OPEN ACCESS
International Journal of Innovations in Science & Technology
RESEARCH & INNOVATION DIVISION
IJIST

Evaluating Chlorine Dosage for Effective Disinfection and Antimicrobial Resistance Profiling in Drinking Water Under Climate Change Influences

Sanam Bhatti [1], Rasool Bux Mahar [2], Tanveer A. Gadhi[1], Bahadur Ali[3], Zubair Ahmed[1], Naila Gul[1], Kamran Ansari[1]

[1]U.S.-Pakistan Center for Advanced Studies in Water, Mehran University of Engineering and Technology, Jamshoro.

[2]Benazir Bhutto Shaheed University of Technology and Skilled Development, Khairpur Mirs.

[3]HANDS (Health and Nutrition Development Society) WGS (Water Governance for Sindh Activity) Karachi.

***Correspondance:** sanam47mb@gmail.com

Citation | Bhatti. S, Mahar. R. B, Gadhi. T. A, Ali. B, Ahmad. Z, Gul. N, Ansari. K, “Evaluating Chlorine Dosage for Effective Disinfection and Antimicrobial Resistance Profiling in Drinking Water Under Climate Change Influences”, IJIST, Special Issue pp. 502-508, June 2024

Received | June 14, 2024 **Revised |** June 18, 2024 **Accepted |** June 23, 2024 **Published |** June 29, 2024.

Introduction/Importance of Study: Climate patterns, such as heavy rainfall and flooding, can introduce contaminants into water sources, leading to increased microbial loads. Chlorine disinfection is essential in mitigating these risks by effectively destroying pathogens.

Novelty Statement: This study investigates the effectiveness of different chlorine disinfectant dosages in eliminating disease-causing microorganisms and assessing antimicrobial resistance (AMR) in drinking water.

Material and Method: A biofilm annular reactor (BAR) setup was utilized to assess the impact of chlorination on pathogenic microorganisms. Three chlorine doses were tested: 0.5 mg/L, 1 mg/L, and 1.5 mg/L. Samples were collected and analyzed for AMR. Five selective bacterial strains were isolated using the membrane filtration method, and antibiotic sensitivity was evaluated using the standardized Kirby-Bauer disc diffusion test.

Result and Discussion: The study isolated five gram-negative bacteria on selective agar: E. coli, Salmonella, Shigella, Pseudomonas, and Vibrio cholerae. Their antimicrobial resistance to five antibiotics (amoxicillin, AML 5 µg; ampicillin, AMP 10 µg; Azithromycin, AZM 15 µg; ceftriaxone, CRO 30 µg; and imipenem, IPM 10 µg) was tested on Mueller-Hinton (MH) media. Azithromycin demonstrated the highest activity against all isolates. The optimal chlorine concentration for removing these bacteria from water was 1.5 mg/L, due to chlorine’s high reactivity.

Concluding Remarks: The study concludes that a chlorine concentration of 1.5 mg/L is optimal for pathogen removal from water, and Azithromycin exhibited exceptional effectiveness against all resistant gram-negative bacterial isolates.

Keywords: Climate Change; Drinking Water; Antimicrobial Resistance (AMR); Chlorination; Biofilm Annular Reactor (BAR); Disease-Causing Microorganisms.

Introduction:

Effects of climate change and extreme weather conditions influence the emergence and transmission of infectious diseases worldwide. As the global climate undergoes significant shifts, changes in environmental factors, such as water temperature, have substantial implications for the effectiveness of disinfection methods used to ensure drinking water safety. Chlorine, one of the most widely used disinfectants, plays a crucial role in protecting the public from waterborne diseases. However, its efficacy in water treatment is directly affected by climate change. A key factor influenced by climate change is water temperature, which impacts the concentration of active chlorine in treated water. Rising temperatures can cause fluctuations in active chlorine concentration, potentially diminishing its ability to effectively eliminate harmful microorganisms. Strict limits are set to control microbial concentrations in water and protect human health. These limits aim to mitigate the risks associated with waterborne pathogens. Despite this, studies have shown that certain bacteria, such as Shigella, enterotoxigenic Escherichia coli (ETEC), and Klebsiella, may persist in water even at chlorine levels recommended for effective disinfection [9][10][11].

