Available online at www.equijost.com

Equity Journal of Science and Technology, 2022 9(2): 54 - 59

ISSN 2354-1814; E-ISSN 2683-5961

EQUIJOST *An Official Publication of Kebbi State University of Science and Technology, Aliero, Nigeria*

Distribution of antibiotic-resistance genes in ultraviolet-treated water samples

Busayo M. Olowe

Department of Biological Sciences, Microbiology Unit, Bamidele Olumilua University of Education, Science and Technology, Ikere Ekiti, Nigeria

*Corresponding Author's email: Olowe.busayo@boesti.edu.ng

Abstract

Antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARG) are a global health problem. Their detection in drinking water samples speaks volumes because water is an essential prerequisite for a healthy life. This study aimed to evaluate the ARG distribution in ultraviolet (UV) treated water samples. One liter each of the thirty well water and borehole water samples were obtained from different dormitories at Ekiti State University in Ekiti State. Each water sample was treated with ultraviolet at 254 nm for 30 min. Bacterial detection and isolation were performed on UV-treated water samples using the standard pour plate technique, and the identification of isolates was performed using standard biochemical methods. The isolates were tested for antibiotic sensitivity according to the Institute of Clinical and Laboratory Standards' procedures. Five ARBs exhibiting resistance to multiple antibiotics were selected and subsequently detected at the molecular level of ARG including tetA, ctx-M and sul1 by PCR technique. Twenty different bacteria belonging to seven species were identified: *Escherichia coli* (25%), *Klebsiella aerogenes* (5%), *Enterobacter aerogenes* (10%), *Staphylococcus aureus* (25%), *Proteus spp.* (5%), *Bacillus lichenifomis* (10%) and *Bacillus cereus* (20%). A remarkable resistance profile was observed among the bacteria and tetA gene was present in *Staphylococcus aureus* and *E. coli*. Only *Enterobacter aerogenes* was positive for sul1 genes and none of the bacterial isolates was positive for ctx-M. The study showed that ARG persisted despite UV treatment, indicating that water treatment did not completely remove ARG. Hence the spread of ARG in the environment.

Keywords: Antibiotic-resistant genes, Bacteria, Multidrug-resistance, Ultraviolet treatment, Water samples

1. Introduction

The society today is concerned with several incidences of waterborne infections which has resulted into health issues and loss of lives globally [1]. This global health concern results into application of different technologies to disinfect water before consumption. Drinking water are now being treated chemically and physically to ensure removal of pathogenic microbes. Among many physical method of water treatments is Ultraviolet (UV) radiation treatment. Ultraviolet disinfection of drinking water is a global water purification technology [2]. Its radiation has many benefits, including inactivation of bacteria, viruses and protozoa; no taste and odour in the water; no sterilization by-products; no overdose effect; practical use; relatively fast processing method/speed compared to some filters such as sand and ceramic filters; and reduce maintenance requirements [3-5]. This technology has been adopted largely for its advantages and is therefore available in small UV systems, including commercially available household systems and locally manufactured UV tube systems which has become an appropriate treatment option for developing communities [6].

The effectiveness of UV technology depends on a number of parameters, such as: UV intensity, exposure time, surface, water transparency, among others [7]. The optimal effectiveness of germicidal UV light is at 254-260 nm [8]. UV light deactivates microorganisms by damaging the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) of microbial cells. When microbial cells are exposed to UV light, their DNA and RNA absorb the UV light and form dimers (covalent bonds between identical nucleic acids) leading to cell damage. The dimer causes defects in the transcription of information from DNA to RNA, thereby disrupting microbial replication. The microorganisms are still alive but cannot multiply and are therefore not infectious [9, 10]. Despite the effectiveness of this technology, one report indicates that UV-inactivated cells can regain viability through repair mechanisms [11]. For example, reactivation of light by simultaneous or sequential irradiation with near-ultraviolet light and visible light (300–500 nm), often referred to as "repair light" [12]. In addition, nucleotide or base excision repair is often referred to as "dark repair" [13]. They also contribute to the persistence of pathogens and especially antibiotic-resistant bacteria, of world importance in the clinical field. UV-sterilization has been found to increase bacterial resistance to sulfadiazine, vancomycin, rifampicin, tetracycline and chloramphenicol [12, 14-16]. Therefore, the distribution of antibiotic resistance genes in drinking water after disinfection is an important public health issue. In view of this, the study aimed to investigate the distribution of some antibiotic resistance genes in UV-treated water.

