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Antimicrobial Resistant and Biofilm forming Coliforms in Drinking Water Systems of Mumbai's Harbour Line Railway Stations

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Abstract

This study aimed to assess the microbiological quality of drinking water stored at railway station tanks in Mumbai and evaluate the prevalence of antibiotic-resistant bacteria. A total of 88 water samples were collected and analysed for presence of coliforms using standard methods. The positive samples were further tested on selective media for bacterial identification. The antibiotic resistance profiles of the isolates were assessed using a panel of 15 broad spectrum antibiotics. The presence of plasmids and biofilm forming ability of the isolates was also evaluated. The analysis revealed that all samples were contaminated with coliform and non-coliform bacteria. Among the isolates, *Pseudomonas* (28 %), *Enterobacter* (27 %), *Escherichia* (8 %), and *Klebsiella* (7 %) were the most prevalent genera. Alarmingly, all isolates produced biofilms and 82 % exhibited resistance to multiple antibiotics with 12 % harboring plasmids. These findings highlight the critical need for more stringent monitoring and management of drinking water systems to prevent the spread of AMR through water systems.

Keywords: Drinking water; Water quality; Antibiotic sensitivity test; Antimicrobial resistance; Biofilms, Plasmids.

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1. Introduction

Water is a critical resource in public, domestic and commercial settings. Ensuring the quality of drinking water available at public places such as railway stations, bus stations, schools and similar areas, is of paramount importance. Safe, accessible, and good-quality drinking water is also vital for preventing waterborne diseases [1]. The Centers for Disease Control and Prevention (CDC) defines source water as bodies of water that provide safe drinking water for public supply and private wells. This includes surface water, groundwater, and recycled water [2]. In a densely populated metropolitan city like Mumbai, water is stored for extended period, which may compromise its quality. The improper storage conditions, inadequate control measures and low maintenance may further

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deteriorate water quality [3]. Along with water-borne diseases, this scenario poses a significant risk of spread of Antimicrobial Resistance (AMR), since water systems act as key microbial reservoirs. Hence close monitoring of water quality for chemical and microbiological contamination and improved water management practices are essential for mitigating the spread of AMR [4].

According to the World Health Organization (WHO) report in 2022, over 2 billion people consume drinking water contaminated with faeces; thus, leading to a significant risk of waterborne diseases [5]. To prevent waterborne infections, the WHO has established guidelines for safe drinking water, emphasizing the importance of setting health-based targets, implementing water safety plans, and conducting surveillance for risk prevention and management. The improved water quality sanitation, and hygiene could prevent approximately 9.1 % of the global disease burden and 6.3 % of all deaths [6].

Among waterborne diseases, acute diarrheal infections are prevalent in underdeveloped as well as developed countries [2]. Although, deaths due to diarrhoea have declined between 1990 and 2019, particularly among children, the condition still poses a major threat to children under five years of age and the elders over 70 years [7]. This problem has aggravated in recent years due to the increasing prevalence of antibiotic-resistant bacteria in water systems. Precisely, the accumulation of antibiotics in wastewater and drinking water has driven the rise of AMR [8]. A recent World Bank report on AMR associated drug resistance as a major contributor to mortality, globally. Approximately 5 million deaths are linked to infections with 1.3 million directly attributed to AMR. The threat of AMR extends beyond humans and affects domestic animals, aquatic environments, and agriculture. Without preventive measures, AMR is projected to cause 10 million deaths annually by 2050, along with 3.8 % loss of the domestic product; significantly impacting the global economy [9].

India is one of the countries severely affected by AMR, with high rates of occurrence of resistant bacterial genes in drinking and waste-waters [9]. Studies indicate that infectious diseases are prevalent in India. Poor sanitation, malnutrition, and the rampant use of antibiotics to treat infections have collectively exacerbated the problem [10]. Another significant factor contributing to bacterial resistance is the production of biofilms, which are extracellular complexes of polysaccharides, proteins, and DNA, by these pathogens. The biofilms impede penetration of the antibiotics, enhancing bacterial resilience and persistence in water systems [11].