Microorganisms in drinking water distribution systems (DWDS) can pose health risks to consumers. Therefore, regular DWDS control aims to maintain drinking water quality within prescribed limits throughout the entire network. Ensuring a safe drinking water supply helps prevent waterborne diseases and microbial contamination. Drinking water should adhere to national and international guidelines and regulations. Common treatment technologies include chemical dosing, such as coagulation followed by sedimentation, and advanced methods like magnetic coagulation with powder-activated carbon, electrocoagulation, or ozone with advanced oxidation processes. These treatments are designed to disinfect drinking water and kill pathogens. Signal disinfection methods like ozone, chlorine, UV-light, and pulse chemical disinfection are applied during treatment. Conventional methods manage various water sources, such as surface water or groundwater [1][2][3]. Maintaining free chlorine at consumption sites within safety limits is typically achieved through post-chlorination with a specified chlorine dosage.

A water loss and temperature-dependent chlorine decay rate constant in generic water networks under unsteady states during hot climates can be computed using a generic chlorine OxPred model. Reactions between free chlorine and organic compounds may lead to the formation of harmful disinfectant by-products, such as trichloromethane (THM), which can deteriorate drinking water quality. Using chloramines instead of free chlorine can mitigate this issue. Operational costs, whether from post-chlorination or additional water quality control measures, are important for both consumers and DWDS suppliers. Climate change necessitates adjustments to demand forecasts [8]. These findings highlight the need for a deeper understanding of the complex relationship between climate change, water disinfection, and bacterial resilience. By understanding how microorganisms adapt and resist disinfection treatments, researchers can develop innovative strategies to combat resistance and mitigate risks to public health [4][5].

The impact of climate change on surface water quality is concerning due to the presence of antibiotic-resistant genes and bacteria. The prevalence of these resistant genes and bacteria has increased significantly with changing climatic conditions, posing a serious threat to the environment and human health. Rising temperatures and shifting precipitation patterns contribute to the impact on surface water quality. Increased runoff from agricultural and urban areas carries pollutants, including antibiotic-resistant genes and bacteria from fertilizers, pesticides, and human waste, into rivers, lakes, and other surface water bodies. These pollutants can transfer resistance to harmless bacteria, reducing the effectiveness of antibiotics. The presence of antibiotic-resistant bacteria in surface waters also threatens aquatic organisms, as they may spread diseases that cause widespread mortality. Additionally, changes in water temperature and quality can disrupt ecosystems, affecting the survival and reproduction of aquatic life and impacting food chains and biodiversity. Increased chlorine dosing in drinking water may further contribute to antimicrobial resistance in pathogens. The complex issue of climate change and surface water quality requires urgent measures to mitigate its effects and preserve water quality for future generations [6][7][8].

This study aims to evaluate the impact of varying chlorine dosages on disinfection efficacy and antimicrobial resistance in drinking water. The findings will provide valuable insights into the risks associated with increased chlorine levels amid climate change. Understanding the relationship between chlorine dosage, disinfection efficacy, and antimicrobial resistance is crucial for developing effective strategies to ensure a safe drinking water supply in changing environmental conditions. Climate change-induced events like heavy rainfall and flooding significantly increase microbial contamination in water sources, posing serious public health risks [14]. This study investigates the efficacy of chlorine disinfection in eliminating pathogens and addressing antimicrobial resistance (AMR) in drinking water, a critical aspect of ensuring safe water supply amid rising climate variability. Using a biofilm annular reactor (BAR) to simulate real-world conditions and systematically test varying chlorine dosages highlights the need for optimized disinfection strategies to combat pathogens and AMR, a growing concern intensified by climate change. The main objective of this study is to optimize chlorine dosage for bacterial disinfection in bulk water and to identify antimicrobial-resistant bacteria.