2. Materials and Methods

2. 1 Sample collection

One Litre each of thirty (30) borehole and well water samples were collected from different hostels in Ekiti State University, Ekiti State using standard sampling technique demonstrated by Tersagh et al. [17]

2.2 Treatment of water sample with UV light

The UV lamp type used was low pressure mercury lamp producing monochromatic UV light at 254nm. Each water sample was exposed to Ultraviolet (UV) treatment at room temperature for 30 mins. And control water sample was not exposed to UV treatment.

2.3 Isolation and identification of bacteria from UV-treated water samples

Bacterial detection and isolation were performed on the UV-treated water samples using standard pour plate technique and identification of the isolates were performed using standard biochemical methods.

2.4 Antibiotic susceptibility testing

Bacterial isolates from UV-treated water samples were then subjected to antibiotic sensitivity testing following Clinical and Laboratory Standards Institute procedure [18]. The antibiotics disc used included TET-tetracycline, COT-trimethoprim/sulfamethoxazole, GEN-gentamicin, CRX-cefuroxime, CHL-chloramphenicol, CTR-ceftriaxone, CTX-cefotaxime, CIP-ciprofloxacin, AMK-Amikacin, MEM-meropenem, VAN-vancomycin and CPZ-cefoperazone

2.5 Determination of Multiple antibiotic resistance

This was determined by considering resistance of bacterial isolates to more than 2 classes of antibiotics [19].

2.6 Molecular Detection of antibiotic resistance genes (ARGs)

Five (5) isolates which demonstrated multiple resistance were selected and then subjected to molecular detection of ARGs which included tetA, ctx-M and sul1 using PCR technique. The primer used are shown in Table 2.1.

Table 2.1: Primers used for molecular detection of ARG

Antibiotic	Gene	Primer	Primer sequence	Reference
Tetracycline	tetA	tetA-F	GCTACATCCTGCTTGCCTTC	[20]
		tetA-R	CATAGATCGCCGTGAAGAGG	
Beta Lactamase	ctx-M	ctx-MF	CGCTTTGCGATGTGCAG	[21]
		ctx-MR	ACCGCGATATCGTTGGT	
Sulfamethoxazole	sul1	sul1-F	TGA GAT CAG ACG TAT TGC GC	[22]
		sul1-R	TTG AAG GTT CGA CAG CAC GT	

3. Results and Discussion

Result in Table 3.1 shows the distribution of bacteria recovered from UV-treated water samples. Twenty bacterial isolates were identified belonging to seven groups which included *Escherichia coli* (25%), *Klebsiella aerogenes* (5%), *Enterobacter aerogenes* (10%), *Staphylococcus aureus* (25%), *Proteus spp.* (5%), *Bacillus lichenifomis* (10%) and *Bacillus cereus* (20%). It was observed that the Gram-positive bacteria are more resistant to the UV-treatment than the Gram-negative bacteria in terms of numbers of isolates recovered after treatment (Table 3.1). Figure 3.2 shows the percentage resistance pattern among the isolates. The figure depicts that all the isolates showed resistance to tetracycline, ceftriaxone and cefotaxime with least resistance to ciprofloxacin and amikacin. *Staphylococcus aureus* were resistant to all the antibiotics except amikacin while *Klebsiella pneumoniae* were susceptible to all the antibiotics except tetracycline, cefuroxime, chloramphenicol, ceftriaxone and cefotaxime. *Proteus vulgaris* showed a notable resistance (100%) to all the antibiotics except chloramphenicol, ciprofloxacin and amikacin. Likewise, *Klebsiella pneumoniae* showed 100% resistance to tetracycline, cefuroxime, chloramphenicol, ceftriaxone and cefotaxime. All other bacteria showed considerable resistance to many of the antibiotics. The bacterial isolates demonstrated multiple antibiotic-resistance (MAR) to the different groups of the antibiotics used. The MAR-Index (MARI) for each bacterial isolate is shown in Figure 3.2. It was observed that all the isolates that demonstrated multidrug resistance (i.e resistance to ≥ 3 classes of antibiotics) have MARI > 0.2 which is significant (Fig. 3.2). *Staphylococcus aureus* had the highest MAR-index of 0.9 while *Klebsiella pneumoniae* and *Bacillus lichenifomis* had the least MAR-index of 0.4.