The Mumbai suburban railway network spanning over 376 km runs across three routes; Western, Central, and Harbour- Trans harbour lines catering to approximately 80 lakh commuters daily [12]. The Harbour line, running between Chhatrapati Shivaji Maharaj Terminus and Panvel has more than 28 stations, and has been recently extended up to Goregaon [13]. This extensive network can become a hub for transmission of pathogens if stringent quality measures are not implemented. This study aimed to assess the bacteriological quality of drinking water from public outlets and storage tanks available on the railway station platforms. The main objectives included identifying pathogens evaluating their antibiotic resistance profile, and detecting the presence of plasmids. These

findings are critical for ensuring public health and addressing AMR challenges in high-density urban environments.

2. Materials and Methods

2.1. *Sample collection*

Water samples were collected in sterile 300 mL glass bottles by following the APHA guidelines for water sampling. Taps were fully opened and water was allowed to run for 2-3 min before sample collection to flush the stagnant water in pipes. Samples were transported to the laboratory in a thermal stabilizer box and analyzed within 6 h [14]. A total of 88 water samples were collected from 25 harbor line railway stations listed in Table 1.

Table 1. Summary of water sampling sites.

Sr. no.	Station name	Station Code	No. of taps	Sr. no.	Station name	Station Code	No. of taps
1	Chhatrapati Shivaji Maharaj terminus	CST	4	14	Govandi	G	4
2	Masjid	MB	4	15	Mankhurd	MK	2
3	Sandhurst Road	SDR	4	16	Vashi	V	9
4	Dockyard Road	DYR	2	17	Sanpada	SP	2
5	Reay Road	RR	2	18	Juinagar	JN	6
6	Cotton Green	CG	2	19	Nerul	N	2
7	Sewri	SW	2	20	Seawood Darave	SD	2
8	Vadala Road	VR	2	21	Belapur	CBD	3
9	GTB Nagar	GTB	3	22	Kharghar	KG	9
10	Chunabhatti	CH	2	23	Manasarovar	MSV	2
11	Kurla	K	3	24	Khandeshwar	KH	7
12	Tilak nagar	TN	3	25	Panvel	P	5
13	Chembur	CB	2				

2.2. *Microbiological analysis of the water samples*

All analyses were performed following standard protocols described in APHA guidelines [15].

2.2.1. *Multiple tube fermentation technique/Total coliform count/Presumptive tests*

The five-tube method was used with lauryl tryptone broth inoculated with water samples for detection of coliforms. The tubes were incubated at 37 °C ± 0.5 °C for 24 h and observed for the acid and gas formation. Tubes showing no reaction were further incubated for 48 h. Acid and gas production indicated a positive presumptive test. For confirmatory test, the brilliant green lactose bile broth was inoculated with water samples that showed a positive presumptive test. The inoculated tubes were incubated at 37 °C ± 0.5 °C for 24 h and

observed for the gas formation, which indicated a positive test. The completed test was then carried out by streaking the samples on Eosin Methylene Blue (EMB) agar plates, and incubated at 37 °C ± 0.5 °C for 24 h. The positive results showed nucleated colonies with or without metallic sheen. These colonies were re-inoculated into the secondary fermentation tubes of lauryl tryptone broth with an inverted vial and on Nutrient agar slants. The tubes were incubated at 35 °C±0.5 °C for 24-48 h. Gas formation in fermentation tubes and the presence of gram-negative, non-spore-forming, rod-shaped bacteria on the plates confirmed the presence of coliform bacteria in the analysed samples [16].

2.2.2. *Biochemical identification of the isolates*

The isolates were identified based on standard biochemical tests including sugar fermentations, oxidase and catalase test, triple sugar iron (TSI), phenylpyruvate deaminase (PPA) and IMViC [17].