Methodology:

Biofilm Annular Reactor Operation:

Two annular reactors (ARs) with a stationary outer cylinder and a rotating inner cylinder (Model 1320 LJ, BioSurface Technologies Corporation, Bozeman, MT, USA) were operated at room temperature at Mehran University of Engineering and Technology, Jamshoro, Pakistan. Various material slides, measuring 18.75 cm^2, were mounted on the inner cylinders of the ARs. Process water entered the inner cylinder, where the slides moved between the two cylinders and a variable speed motor. To simulate actual pipe flow conditions and shear stress in the drinking water system, the inner cylinder was set to rotate at 30 rpm (Reynolds number RE = 960 and shear stress of 0.007 N/m^2) [12][15]. The reactor ran for one month, starting with a first-week acclimatization phase without chlorine. Chlorine was then introduced at a constant concentration of 0.5 mg/L during the second week, 1 mg/L during the third week, and 1.5 mg/L during the fourth week.

Collection and Physicochemical Analysis of Inlet and Outlet Water Samples:

Inlet and outlet water samples were collected weekly in sterile plastic bags. All glassware was thoroughly washed, rinsed with distilled water, and sterilized at 121°C for 15 minutes. The physicochemical parameters of the flowing bulk water—pH, temperature, total dissolved solids (TDS), and electrical conductivity (EC)—were analyzed using a multi-parameter 3630 IDS (Xylem Analytics, Weilheim, Germany). Turbidity was measured with a Lovibond TB 210 IR (Lovibond, Dortmund, Germany) [13].

Bacterial Counts in Water Samples:

Selective bacteria detection in the bulk water samples was conducted using five different selective media agars: Rapid E. coli, XLD (Oxoid Limited, Thermo Fisher Scientific Inc., Loughborough, UK), cetrimide (Oxoid Limited, Thermo Fisher Scientific Inc., Loughborough, UK), and thiosulfate–citrate–bile salts–sucrose (TCBS, Oxoid Limited, Thermo Fisher Scientific Inc., Loughborough, UK). These agars were used to detect and identify E. coli, Salmonella/Shigella, Pseudomonas, and Vibrio cholerae. A 100 mL volume from both inlet and outlet water samples was plated on the agar plates using the membrane filtration technique. All agar media plates were incubated at 37°C for 24 hours. Following incubation, bacterial colonies were counted and recorded as CFU/100 mL for the bulk water samples. Figure 1 shows the flow diagram of the methodology used in this study.