Nanodrop quantification showing the DNA quantity and quality extracted from each bacteria isolate is shown in Table 3.2. Result in this table shows that *Enterobacter aerogenosa* had the highest quantity of nucleic acid while *Escherichia coli* (B) had the least quantity. The absorbance quotient for nucleic acid quality was between 1.85 and 1.95. This is indicative of a good and purified Nucleic acid (Table 3.2). Figure 3.3 shows the integrity of DNA extracted from all the bacterial samples with different intensity bands. Organisms in lane 1, 2, and 3 show high intensity band while organisms in lane 4 and 5 show low intensity band. The agarose gel electrophoresis for the detection of antibiotic resistance genes is shown in Figure 3.4-3.6. Figure 3.4 indicates that *Staphylococcus aureus* and *Escherichia coli* (A) were positive for the presence

of TetA genes of approximately 210 bp. There was no organism carrying Ctx-M gene (Figure 3.5) while only *Enterobacter aerogenes* was positive for the presence of Sul1 genes of 330bp (Figure 3.6).

Table 3.1: Distribution of bacteria recovered from UV-treated water samples.

S/N	Isolates	Freq.	(%)
1	*Escherichia coli*	5	25
2	*Klebsiella pneumoniae*	1	5
3	*Enterobacter aerogenes*	2	10
4	*Staphylococcus aureus*	5	25
5	*Proteus vulgaris*	1	5
6	*Bacillus lichenifomis*	2	10
7	*Bacillus cereus*	4	20
	Total	**20**	**100**

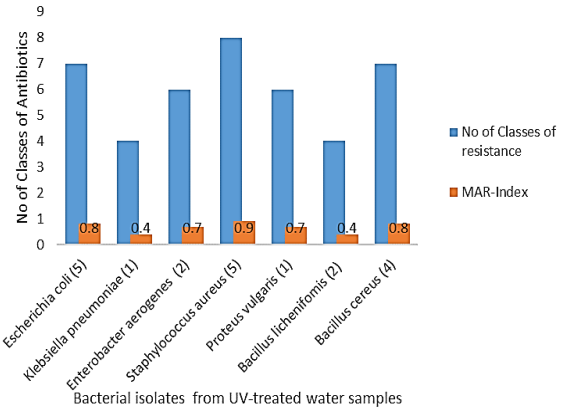

Figure 3.1: Multiple antibiotic resistance-index of antibiotic-resistant bacterial isolates from UV-treated water samples

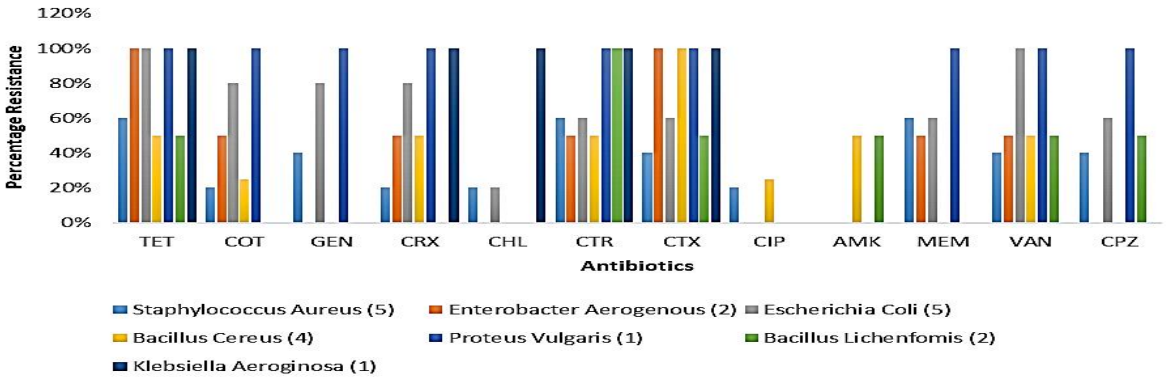

Figure 3.2**:** The percentage resistance pattern of bacteria recovered from well and borehole water sample

