Proceedings of the Pakistan Academy of Sciences: B Life and Environmental Sciences 60(S): 45-53 (2023)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(60-sp1)783

Pakistan Academy of Sciences

Research Article

High Burden of Multidrug-Resistant Bacteria Detected in Different Water Sources can Spread the Antibiotic Resistance Genes in the Environment

Hafiza Jawairia Hashmi[1, 2*], and Nazia Jamil[1]

[1]Institute of Microbiology & Molecular Genetics, University of the Punjab, Lahore
[2]Soil & Environmental Biotechnology Division,
National Institute for Biotechnology and Genetic Engineering, Faisalabad

Abstract: Antibiotic-resistant bacterial infections are of global concern nowadays. Environmental sources like water and soil are playing a key role in spreading antibiotic-resistance genes to humans, animals, and other environments. Objective: The purpose of this study was to identify and report the presence of multidrug-resistant bacteria (MDRs) in environmental water sources that can direct the spread of resistant genes to other bacteria/environments. Methodology: Environmental water samples were collected from 2 livestock farms and a fish pond. Bacterial isolation and identification were carried out by following Burgey's manual of systematic bacteriology. Antibiotic susceptibility testing was done using the disk diffusion method and CLSI guidelines. Multiple antibiotic-resistant indexes were calculated. Whole genome sequences of previously reported bacteria were downloaded from NCBI to detect the resistance genes associated with phenotypic drug resistance and compared using the bioinformatics approach. Results: Microbial load was significantly high in all water sources. Following Genera were the most common: *Klebsiella, Escherichia, Proteus, Serratia, Acinetobacter, Enterobacter, Pseudomonas, Bacillus, Lactobacillus*, and *Staphylococcus*. Out of 10 classes of antibiotics, resistance against 8 classes were identified. Multiple Antibiotic Resistance (MAR) index range of isolated strains was between 0.4 and 0.9. Key Findings: Resistance against beta-lactam antibiotics was highest in our isolated strains with a MAR index of greater than 0.4 altogether. Conclusion: High burden of multidrug-resistant bacteria were detected in all water samples which can trigger the silent pandemic of antibacterial resistance.

Keywords: Antibiotic Resistance, Antibiotic Resistant Bacteria (ARBs), Antibiotic Resistance Genes (ARGs), Beta-lactam antibiotics, ESKAPE pathogens, MAR Index, Penicillins.

1. INTRODUCTION

Antimicrobial resistance (AMR) is increasing at an alarming pace in bacteria causing a major threat to existing options for antibiotics treatment. An enormous increase in antibiotic-resistant bacteria and antibiotic-resistance genes (ARGs) are universally found in human and animal infections and also in contaminated environments. This led to the emergence of a new term "Silent Pandemic of Antibiotic Resistance" [1]. Resistance to even the last regimes of antibiotics has been developed leaving very limited options or on occasion with no options at all making it impossible to treat antibiotic-resistant bacterial infections globally [2]. With each passing year, the number of deaths occurred by antibiotic-resistant bacterial infections is increasing significantly [3].

In 2019, nearly 4.95 million deaths have occurred due to antibiotic-resistant bacterial infections solely [4]. This number will increase exponentially in coming years and according to Balasegaram, this rise in infections due to antibiotic-resistant superbugs will leave humans with no choice even to treat very common bacterial infections in near future [5]. According to O'Neill, 2016, this silent pandemic will lead to more and more loss of precious human lives. In absence of any effective control measures, this pandemic

Received: December 2022; Accepted: January 2023
*Corresponding Author: Hafiza Jawairia Hashmi <hafizajawairiahashmi@gmail.com; jawairia.phd.mmg@pu.edu.pk>

will lead to around 10 million deaths and over 100 trillion dollars in monetary loss globally by 2050 [1]. According to research done by RAND Cooperation, the world population would have been 11-444 million more in absence of AMR as it would be in presence of drug-resistant superbugs in 2050 [1].