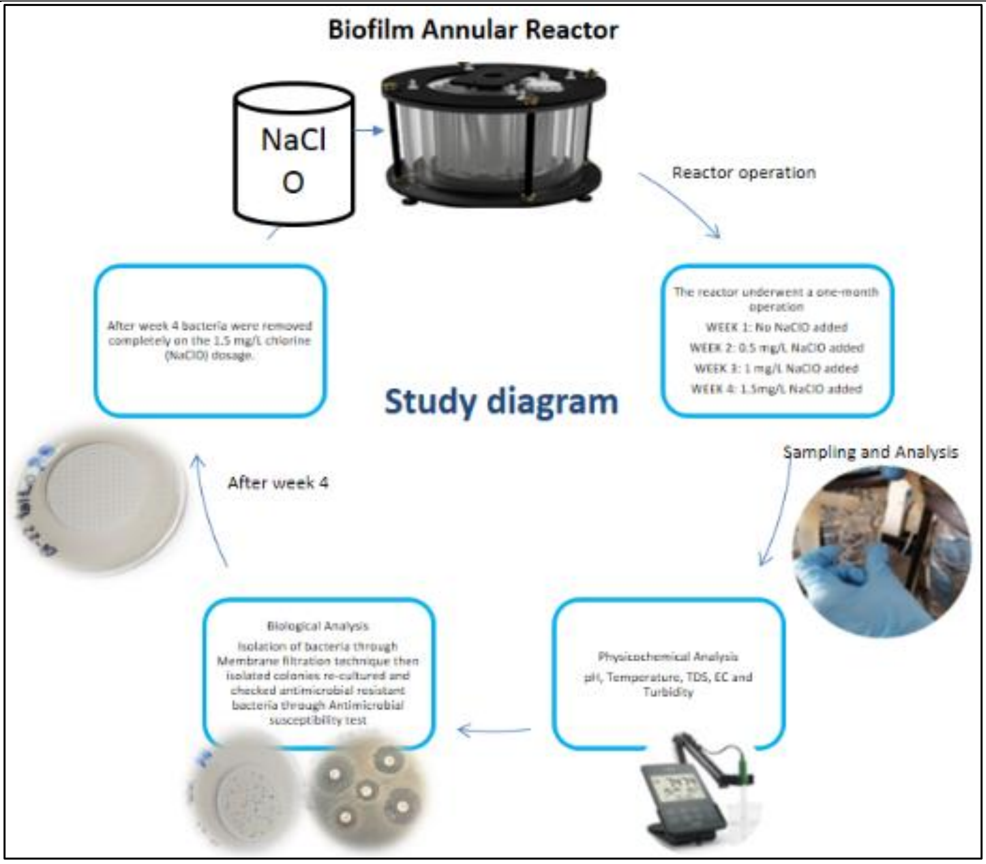

Figure 1: Flow of study Diagram

Antibiotic Resistance Test:

Antimicrobial-resistant bacteria (ARBs) were quantified, and zones of inhibition for different antibiotics were determined using the disc diffusion method. Antibiotic resistance in the identified bacteria was assessed using the Kirby-Bauer disk assay. The bacteria were incubated at 37°C for 24 hours and analyzed at an OD of 360 nm using a spectrophotometer (Lambda). Bacterial lawns were prepared on Mueller-Hinton agar plates, and various antibiotic discs were placed on the agar. Eleven antibiotics were tested: Azithromycin (AZM 15 µg), Ampicillin (AMP 10 µg), Amoxicillin (AML 5 µg), Ceftriaxone (CRO 30 µg), and Imipenem (IPM 10 µg). The plates were incubated at 37°C for 24 hours. The inhibition zones were measured in millimeters using a vernier caliper for each antibiotic disc. The sizes of the inhibition zones were compared using the chart in Leboffe and Pierce (Table 1).

Table 1: Selective antibiotics zone of inhibition (mm)

Name of Antibiotics	Disc content	Sensitive (S)	Moderately Sensitive (MS)	Resistant (R)
Amoxicillin	20/10 ug	≥18	14-17	≤ 13
Ampicillin	10 ug	≥15	12–14	≤ 11
Azithromycin	15 ug	≥18	14-17	≤13
Ceftriaxone	30 ug	≥21	14–20	≤ 13
Imipenem	10 ug	≥16	14–15	≤13

Results and Discussion:

Bacterial Isolates CFU/mL and Chlorine Dosage Optimization:

The research assessed the effectiveness of various chlorine dosages in eliminating five bacterial isolates from a biofilm annular reactor (BAR) [15] outlet water sample. The isolates tested were E. coli, Salmonella, Shigella, Pseudomonas, and Vibrio cholerae. Initial observations revealed a high CFU count for these bacteria, indicating significant bacterial presence. In the second week, a chlorine dose of 0.5 mg/L was applied, which proved inadequate as many CFUs remained detectable. The dosage was then increased to 1 mg/L, which improved the bacterial removal rate but did not achieve complete eradication. The presence of CFUs at this concentration suggested that 1 mg/L of chlorine is only partially effective in a biofilm environment. Finally, the chlorine concentration was raised to 1.5 mg/L, resulting in the complete removal of all five bacterial isolates. This demonstrates that a chlorine dose of 1.5 mg/L is effective in eradicating these bacteria from the BAR outlet water sample. The results (Figure. 1) illustrate a clear dose-response relationship between chlorine concentration and bacterial removal efficiency. Although physicochemical parameters showed slight variations after chlorine dosing, the findings underscore the importance of appropriate chlorine dosing in water treatment processes to effectively eliminate harmful bacterial contaminants. Figure 2 depicts the counts (CFUs/mL) of E. coli, Salmonella, Shigella, Pseudomonas, and Vibrio cholerae in water samples treated with chlorine doses of 0.5, 1.0, and 1.5 mg/L.