2.2.3. *Antibiotic Sensitivity test*

The antimicrobial susceptibility pattern of each isolate was studied using the Octa Disc (Himedia laboratories) diffusion method according to National Committee Laboratory Standards (NCCLS) recommendations [18]. The results were interpreted using the Kirby Bauer chart [19].

2.2.4. *Quantitative detection of biofilm by tube method*

The cultures were inoculated in sterile 10 mL Trypticase soya broth and incubated at 37 °C±0.5 °C for 24 h. After incubation, the tubes were decanted, washed with Phosphate Buffered Saline (pH 7.3), and dried. The tubes were stained with 0.1 % crystal violet. Excess stain was removed, and tubes were washed again with PBS (pH 7.3), and dried. The tubes were scored based on the intensity of purple stain [20]. To quantify biofilm production, 5 mL of 30 % acetic acid was added to each tube and absorbance was measured at 550 nm [21].

2.2.5. *Extraction of plasmid by Alkaline Lysis method*

Plasmid DNA was extracted using the Alkaline Lysis Miniprep Method. The extracted plasmid DNA was run on 1.2 % agarose gel stained with ethidium bromide to visualize the plasmid on a UV trans-illuminator [22].

3. Results and Discussion

3.1. *Microbial contamination in water samples*

The analysis of water samples revealed presence of coliforms in 57.15 % samples (based on presumptive positive test), while 42.85 % showed absence of coliforms (<2 MPN index), suggesting relatively good hygiene and sanitation at some sampling sites. The positive samples were grossly contaminated (>1600 MPN index) with all the tubes showing acid and gas production. The findings of this study are similar to those reported from Tripura in 2023, showing variability in observations at different railway stations. They further reported the presence of coliforms in almost all the stored samples, indicating low level of sanitation employed for storage tanks [23]. Similar contamination levels were reported along the Central railway line from Nagpur to Bhusawal (stretching across 43 stations) in Maharashtra, where 76 % of water was severely contaminated and unfit for consumption [24]. These observations are alarming, considering the high morbidity rate associated with contaminated water. A study carried out in Ecuador highlighted the highest morbidity rate of 79 % due to waterborne diseases with the young and elderly population being affected the most [5].

3.2. *Identification of isolates*

Biochemical tests identified *Pseudomonas spp*., as the most prevalent genus (55 %) followed by *Enterobacter spp*. (25 %), *Escherichia spp*. (13 %), and *Klebsiella spp*. (8 %). The dominance of *Pseudomonas spp*., in this study, is particularly concerning due to its ability to form biofilms, which not only protect the bacteria from antimicrobial agents and chemical disinfectants, but also contribute to persistent contamination in water distribution systems, leading to their widespread dissemination [25]. Our observations align with the findings reported by Penna *et al*. [26]. Their study described the prevalence of *Pseudomonas spp.* in drinking and purified water systems in thirteen collection points. In contrast, a study from Africa reported the prevalence of *Escherichia spp*., in 60 % of the drinking water samples [27].

3.3. *Antibiotic resistance*

The antibiotic resistance observed among isolates is represented in Fig. 1. Our study indicated that 74 % of the positive samples exhibited resistance to at least one antibiotic. Most isolates were resistant to ampicillin, followed by ceftoxitin, ceftriaxone, and chloramphenicol. The widespread environmental dissemination of antibiotic resistance has led to referring of present time as the post-antibiotic era. The literature highlights the current antibiotic resistance trends to beta-lactams, ciprofloxacin, and fluoroquinolones among the bacterial flora in Indian water systems [10]. A study on antibiotic profiling of the bacterial flora in Yamuna River surface waters (Delhi) also indicated high rate of resistance to ofloxacin, amoxicillin, and erythromycin [28]. The presence of antibiotic-resistant genes (ARGs), which can be transferred between bacteria via horizontal gene transfer, poses an additional risk by spreading resistance beyond the immediate environment and leading to wider dissemination risks [4].