Table 3.2: Nanodrop quantification showing the DNA quantity and quality extracted from each bacteria isolate

Sample ID	Sample bacteria	Nucleic Acid (ng/µl)	A260 (Abs)	A280 (Abs)	260/280
$\boldsymbol{sdH_2O}$	sdH_2O	0.1	0.002	0.011	0.54
	Staphylococcus aureus	730.5	0.609	0.33	1.85
	Escherichia coli (A)	39.6	0.191	0.104	1.85
	Enterobacter aerogenosa	913	0.26	0.135	1.94
	Escherichia coli (B)	446.9	0.437	0.269	1.90
	Bacillus cereus	531.7	0.619	0.353	1.95
	Buffer control	554.9	0.197	0.124	1.95

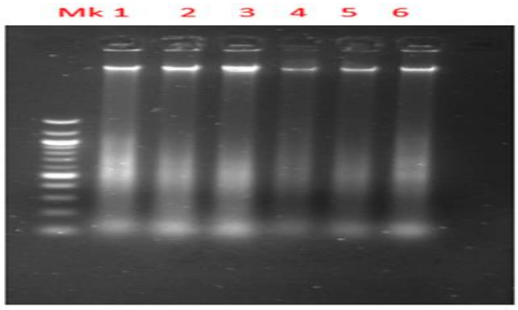

Figure 3.3: DNA integrity showing the presence of extracted DNA from bacteria samples
PCR Loading arrangement: Mk-marker, 1- *Staphylococcus aureus*, 2 - *Escherichia coli* (A), 3- *Enterobacter aerogenosa*, 4-*Escherichia coli (B)*, 5- *Bacillus cereus* and 6 -Buffer control

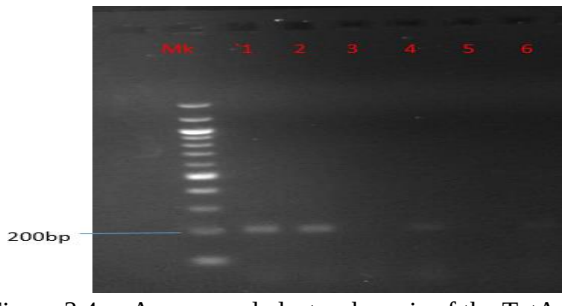

Figure 3.4: Agarose gel electrophoresis of the TetA gene (approximately 210 bp)
PCR Loading arrangement: Mk-marker, 1- *Staphylococcus aureus*, 2 - *E. coli*, 3- *Enterobacter aerogenosa*, 4-*Escherichia coli*, 5- *Bacillus cereus* and 6 Buffer control

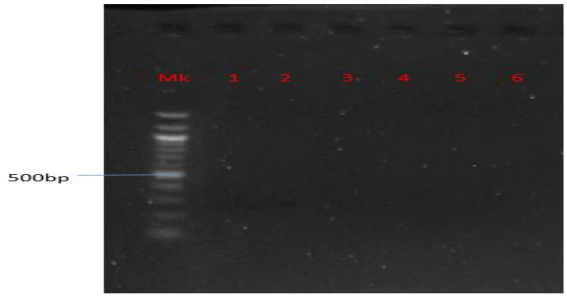

Figure 3.5: Agarose gel electrophoresis of the PCR products of Ctx-m gene (≈ 550bp)
PCR Loading arrangement: Mk-marker, 1- *Staphylococcus aureus*, 2 - *E. coli*, 3- *Enterobacter aerogenosa*, 4-*Escherichia coli*, 5- *Bacillus cereus* and 6 Buffer control.