Figure 2: Five selective bacteria E.Coli, Salmonella, Shigella, Pseudomonas, and Vibrio Cholera counts (CFUs/mL) in water samples and Applied 0.5, 1.0, 1.5 mg/L chlorine doses.

Table 2: Physicochemical analysis from Bulk water's inlet and outlet samples

Disinfection Dose	Sample Collection	Temperature	pH	TDS	EC (mics/cm)	Salinity (ppt)	Turbidity (NTU)
13-6-019	Bulk water injected in Reactor	32°C	7.5	583.7	898	0.4	16.8
	Outlet	30°C	8.1	367.9	566	0.2	3.52
0.5 mg/L	Inlet	31°C	7.9	365.3	562	0.2	3.87
	Outlet	31°C	7.8	352.3	542	0.2	2.98
1 mg/L	Inlet	30°C	8	388.7	598	0.3	3.98
	Outlet	30°C	8	368.55	567	0.3	3.22
1.5 mg/L	Inlet	24°C	7.9	526.5	810	0.5	5.94
	Outlet	24°C	7.9	917.8	1412	0.5	4.94

Antimicrobial Resistance Profiling:

Five bacterial pathogens were tested for antibiotic resistance using the disc diffusion method. The results are detailed in [Table 3]. E. coli showed resistance rates of 98% to Ampicillin, 95% to Amoxicillin, 30% to Azithromycin, 14% to Ceftriaxone, and 15% to Imipenem. Pseudomonas exhibited resistance at 96% to Ampicillin, 94% to Amoxicillin, 28% to Azithromycin, 14% to Ceftriaxone, and 12% to Imipenem. Vibrio cholerae was resistant to Amoxicillin (96%), Ampicillin (94%), Azithromycin (28%), Imipenem (28%), and Ceftriaxone (24%). Salmonella showed resistance rates of 42.5% to Amoxicillin, 35% to Ampicillin, 27.5%

to Imipenem, 22.5% to Azithromycin, and 15% to Ceftriaxone. Shigella demonstrated resistance of 100% to Amoxicillin, 68% to Imipenem, 12% to Azithromycin, and 14% to Ceftriaxone. These results highlight the varying levels of resistance among the tested bacterial pathogens to the five antibiotics.

Table 3: Susceptibility Percentage of five selective bacteria against five antibiotics

SNO.	Isolates	Antibiotics
1	E.Coli	Ampicillin (98%), Amoxicillin (95%), Azithromycin (30%), Ceftriaxone (14%), and Imipenem (15%).
2	Salmonella	Amoxicillin (42.5%), Ampicillin (35%), Imipenem (27.5%), Azithromycin (22.5%), and Ceftriaxone (15%).
3	Shigella	Amoxicillin (100%), Ampicillin (98%), Imipenem (68%), Azithromycin (12%), and Ceftriaxone (14%).
4	Pseudomonas	Ampicillin (96%), Amoxicillin (94%), Azithromycin (28%), Ceftriaxone (14%), and Imipenem (12%).
5	Vibrio cholerae	Amoxicillin (96%), Ampicillin (94%), Azithromycin (28%), Imipenem (28%), and Ceftriaxone (24%)

Discussion:

After identifying the bacteria in water samples, efficacy trials were conducted to assess the impact of different chlorine doses and contact times on disinfection. The concentration of disinfectant and the contact time significantly affected the CFU/mL counts of E. coli, Salmonella, Shigella, Pseudomonas, and Vibrio cholerae in all samples. Bacterial counts decreased over time, from day 0 to day 7, at all chlorine doses. Initially, the bacterial counts were high: E. coli had 187 CFU/100 mL, Salmonella 58 CFU/100 mL, Shigella 131 CFU/100 mL, Pseudomonas 141 CFU/100 mL, and Vibrio cholerae 212 CFU/100 mL, as shown in Figure. 1. The 0.5 mg/L chlorine dose had the least effect on reducing bacterial counts, whereas the 1.5 mg/L dose achieved the most significant reduction for the selected pathogens. Literature supports that chlorine is effective at killing harmful bacteria and other pathogens by disrupting their cell walls and metabolism, thereby preventing the spread of waterborne diseases. As depicted in Figure. 1, the most effective bacterial removal was observed at the 1.5 mg/L dose, with E. coli, Salmonella, Shigella, Pseudomonas, and Vibrio cholerae being significantly reduced seven days after disinfection.