Mostly six (6) bacteria are involved in causing the deaths of humans in clinical cases named ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species). In 2019, ESKAPE pathogens have caused more than 1 million human deaths worldwide. [6]. The acquisition of resistance genes by these bacteria have been reported internationally to reduce the available antibiotic options to treat clinical infections [7]. These bacteria acquire resistance genes mostly through horizontal gene transfer mechanisms when present in a favorable environment along with a little role played by vertical transmission of genes from parents to daughter cells [5].

Intensive use of antibiotics as growth promoters and prophylactic use in animal farming, aquacultures, and clinical use of antibiotics in humans has been proposed to be the most common means of antibiotic resistance development and dissemination [1]. COVID-19 pandemic has also played a vital role in elevating drug resistance, as excessive and unnecessary use of antiseptics and sterilizers expedited the ARB propagation [8].

The unnecessary use and incomplete regimens of antibiotics in animals and humans lead to the survival of ARBs in presence of sub-lethal doses of antibiotics favoring the resistant superbugs to survive. These superbugs can then spread to humans through animal waste contaminated water, soil, and other environments where humans are in contact with animals and also by contaminated food animals like fish, cattle, etc. [1].

Epidemiological records of AMR are very imperative for AMR surveillance, for policy makers to ploy effective strategies, and to check the success of control measures adopted to combat AMR. Most of the recent and past studies are focused on clinical AMR cases. For effective surveillance of AMR, detection of ARBs and their antibiotic resistance patterns in different environmental sources is imperative to control the antibiotic resistance pandemic in the future. In this paper, we have investigated the presence of ARBs in contaminated water sources including fish pond water and drinking water of livestock rearing farms in Lahore Pakistan. This paper has helped us in pinpointing the presence of similar ARGs in pathogenic as well as in non-pathogenic bacteria confirming the spread of ARGs from environmental bacteria to human/ animal pathogens through HGT events.

2. MATERIALS AND METHODS

2.1 Water Sampling Sites

Water samples were collected from 3 different sources. One sample was collected from a fish pond located in Lahore while the other 2 samples were collected from small local livestock farms (drinking water samples of cattle) located at two different localities in the outskirts of Lahore. Topographical plots of all the sample collection sites (Figure 1).

2.2 Physical and Chemical Parameters of Water Samples

The sample's pH, temperature, colour, and odour were checked at sampling sites while taking samples. Sampling bottles containing samples were kept in ice containers before shipping to the lab for further analysis. On reaching the lab, the sample's electrical conductance (E.C), turbidity, and total dissolved solids were checked.

Chemical parameters of water quality testing were also checked to determine the safety of water samples for drinking by livestock animals.

2.3 Microbiological Testing

For microbiological analysis, samples were collected in sterile bottles. Serial dilutions of each sample were made and 100 µl of each dilution was spread on N-Agar plates in triplicate. These plates were incubated at 37 °C for 24 hours. After incubation, the number of colonies in each plate was recorded and the average at each dilution was calculated. Colony morphology of each unique and single colony on all plates was recorded and these marked colonies were further purified by using the quadrant streaking method.

2.4 Gram Staining and Biochemical Tests

Gram staining was done to find out the bacterial shape and gram's reaction. Smears were made, stained, and analyzed under a light microscope using an oil immersion lens. Based on gram staining results, each isolated strain was identified biochemically up to the genus level using Bergey's manual of systematic bacteriology.

2.5 Antibiotic Susceptibility Testing (AST)

Each identified strain was tested against at least 4 or more antibiotics classes following the Kirby Bauer disk diffusion assay. Each strain was diluted in accordance with 0.5 McFarland standard and then sterile cotton swabs were used for swabbing on Muller Hinton agar. After 16-18 hours of incubation at 37 °C, zones of inhibition diameters were measured and strains were categorized as resistant, intermediate, and sensitive against each tested antibiotic using the Clinical and Laboratory Standards Institute's (CLSI) guidelines. Multiple antibiotic resistance index was also calculated by dividing the no. of antibiotics to which a specific bacterial strain had shown resistance by the number of total tested antibiotics [9].