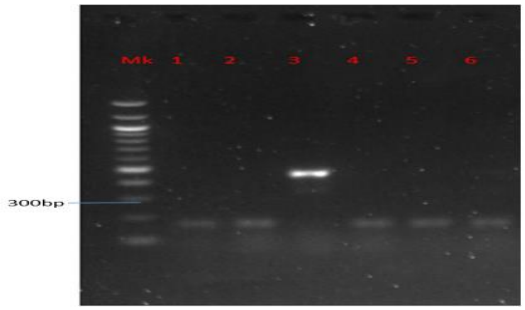

Figure 3.6: Agarose gel electrophoresis of the PCR products of sul1 gene in selected bacteria isolates, (330bp).
PCR Loading arrangement: Mk-marker, 1- *Staphylococcus aureus*, 2 - *E. coli*, 3- *Enterobacter aerogenosa*, 4 *Escherichia coli*, 5- *Bacillus cereus* and 6 Buffer control.

Research shows that certain groups of organisms persist in water even after 30 minutes of UV exposure. This reinforces the results obtained in different studies reporting the survival of different types of bacteria after UV water treatment [23-25]. The resistance of bacterial strains to tetracycline, ceftriaxone and cefotaxime was similar to that found by Adesoji et al. [26], who also reported high bacterial resistance to older generation antibiotics such as tetracycline (0-100%), streptomycin (17-100). %) and sulfamethoxazole (33-100%). They also reported similar bacteria including *Proteus, Klebsiella, Alcaligenes, Aeromonae* which demonstrated MDR [26]. The results of this study agree with the findings of Ahmed et al. [27] who documented 115 *E. coli* from tap and groundwater samples, most were resistant to cefuroxime (88.7%), trimethoprim-sulfamethoxazole (62.6%), and amoxicillin-clavulanic acid (52.2%). Same authors further reported that multidrug resistance was mainly observed in *E. coli* (58%) [27].

The spread of antibiotic resistance genes in UV treated water samples is a major risk. Although there is no fully developed link between the release of resistance genes in the environment and the emergence of new resistant pathogens in the clinic, resistant bacteria can transfer resistance genes to other species causing disease in humans. Thereby reducing the effectiveness of antibiotic treatment [28, 29]. Therefore, some studies recommend minimizing practices that lead to the release of antibiotic resistance into the environment [30-32]. Urban wastewater treatment plants have been identified as a major source of antibiotic-resistant bacteria and their associated antibiotic resistance genes (ARGs) in the environment, leading to increased levels of antibiotic and bacterial resistance genes downstream of wastewater discharge, even after treatment [33, 34]. The present study examined the presence of antibiotic resistance genes, the TetA gene, the Ctx-M gene and the Sul1 gene and only the TetA and Sul1 genes for tetracycline and sulfamethoxazole resistance, respectively, were detected in few of isolates recovered from UV treated water. This finding corroborates the findings of Tavares et al. [35] who identified 9 of the 20 antibiotic resistance genes studied [sul1, sul2, sul3, tet(A), tet(B), blaOXA-1-like, aacA4, aacA4-cr and qnrS1] using PCR screening with sul1, sul2 and tet(A) being predominate. The absence of the Ctx-M gene encoding beta-lactamase resistance in this study contradicts the conclusion of Oliveira et al. [36] that the study demonstrated the frequent presence of ESBL-producing *E. coli* and a series of ARG markers (including ESBL genes blaCTX-M and blaTEM) in both tributaries and wastewater of four wastewater treatment plants in Spain. The results of the present study, along with other similar findings, suggest that wastewater and wastewater treatment plants play an important role in the diffusion of ARBs and ARGs, which are considered hotspots for transmission of antibiotic resistance [33].

4. Conclusion

The study reveals that UV treatment of water, though, reduces heterotrophic plate count but does not completely eradicate antibiotic resistant bacteria carrying resistance genes which can still be propagated in the environment. Thereby contributing to increasing risk of infection with these organisms and treatment failure in the clinical sectors. It is therefore worthy of note that more extensive studies are needed to provide modification in the disinfection process. Which will help to reduce the risk of persistence of antibiotic resistance genes in water after treatment.

5. Acknowledgements

Many thanks go to the Laboratory Technologists of the Department of Science Laboratory Technology, Microbiology Unit, Federal Polytechnic Ado-Ekiti, who rendered technical assistance during the research activities in their laboratory. Also, appreciation goes to my students: Lola and Seun Joy of the Department of Microbiology who also assisted in the bench work.