One study evaluated chlorine disinfection efficacy using flow cytometry (FCM) under various conditions to gain insights into disinfection processes. The inactivation rates for E. coli and microorganisms in treated water from operational water treatment works (WTWs) were assessed. The study revealed a dose-dependent increase in inactivation rate (k), ranging from 0.03 to 0.26 L/mg·min for WTW bacteria and 0.32 to 3.14 L/mg·min for E. coli. After 2 minutes of treatment, E. coli was reduced by 2 log for all chlorine doses (0.12 to 1.00 mg/L). For WTW filtrate bacteria, log reductions after 2 minutes ranged from 0.54 to 1.14, increasing to 1.32 to 2.33 after 30 minutes with higher chlorine concentrations. The study also found that disinfection efficacy decreased as the temperature dropped from 19°C to 5°C for both microbial populations. Additionally, chlorination at different pH levels (pH 6, 7, 8) showed more membrane damage at higher pH, which was inconsistent with the greater disinfection efficacy observed at lower pH using culture-based methods [16].

The compiled results indicated that a disinfectant dose of 1.5 mg/L was effective in removing bacteria from water samples. Chlorine levels in drinking water are considered safe for lifelong human consumption if they do not exceed the WHO's recommended guideline value of 4–5 mg/L [17]. However, some studies suggest that bacteria may develop resistance to chlorine at higher doses [18].

Conclusion:

This study evaluates the impact of climate change on surface water quality and demonstrates that chlorine disinfectant can effectively eliminate pathogens during bulk water distribution or emergency flood situations. In the annular reactor, testing continuous and bulk water flows revealed that among the three chlorine dosages (0.5, 1, and 1.5 mg/L), a dosage of 1.5 mg/L was the most effective at killing disease-causing microorganisms such as E. coli, Salmonella, Shigella, Pseudomonas, and Vibrio cholerae. Additionally, the identified gram-negative bacterial isolates exhibited high antibiotic resistance to Azithromycin, but were successfully disinfected by chlorine.

Acknowledgment: The Higher Education Commission of Pakistan supports this study through the Center of Excellence Research Grants (HEC-COE-37) and capacity-building initiatives at Mehran University of

Engineering & Technology to address drinking water issues in Pakistan. The authors also acknowledge the HANDS (Health and Nutrition Development Society) Pakistan and the USAID-funded WGS (Water Governance for Sindh Activity) project for their administrative support.

References:

[1] K. Guo, Z. Wu, C. Chen, and J. Fang, "UV/Chlorine Process: An Efficient Advanced Oxidation Process with Multiple Radicals and Functions in Water Treatment," Acc. Chem. Res., vol. 55, no. 3, pp. 286–297, Feb. 2022, doi: 10.1021/ACS.ACCOUNTS.1C00269.

[2] L. E. Anderson et al., "A review of long-term change in surface water natural organic matter concentration in the northern hemisphere and the implications for drinking water treatment," Sci. Total Environ., vol. 858, no. Pt 1, Feb. 2023, doi: 10.1016/J.SCITOTENV.2022.159699.

[3] S. Kataki, S. Chatterjee, M. G. Vairale, S. Sharma, and S. K. Dwivedi, "Concerns and strategies for wastewater treatment during COVID-19 pandemic to stop plausible transmission," Resour. Conserv. Recycl., vol. 164, p. 105156, Jan. 2021, doi: 10.1016/J.RESCONREC.2020.105156.

[4] E. Y. Y. Chan et al., "Narrative Review of Primary Preventive Interventions against Water-Borne Diseases: Scientific Evidence of Health-EDRM in Contexts with Inadequate Safe Drinking Water," Int. J. Environ. Res. Public Health, vol. 18, no. 23, Dec. 2021, doi: 10.3390/IJERPH182312268.