3. RESULTS

3.1 Physical and Chemical Parameters of Water Quality Testing

All the Physical parameters of water quality testing were normal for both drinking water samples of livestock farms whereas the fish pond water sample had a slightly unpleasant smell. Fish pond water samples also had a higher TDS and hence higher temperature and E.C. as well in comparison to other water samples (Table 1). All the chemical parameters of water samples were also in an acceptable range of drinking water (Table 2).

3.2 Microbiological Testing

There was a very high number of bacteria in the fish pond water sample as compared to the other 2 samples. Water sample from farm 1 has a lower colony count as compared to the sample from farm 2. Diversity of bacteria in drinking water samples from both farms was very low. Similar colonies in variable numbers were present on all petri dishes (Table S1). From the fish pond sample, 12 (52 %) colonies were selected for further study and characterization whereas, from livestock drinking water samples, 6 (26 %) and 5 (22 %) colonies were selected from farms 1 & 2 respectively. Colony morphology of each selected strain is summarized in Table S2. Pigmentation of selected and isolated strains varies from off-white, and white to yellow and orange. Their sizes range from pinpoint colonies to 35 mm. Elevation, texture, surface appearance, shape, margins, and opacity are also summarized in Table S2.

3.3 Gram Staining and Biochemical Characterization

In gram staining results, 9 % of strains were identified as gram-positive cocci, 13 % as gram-positive rods, and 78 % as gram-negative rods making them the most prevalent bacterial type. We have observed not a single gram-negative coccus from all three samples.

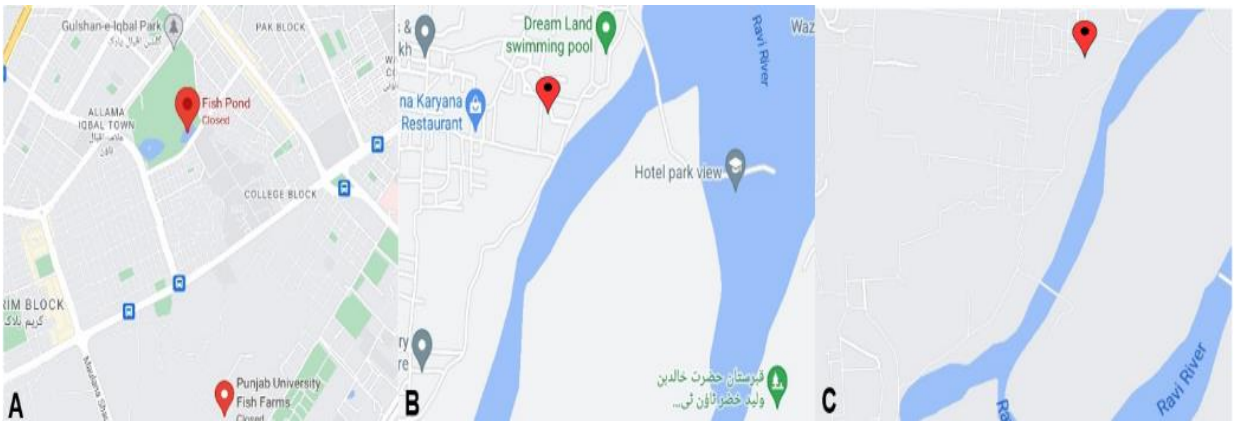

Fig 1. Topographical plots of the sample collection sites; A: Fish Pond water sample, B: Cattle farm 1 sample, C: Cattle farm 2 sample.

Table 1. Physical Parameters of Water Quality

Parameters	Units	Detection Limit	Reference Method	Permissible limits (PSQCA/NSDWQ, 2010)	Permissible limits (WHO)	Fish Pond	Cattle farm 1	Cattle farm 2
Color	-----	-----	Sensory Evaluation	5	5	Colorless	Colorless	Colorless
Odor	-----	-----	Sensory Evaluation	Odorless	Odorless	Pungent	Odorless	Odorless
Temperature	°F	41	Thermometer	NGVS	NGVS	68	62	60.6
Electrical Conductivity (E.C)	(µS/cm)	0.11	APHA, 22nd Edition	NGVS	NGVS	876	282	234
pH	-----	0.03	APHA, 22nd Edition	6.5-8.5	6.5-8.5	7.9	7.3	6.9
Turbidity	NTU	0.31	APHA, 22nd Edition	<5	0.5	7.1	1.25	0.9
Total Dissolved Solids (TDS)	ppm	-----	APHA, 22nd Edition	1000	1000	672	172	154