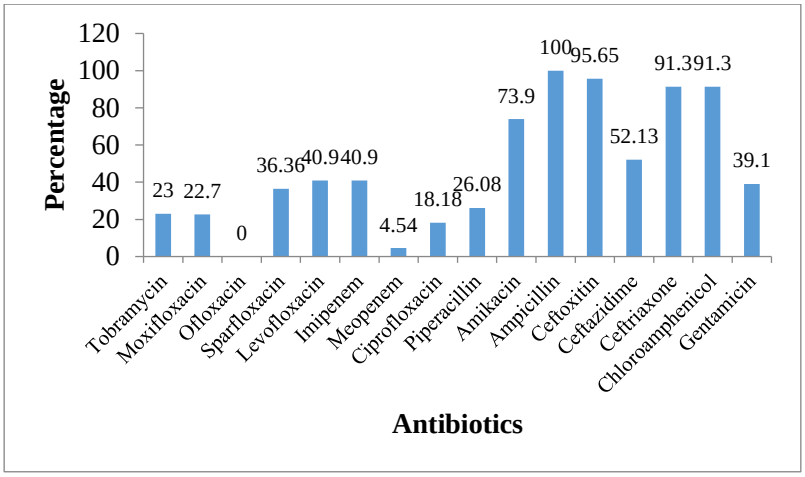

Fig. 1. Antibiotic resistance among isolates.

3.4. *Biofilm formation*

Biofilms were produced by all isolates obtained from MPN tubes (n=53). The density of biofilms in tubes inoculated with each of these isolates is represented in Fig. 2. Biofilm formation is a significant advantage for antibiotic resistant organisms, with biofilm-associated bacteria being more resilient than their planktonic counterparts [11]. Biofilms in stored water systems and pipes pose a substantial challenge to drinking water safety [29]. The exopolysaccharides alone in the biofilms can retain at least 25 % of the antibiotics, in addition to which biofilm forming bacteria follows other resistance mechanisms such as quorum sensing [30]. Similar to this study, Koskeroglu *et al.* [31] showed that 53.3 % of the extended-spectrum beta-lactamase (ESBL) isolates were biofilm producers.

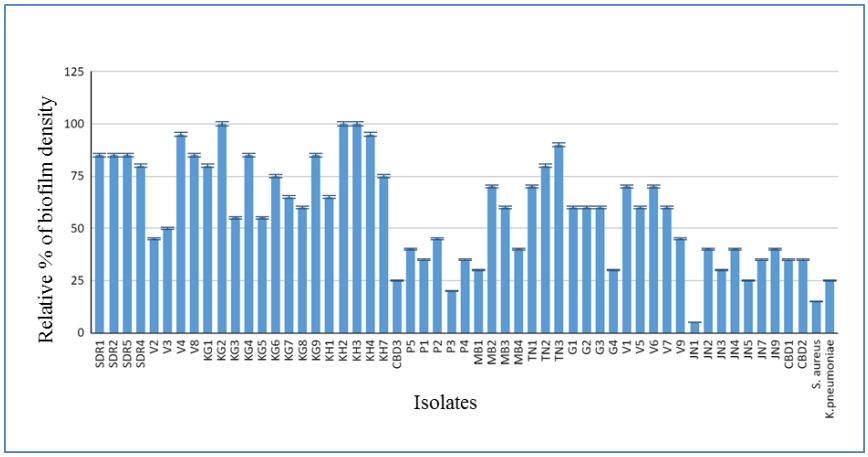

Fig. 2. Relative density of biofilms produced by test isolates.