[5] "FAQs: Microbial Contamination | Mass.gov." Accessed: Jul. 01, 2024. [Online]. Available: https://www.mass.gov/info-details/faqs-microbial-contamination

[6] W. M. Manetu and A. M. Karanja, "Waterborne Disease Risk Factors and Intervention Practices: A Review," OALib, vol. 08, no. 05, pp. 1–11, 2021, doi: 10.4236/OALIB.1107401.

[7] R. Wang, M. Ji, H. Zhai, Y. Guo, and Y. Liu, "Occurrence of antibiotics and antibiotic resistance genes in WWTP effluent-receiving water bodies and reclaimed wastewater treatment plants," Sci. Total Environ., vol. 796, p. 148919, Nov. 2021, doi: 10.1016/J.SCITOTENV.2021.148919.

[8] S. Cheng et al., "Developing a restricted chlorine-dosing strategy for UV/chlorine and post-chlorination under different pH and UV irradiation wavelength conditions," Chemosphere, vol. 258, p. 127393, Nov. 2020, doi: 10.1016/J.CHEMOSPHERE.2020.127393.

[9] X. Zhang, P. Ren, J. Zhou, J. Li, Z. Li, and D. Wang, "Formation of disinfection byproducts in an ammonia-polluted source water with UV/chlorine treatment followed by post-chlorination: A pilot-scale study," Environ. Technol. Innov., vol. 26, p. 102266, May 2022, doi: 10.1016/J.ETI.2021.102266.

[10] D. L. Wu et al., "Contamination profile of antibiotic resistance genes in ground water in comparison with surface water," Sci. Total Environ., vol. 715, May 2020, doi: 10.1016/J.SCITOTENV.2020.136975.

[11] A. M. Voigt et al., "The investigation of antibiotic residues, antibiotic resistance genes and antibiotic-resistant organisms in a drinking water reservoir system in Germany," Int. J. Hyg. Environ. Health, vol. 224, Mar. 2020, doi: 10.1016/J.IJHEH.2020.113449.

[12] R. G. Skaland et al., "Impacts of climate change on drinking water quality in Norway," J. Water Health, vol. 20, no. 3, pp. 539–550, Mar. 2022, doi: 10.2166/WH.2022.264.

[13] Z. Zhu, C. Wu, D. Zhong, Y. Yuan, L. Shan, and J. Zhang, "Effects of pipe materials on chlorine-resistant biofilm formation under long-term high chlorine level," Appl. Biochem. Biotechnol., vol. 173, no. 6, pp. 1564–1578, 2014, doi: 10.1007/S12010-014-0935-X.

[14] S. Bhatti et al., "The Identification of Selective Pathogenic Microbial Community Biofilms in Different Distribution Pipeline Materials and Their Disinfection Kinetics," Water (Switzerland), vol. 15, no. 23, p. 4099, Dec. 2023, doi: 10.3390/W15234099/S1.

[15] J. Hyun-Jung, Y. J. Choi, and J. O. Ka, "Effects of diverse water pipe materials on bacterial communities and water quality in the annular reactor," J. Microbiol. Biotechnol., vol. 21, no. 2, pp. 115–123, Feb. 2011, doi: 10.4014/JMB.1010.10012.

[16] R. Cheswick, A. Nocker, G. Moore, B. Jefferson, and P. Jarvis, "Exploring the use of flow cytometry for understanding the efficacy of disinfection in chlorine contact tanks," Water Res., vol. 217, Jun. 2022, doi: 10.1016/J.WATRES.2022.118420.

[17] Jia,S.;Jia,R.;Zhang,K.;Sun,S.;Lu,N.;Wang,M.;Zhao,Q.Disinfection Characteristics of Pseudomonas Peli, a Chlorine-Resistant Bacterium Isolated from a Water Supply Network. Environ. Res. 2020, 185, 109417. [CrossRef]

[18] Zhu, Z.; Wu, C.; Zhong, D.; Yuan, Y.; Shan, L.; Zhang, J. Effects of Pipe Materials on Chlorine-Resistant Biofilm Formation under Long-Term High Chlorine Level. Appl. Biochem. Biotechnol. 2014, 173, 1564–1578. [CrossRef] [PubMed]