References

1. Paudel S, Kumar P, Dasgupta R, Johnson BA, Avtar R, Shaw R, Mishra BK, Kanbara S. Nexus between water security framework and public health: A comprehensive scientific review. *Water.* 2021; 13(10):1365.

2. Jarvis P, Autin O, Goslan EH, Hassard F. Application of ultraviolet light-emitting diodes (Uv-Led) to full-scale drinking-water disinfection. *Water*. 2019; 11(9):1894.
3. Davarcioglu B. An Overview of the Water Disinfection with Ultraviolet Radiation Nowadays: New Approaches. *International Journal of Innovative Science, Engineering & Technology.* 2015; 2(7):577-587.
4. Srikanth B. The Basic benefits of ultraviolet technology. *Water Cond Purif*. 1995:26-27.
5. Chatterley C, Linden K. Demonstration and evaluation of germicidal UV-Leds for point-of-use water disinfection. *Journal of Water and Health.* 2010; 8(3):479-486.
6. Gruber JS. Study design, outcome measurement, and parameter estimation for the evaluation of an ultraviolet based household water treatment and safe storage intervention. University of California, Berkeley 2013.
7. Barnes PW, Williamson CE, Lucas RM, Robinson SA, Madronich S, Paul ND, Bornman JF, Bais AF, Sulzberger B, Wilson SR. Ozone depletion, ultraviolet radiation, climate change and prospects for a sustainable future. *Nature Sustainability*. 2019; 2(7):569-579.
8. Nishigori C, Yamano N, Kunisada M, Nishiaki-Sawada A, Ohashi H, Igarashi T. biological impact of shorter wavelength ultraviolet radiation-C. *Photochemistry and Photobiology.* 2022.
9. Stange C, Sidhu J, Toze S, Tiehm A. Comparative Removal of Antibiotic Resistance Genes During Chlorination, Ozonation, and UV Treatment. *International Journal of Hygiene and Environmental Health*. 2019; 222(3):541-548.
10. Raeiszadeh OM. Studying microbial inactivation by a New UV radiation source: Micro plasma UV. 2020, University of British Columbia.
11. Gmurek M, Borowska E, Schwartz T, Horn H. Does Light-based tertiary treatment prevent the spread of antibiotic resistance genes? performance, regrowth and future direction. *Science of The Total Environment*. 2022:153001. Available from: Doi: 10.1016/j.scitotenv.2022.153001
12. Destiani R, Templeton M. Chlorination and ultraviolet disinfection of antibiotic-resistant bacteria and antibiotic resistance genes in drinking water. *AIMS Environmental Science*. 2019; 6(3):222-241.
13. Strzałka W, Zgłobicki P, Kowalska E, Dziga D, Banaś AK. The Dark side of Uv-Induced DNA lesion repair. *Genes*. 2020; 11(12):1450.
14. Pazda M, Kumirska J, Stepnowski P, Mulkiewicz E. Antibiotic resistance genes identified in wastewater treatment plant systems: A review. *Science of the Total Environment*. 2019; 697: 134023.doi: 10.1016/j.scitotenv.2019.134023
15. Zhang G, Li W, Chen S, Zhou W, Chen J. Problems of conventional disinfection and new sterilization methods for antibiotic resistance control. *Chemosphere.* 2020; 254:126831. Available from: Doi: 10.1016/j.chemosphere.2020.126831
16. Yadav S, Arora U, Zaman K. Antibiotics, antibiotic-resistant bacteria, and the environment, in degradation of antibiotics and antibiotic-resistant bacteria from various sources. *Elsevier*. 2023:117-142.
17. Tersagh I, Dooshima N, Peter OJ. Efficacy of water guard disinfectant as a domestic stored water treatment method in Makurdi metropolis. *Research Journal of Environmental Sciences*. 2015; 9(8):364.
18. Weinstein MP, Lewis JS. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *Journal of Clinical Microbiology*. 2020; 58(3): e01864-19. Available from: doi:10.1128/JCM.01864-19
19. Cabral L, Rodrigues L, Tavares AH, Tomé G, Caetano M, Chaves C, Afreixo V. Analysis of potential risk factors for multidrug-resistance at a burn unit. *European Burn Journal*. 2023; 4(1):9-17.
20. Ng L-K, Martin I, Alfa M, Mulvey M. Multiplex Pcr for the detection of tetracycline resistant genes. *Molecular and cellular probes*. 2001; 15(4):209-215.
21. Bonnet R, Recule C, Baraduc R, Chanal C, Sirot D, De Champs C, Sirot J. Effect of D240g Substitution in a Novel Esbl Ctx-M-27. *Journal of Antimicrobial Chemotherapy*. 2003; 52(1):29-35.
22. Hendriksen RS, Munk P, Njage P, Van Bunnik B, McNally L, Lukjancenko O, Röder T, Nieuwenhuijse D, Pedersen SK, Kjeldgaard J. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nature communications*. 2019; 10(1):11 - 24.
23. Shekhawat SS, Kulshreshtha NM, Vivekanand V, Gupta AB. Impact of combined chlorine and UV technology on the bacterial diversity, antibiotic resistance genes and disinfection by-products in treated sewage. *Bioresource Technology*. 2021; 339:125615.