APHA: American Public Health Association
NGVS: No Guideline value set
NSDWQ: National Standards for Drinking Water Quality
PSQCA: Pakistan Standard Quality Control Authority
WHO: World Health Organization

Table 2. Chemical Parameters of Water Quality

Parameters	Units	Detection Limit	Reference Method	Permissible limits (PSQCA/NSDWQ, 2010)	Permissible limits (WHO)
Alkalinity as CaCO3	ppm	----------	APHA, 22nd Edition	NGVS	NGVS
Bicarbonate	ppm	5.0	APHA, 22nd Edition	NGVS	NGVS
Calcium	ppm	2.0	APHA, 22nd Edition	NGVS	200
Carbonate	ppm	5.0	APHA, 22nd Edition	NGVS	NGVS
Chloride	ppm	2.0	APHA, 22nd Edition	250	250
Hardness	ppm	5.0	APHA, 22nd Edition	500	500
Magnesium	ppm	1.0	APHA, 22nd Edition	NGVS	150
Potassium	ppm	0.02	APHA, 22nd Edition	NGVS	30
Sodium	ppm	1.57	APHA, 22nd Edition	NGVS	200
Sulphate	ppm	0.24	APHA, 22nd Edition	NGVS	250
Nitrate	ppm	0.03	APHA, 22nd Edition	10	10

APHA: American Public Health Association
NGVS: No Guideline value set
NSDWQ: National Standards for Drinking Water Quality
PSQCA: Pakistan Standard Quality Control Authority
WHO: World Health Organization

Biochemically, gram-positive cocci were further checked for the presence of catalase enzyme and all the isolates were positive for the catalase test confirming them to be Staphylococcus species and ruling out *Streptococcus species* (100 %). The yellow pigment was not observed in any of the gram-positive cocci colonies, so, ruled out the presence of *Micrococcus* organisms. Spore staining of gram-positive rods was done to find out the *Bacillus* species and 66.67 % of isolates were positive for endospore confirming them to be Bacillus species. No isolate was positive for acid-fast stain ruling out the presence of *Mycobacterium* species. Non-spore formers were further checked for the presence of catalase enzyme and 33 % of isolates were confirmed to be *Lactobacillus* by having catalase enzyme.

Oxidase test was performed for all the gram-negative rods; 33 % of strains were positive for oxidase. These oxidase-positive organisms were further evaluated for glucose fermentation. Approximately, 67 % of isolates were negative for glucose fermentation characterizing them to be *Pseudomonas* species whereas the remaining 33 % of the organism that were negative for glucose fermentation and positive for lactose fermentation were characterized as *Aeromonas* species. None of the isolates required sodium salts for their growth. A single strain that is gram-negative coccobacilli, oxidase negative, catalase positive, and oxidized glucose in OF test was identified to be an *Acinetobacter* specie. Remaining gram-negative isolates were characterized as pathogens of the family *Enterobacteriaceae*. They were further characterized using the API 20E strips. Proteus species were found to be 27 % whereas *Escherichia, Serratia, Enterobacter*, and *Klebsiella* species were all found to be 18 % approximately.

3.4 Antibiotic Susceptibility Testing

Various classes and multiple numbers of antibiotics were used for testing. CLSI guidelines (2019) were followed to characterize the strains as resistant, sensitive, and intermediately resistant to tested antibiotics. Resistance against eight out of 10 classes of antibiotics was observed. All verified strains were resistant to multiple classes of antibiotics confirming them to be multiple drug-resistant (MDR) strains. Out of all the tested strains against amoxicillin, ampicillin, piperacillin, piperacillin-tazobactam, tobramycin, cefuroxime, cefoxitin, meropenem, and linezolid was 100 %. Tested strains showed 100 % sensitivity towards doxycycline and clindamycin. AST results are summarized in Table 3.