3.5. *Analysis of plasmids*

Twenty percent of the resistant isolates harbored plasmids. These included *Pseudomonas spp*., (n=1), *Enterobacter spp*., (n=5), *Escherichia spp*., (n=1), and *Klebsiella spp*., (n=1). The antibiotic resistance profile of plasmid-borne isolates is indicated in Table 2. Maximum resistance was observed for fluoroquinolones followed by carbapenems. Similar scenario was presented in a study from Kenya that highlighted the prevalence of plasmid-mediated quinolone resistance in *Escherichia* and *Klebsiella spp*. [32]. In this study, plasmids were not detected in ampicillin-resistant *Pseudomonas*. This is probably due to chromosomal or integron-mediated resistance mechanisms. Also, unlike *Escherichia*, *Pseudomonas* exhibits a narrow host range for plasmid exchange [33].

Table 2. Antibiotic resistance profile of plasmid-borne isolates.

Name of the isolate	Probable organism	Antibiotic resistance profile	Antibiotic class
MB1	*Pseudomonas spp.*	Sparfloxacin	Fluoroquinolone
		Levofloxacin	Fluoroquinolone
		Imipenem	Carbapenem
		Moxifloxacin	Fluoroquinolone
KH1	*Enterobacter spp.*	Sparfloxacin	Fluoroquinolone
		Levofloxacin	Fluoroquinolone
		Imipenem	Carbapenem
KH2	*Enterobacter spp.*	Tobramycin	Fluoroquinolone
		Moxifloxacin	Fluoroquinolone
		Sparfloxacin	Fluoroquinolone
		Imipenem	Carbapenem
		Ciprofloxacin	Fluoroquinolone
KH3	*Enterobacter spp.*	Levofloxacin	Fluoroquinolone
		Sparfloxacin	Fluoroquinolone
		Imipenem	Carbapenem
V2	*Enterobacter spp.*	Imipenem	Carbapenem
		Ciprofloxacin	Fluoroquinolone
V3	*Enterobacter spp.*	Imipenem	Carbapenem
KG9	*Escherichia spp.*	Sparfloxacin	Fluoroquinolone
SDR2	*Klebsiella spp.*	Sparfloxacin	Fluoroquinolone
		Levofloxacin	Fluoroquinolone
		Imipenem	Carbapenem
		Moxifloxacin	Fluoroquinolone

3.6. *Implications of this study*

Antibiotic resistance is among the top ten challenges faced by the world population. This study indicated that storage water systems at harbour line railway stations in Mumbai are potential reservoirs for coliforms which are antibiotic resistant and form biofilms. The resistance of isolates to beta-lactams, fluoroquinolones, carbapenems, and aminoglycosides is particularly alarming. These findings necessitate improved sanitation practices and

frequent surveillance measures to mitigate the risks associated with AMR and waterborne diseases. Another useful approach can be the up-gradation of water quality guidelines that take into account not only the coliform control measures but also plasmids and other ARGs. Currently, these guidelines primarily focus on preventing fecal contamination and updated measures may help mitigate broader public health crisis.

As suggested earlier, surveillance studies from India have indicated poor drinking water quality at railway stations [23,24]. Unfortunately, this is a global scenario. In the U.S, surveillance studies for the drinking water quality carried out in select states reported the presence of biofilm-producing organisms. They further linked poor drinking water quality with disease outbreaks and spread of AMR [33,34].

This study aligns with the Sustainable Development Goals (SDG) adopted by the UN members in 2015 [35]. The SDG thrives to achieve a universal call to end poverty, protect our planet, and ensure peace and prosperity for its people. The sixth SDG is to ensure the availability and sustainable management of water and sanitation for all. These measures will be useful to combat the anticipated pandemic of antibiotic resistance by 2050.

4. Conclusion

This study highlights concerns regarding the quality of drinking water at railway stations along the harbour line in Mumbai. By integrating the detection and monitoring of AMR and ARGs into routine water quality assessments, we can better protect public health and reduce the spread of resistance. Further research is required to develop innovative methods for disinfecting and maintaining water quality in storage systems, especially in settings with frequent water handling, such as railway stations.

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