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Tabassum, Sumaya

School of Business and Entrepreneurship, Independent University, Bangladesh (IUB)

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Occurrence and Removal Efficiency of Multiple Antibiotic-Resistant Bacteria in Rajshahi's Water Supply System

Sumaya Tabassum
Department of Civil Engineering, Rajshahi University of Engineering & Technology, Rajshahi, Bangladesh
sumaya@ce.ruet.ac.bd

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Abstract - **Multiple antibiotic-resistant (MAR) bacteria have recently been detected in Dhaka's municipal water, prompting concerns about water safety in Bangladesh's other cities. Rajshahi Water Supply & Sewerage Authority (RWASA) meets 91 % of its demand from groundwater and 9 % from the Padma River, into which untreated urban drains discharge. In this study, we first identified the thirteen most commonly used antibiotics in Rajshahi, then collected water samples from the raw river intake, each treatment-plant unit process, finished surface water ready for distribution, the groundwater pumping station, and household taps served by both networks. Bacterial isolation was performed via membrane filtration, and antibiotic susceptibility was assessed by the disk diffusion method following CLSI guidelines. Isolates from raw-river and intermediate treatment stages exhibited resistance to all selected antibiotics, whereas finished surface water and stagnant river samples were uniformly susceptible. Notably, no culturable bacteria were detected at the intake from Padma after repeated sampling, and post-chlorination groundwater samples showed negligible MAR presence. These findings demonstrate that RWASA's multi-step surface-water treatment train.**

Keywords **- Multiple antibiotic-resistant bacteria, Rajshahi WASA, disk diffusion, surface-water treatment, Padma River**

I.INTRODUCTION

The global proliferation of multiple antibiotic-resistant (MAR) bacteria in water supplies presents a mounting threat to public health, challenging the assumption that conventional treatment reliably delivers safe drinking water. Municipal and environmental waters have been identified as reservoirs and transmission pathways for resistant strains, with selection pressures during disinfection and distribution processes sometimes amplifying MAR prevalence [4] [7]. In Bangladesh, a growing body of evidence highlights widespread contamination of drinking and surface waters with MAR organisms. It is found that 80 % of Dhaka's municipal water samples contained coliforms and 34 % harbored fecal coliforms, with multidrug resistance observed across isolates and cefotaxime remaining one of the few consistently effective agents [10]. Researchers reported ubiquitous ESBL-producing E. coli in drinking water, wastewater, and surface-water bodies, while carbapenem-resistant strains were especially prevalent in effluents from urban markets [5]. Some studies documented 100 % resistance of Pseudomonas aeruginosa in Dhaka surface waters to ampicillin and high susceptibility to third-generation antibiotics, underscoring site-specific resistance profiles with significant zoonotic and environmental health implications [9].

Further investigations reveal alarmingly high MAR indices across diverse settings. Researchers detected resistance to eight of ten antibiotic classes in isolates from livestock-farm and fish-pond waters, with MAR indices ranging from 0.4 to 0.9 [15]. Besides, nearly half of the hospital and market wastewater isolates in Noakhali were observed resistant to at least six antibiotics [11]; Vibrio spp., E. coli, Salmonella spp., and fecal coliforms were found in Dhaka wastewater [13], each exhibiting significant antibiotic resistance patterns. Seasonal and spatial dynamics further complicate the landscape: post-monsoon MAR prevalence in the River Hooghly was reported at 73.6 %, compared to 53.6 % in summer [8], while downstream coliform MAR indices were recorded as 0.43 versus 0.15 upstream [6].

Surface-water bodies and wastewater effluents serve as critical reservoirs and conduits for antibiotic resistance genes, with high loads documented in rivers, urban drains, hospital effluents, and market wastewater across South Asia [5, 14, 2]. Groundwater aquifers, while generally lower in microbial load, can still harbor resistant Pseudomonas aeruginosa and fecal coliforms, with regional variation driven by hydrogeochemical conditions and recharge dynamics [1, 3, 12]

Despite extensive characterization of MAR bacteria in Bangladesh's waters, there is a critical knowledge gap regarding the fate of these organisms within multi-source urban supply systems. Rajshahi Water Supply & Sewerage Authority (RWASA) draws 9 % of its water from the Padma River, into which untreated urban drains discharge, and 91 % from deep aquifers, yet the occurrence, persistence, and removal efficiency of MAR bacteria through each stage of RWASA's treatment and distribution network remain unexamined.