24. Mofidi AA, Rochelle PA, Chou CI, Mehta HM, Linden KG, Malley JP. Bacterial survival after ultraviolet light disinfection: resistance, regrowth and repair. In*: American Water Works Association Water Quality and Technology Conference (AWWA WQTC).* Seattle, Washington; 2002. p. 10-13.

25. Coohill TP, Sagripanti JL. Overview of the inactivation by 254 nm ultraviolet radiation of bacteria with particular relevance to biodefense. *Photochemistry and photobiology*. 2008; 84(5):1084-1090.

26. Adesoji A,Ogunjobi A. Occurrence of multidrug-resistant bacteria in selected water distribution systems in Oyo state, Nigeria. *Global Veterinaria*. 2013; 11(2):214-224.

27. Ahmed H, Zolfo M, Williams A, Ashubwe-Jalemba J, Tweya H, Adeapena W, Labi A-K, Adomako LA, Addico GN, Banu RA. Antibiotic-resistant bacteria in drinking water from the greater Accra region, Ghana: a cross-sectional study, December 2021–March 2022. International Journal of Environmental Research and Public Health. 2022; 19(19):12-30.

28. Peterson E, Kaur P. antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. *Frontiers in microbiology*. 2018; 9:29-38.

29. Sanganyado E, Gwenzi W. Antibiotic resistance in drinking water systems: occurrence, removal, and human health risks. *Science of the Total Environment*. 2019; 669:785-797.

30. Pruden A, Larsson DJ, Amézquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JM, Suzuki S, Silley P, Snape JR. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. *Environmental health perspectives.* 2013; 121(8):878-885.

31. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H. Antibiotic resistance: The need for global solutions. *The Lancet Infectious Diseases*. 2013; 13(12):1057-1098.

32. Larsson DJ, Flach CF. Antibiotic resistance in the environment. *Nature Reviews Microbiology*. 2022; 20(5):257-269.

33. Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy M, Michael I, Fatta-Kassinos D. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Science of the total environment*. 2013; 447:345-360.

34. Zhao F, Wang B, Huang K, Yin J, Ren X, Wang Z, Zhang X-X. Correlations among antibiotic resistance genes, mobile genetic elements, and microbial communities in municipal sewage treatment plants, revealed by high-throughput sequencing. *International Journal of Environmental Research and Public Health*. 2023; 20(4):3593.

35. Tavares RD, Tacão M, Figueiredo AS, Duarte AS, Esposito F, Lincopan N, Manaia CM, Henriques I. Genotypic and phenotypic traits of Blactx-M-Carrying Escherichia Coli Strains from an UV-C-Treated Wastewater Effluent. *Water Research*. 2020; 184:116079.

36. Oliveira M, Truchado P, Cordero-García R, Gil MI, Soler MA, Rancaño A, García F, Álvarez-Ordóñez A, Allende A. Surveillance on Esbl-Escherichia Coli and Indicator Arg in wastewater and reclaimed water of four regions of Spain: impact of different disinfection treatments. *Antibiotics.* 2023; 12(2):400.