential intermediate resistance to Ciprofloxacin 5µg according to the CLSI range of 21-23mm and 19-24mm classified as intermediate resistance for *Campylobacter* spp. and *Pseudomonas* spp. respectively.

The P-value of the ANOVA test was greater than 0.05, indicating no statistical significance, which means the bacterial response to antibiotics for several technical replicates was similar.

## CONCLUSION / RECOMMENDATIONS

Further biochemical testing to obtain species-level identification should be done, followed by molecular identification tests, such as PCR and sequencing of the bacterial samples, to confirm the identification. This research can be expanded throughout the districts to identify trends and patterns from different areas. With different variables, such as the original water source and purification system.

Furthermore, data can be gathered from hospitals on patients contracting antibiotic-resistant strains of bacteria, and a correlation test can be conducted with the results obtained from water samples.

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