This study therefore aims to (1) identify the thirteen most commonly used antibiotics in Rajshahi's clinical settings; (2) quantify MAR bacterial loads at key points including the Padma-river intake, successive treatment-plant unit processes, finished surface water, central groundwater pumping station, and household taps; and (3) evaluate the effectiveness of membrane-filtration and disk-diffusion assays in monitoring MAR organisms. By establishing a comprehensive baseline of MAR incidence and treatment-step removal performance, we seek to inform targeted interventions and operational

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enhancements that will safeguard the microbiological quality of Rajshahi's dual-source water supply.

II. METHODOLOGY

A cross-sectional survey was conducted to assess antibiotic resistance profiles of environmental bacterial isolates in Rajshahi. Five sampling points, including raw Padma-river intake, sequential treatment-plant outlets, a groundwater pumping station, and household taps, were visited between June and August 2025. At each location, 1-L sterile bottles were used to collect triplicate grab samples following APHA guidelines. Samples were transported on ice and processed within four hours of collection.

Each 100-mL aliquot was vacuum-filtered through a 0.45-µm cellulose nitrate membrane (Millipore). Filters were aseptically placed onto nutrient agar and incubated at 37 °C for 24 h. Colonies exhibiting distinct morphologies were sub-cultured to purity. Isolates were preliminarily characterized by Gram staining and colony morphology, then subjected to standard biochemical assays (oxidase, catalase, indole, methyl red, Voges–Proskauer, citrate utilization) according to Bergey's Manual of Systematic Bacteriology.

Pure isolates were tested against 13 antibiotics representing 10 classes: azithromycin (15 µg), chloramphenicol (30 µg), cefotaxime (30 µg), nalidixic acid (30 µg), cotrimoxazole (1.25/23.75 µg), cephalexin (30 µg), cefuroxime (30 µg), tetracycline (30 µg), ampicillin (10 µg), cefixime (5 µg), ciprofloxacin (5 µg), Azithromycin, and erythromycin (15 µg). The Kirby–Bauer disk diffusion method was performed on Mueller–Hinton agar plates following Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. Briefly, 0.5 McFarland suspensions of each isolate were lawn-inoculated, antibiotic disks were applied, and plates were incubated at 35 ± 2 °C for 16–18 h. Zone diameters were measured with a digital caliper and classified as Sensitive (S), Intermediate (I), or Resistant (R) per CLSI breakpoints.

Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used as quality-control strains to validate media performance and disk potency. All tests were performed in duplicate to ensure reproducibility.

III. RESULT

Disk-diffusion testing of 10 environmental isolates against 13 antibiotics revealed widespread resistance across most classes (Table I). Ciprofloxacin retained the greatest activity (90 % sensitive), followed by azithromycin (50 % sensitive). In contrast, first-line β-lactams (ampicillin, cephalexin, cefuroxime) and older broad-spectrum agents (chloramphenicol, tetracycline, cotrimoxazole, cefotaxime, cefixime, erythromycin) all exhibited high resistance rates (70–90 %). Nalidixic acid showed intermediate performance (60 % resistant, 20 % intermediate, 20 % sensitive). Azithromycin was found to be 100% resistant in all samples; the rest are summarized in Table II.

TABLE I
SAMPLE WISE BACTERIA RESISTANCE

Sample	Azithromycin	Chloramphenicol	Cefotaxime	Nalidixic Acid	Cotrimoxazole	Cephalexin	Cefuroxime	Tetracycline	Ampicillin	Cefixime	Ciprofloxacin	Erythromycin	Azithromycin
2-1	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Sensitive	Resistant	Resistant
2-2	Intermediat	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Sensitive	Resistant	Resistant
2-3	Intermediat	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Sensitive	Resistant	Resistant
3-1	Intermediate	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Sensitive	Resistant	Resistant
3-2	Intermediat	Resistant	Resistant	Intermediat	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Sensitive	Resistant	Resistant
4-1	Sensitive	Resistant	Intermediat	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Intermediat	Resistant	Resistant
5-1	Sensitive	Intermediat	Resistant	Intermediat	Intermediat	Resistant	Resistant	Resistant	Resistant	Intermediat	Sensitive	Intermediat	Resistant
5-3	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant

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5-4	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Intermediat	Resistant	Intermediat	Sensitive	Resistant	Resistant
5-5	Sensitive	Intermediat	Intermediat	Sensitive	Resistant	Resistant	Resistant	Intermediat	Resistant	Resistant	Sensitive	Intermediat	Resistant

TABLE II
PERCENTAGE OF BACTERIA RESISTANCE

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Azithromycin	50	40	10
Chloramphenicol	10	20	70
Cefotaxime	10	20	70
Tetracycline	10	20	70
Ampicillin	10	0	90
Cefixime	10	20	70
Ciprofloxacin	10	20	70
Nalidixic Acid	20	20	20
Erythromycin	10	20	70
Cotrimoxazole	20	10	70
Cephalexin	10	0	90
Cefuroxime	10	0	90
Azithromycin	0	0	100

Nine of ten isolates (90 %) were resistant to three or more antibiotic classes, meeting the definition of multiple antibiotic resistance (MAR). One isolate (sample 5-3) remained fully susceptible across all tested agents.

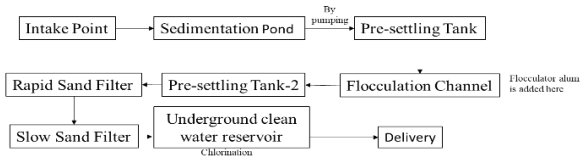


Fig. 1. Stages of water treatment by RWASA

No.1 from the intake point (shown in figure 1), 2 was collected after the rapid sand filter, 3 was the ready to supply water (after the slow sand filter), 4 was stagnant water near the bank of the Padma river, and 5 was tap water (collected from a part of the groundwater supply)

IV. DISCUSSIONS



Fig. 2. Photos of stages of water treatment by RWASA

The high resistance rates to traditional β-lactams, tetracycline, chloramphenicol and cotrimoxazole mirror findings in Dhaka and other South Asian water studies, where MAR indices frequently exceeded 0.4–0.7 [10,11,15]. The retained efficacy of ciprofloxacin aligns with reports of lower fluoroquinolone resistance among Pseudomonas and coliforms in surface waters [9].

At intake, the bacteria were “nil”. After passing several steps shown in Figure 2, the water got contaminated with MAR (which were almost resistant to all antibiotics), and the ready to supply water carrying that water (sample 3) without much alteration in characteristics. Even the water collected from the tap (source groundwater) also contains multiple antibiotic-resistant bacteria. Even the water from the bank of the Padma river, receiving contamination from drains and human activities, is less MAR susceptible to the supplied water of RWASA.

Selective pressures during chlorination and distribution can inadvertently favor resistant strains, as observed by researchers [4] in treated drinking water. Our results suggest that RWASA’s current treatment train, while not reducing overall microbial loads, necessitates enhancements such as UV or advanced oxidation processes to target residual resistant populations.

Limitations of this study include the small isolate number and lack of molecular resistance-gene profiling. Future work should expand sampling across seasons, quantify gene abundances via qPCR or metagenomics, and evaluate the log-removal efficiencies of each treatment unit to pinpoint critical control points for MAR mitigation.

V. CONCLUSIONS

Our comprehensive assessment of Rajshahi WASA’s dual-source supply demonstrates that its multi-barrier treatment train. The selected antibiotics were found to be resistant in all samples; however, they were sensitive in the water from the treatment plant, which was ready for supply, and stagnant water from the Padma River. Most interestingly, the intake point from the Padma river is sufficiently deep, and no bacteria were found are culturing the samples several times.

The source of MAR in the prevailing water supply system is the treatment plant for surface water and

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for groundwater; the contamination may come from the distribution pipeline or storage tank at the user level. Flowing water at the intake source or pumping station of groundwater exhibits almost nil contamination to MAR.

The presence of MAR in water sources like the bank of the Padma showed even less contamination than at stages of the treatment plant, and the results were consistent in later stages of the plant, suggesting that the bacteria removal efficiency is not significant in the prevailing system.

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