3.5 MAR Index

MAR index of all the isolated strains was higher than 0.4 and ranges between 0.4 – 0.9. Bacteria isolated from livestock farms had the highest MAR index as compared to strains isolated from fish pond water samples (Figure 2).

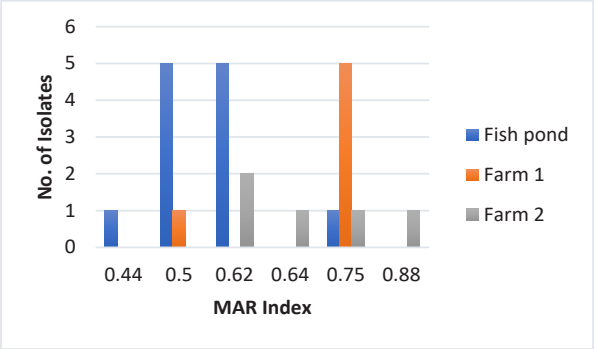

Fig. 2. Comparison of MAR Index of isolated strains

4. DISCUSSION

A large number of bacterial infections are caused by multidrug-resistant (MDR), extremely drug-resistant (XDR), or even total drug-resistant (TDR) organisms worldwide. AMR leads to greater than before morbidity cases, and early deaths in young individuals cause powerlessness. AMR is not only a threat to human lives but it is also a threat to the world economy [10]. To check the presence of ARBs in environments where food animals live, a water sample from a fish pond and 2 livestock farms was collected. Physical and chemical water quality parameters were checked as these were the drinking water samples of livestock animals. Visual impurities were not present in any sample indicated by colorlessness, odourlessness, normal pH range, and E.C. under permissible limits of WHO [11]. Chemical parameters of all the samples were also in the permissible range implying that these water samples are safe to drink by livestock animals [11]. Our main focus was on the biological contamination in water sources in accordance with the WHO plan to monitor and report the environmental ARBs

Table 3. Summary of Antibiotic Susceptibility Testing

Antibiotic Class	Antibiotic Generation	Antibiotic (Conc. In µg)	Abbreviation	No. of Isolates		
				Resistant	Intermediate	Sensitive
Penicillins	2nd	Amoxicillin (2)	A2	14	0	0
	2nd	Amoxicillin + Clavulanic acid (2+1)	AUG3	6	0	1
	3rd	Ampicillin (10)	AM10	7	0	0
	4th	Piperacillin 30	PRL30	9	0	0
	4th	Piperacillin-Tazobactam (30+6)	PTZ36	7	0	0
Aminoglycosides	-----	Gentamycin (10)	GM10	2	2	8
	-----	Tobramycin (10)	TN10	1	0	0
	-----	Amikacin (30)	AK 30	3	2	5
Tetracycline	1st	Tetracycline (30)	TE30	4	2	3
	2nd	Doxycycline (30)	DX30	0	0	1
Quinolones	2nd	Ciprofloxacin (1)	CIP1	10	3	0
	2nd	Levofloxacin (10)	LEV10	6	7	2
	3rd	Gatifloxacin (5)	GAT5	0	3	6
Cephalosporins	2nd	Cefuroxime (5)	CXM5	11	0	0
	2nd	Cefoxitin (10)	FOX10	14	0	0
	3rd	Cefotaxime+Clavulanic Acid (30+10)	CTC40	7	0	9
Macrolides	-----	Azithromycin (15)	ATH15	6	1	2
Carbapenems	-----	Meropenem (10)	MEM10	9	0	0
	-----	Imipenem (10)	IMI10	6	1	0
Lincosamide	-----	Clindamycin (2)	CD2	0	0	2
Chloramphenicol	-----	Chloramphenicol (30)	C30	0	4	5
Oxazolidinones	-----	Linezolid (10)	LZD10	1	0	0

along with clinical cases of ARBs [12]. Bacterial load was high in all samples, particularly in the fish pond water sample. The most frequent genus found in our study was *Pseudomonas* followed by *Proteus* (Figure 3). A study from Ghana also reported the high prevalence of *Pseudomonas* in environmental samples [13]. Another study reported the high prevalence of *Escherichia* and *Klebsiella* isolates [14]. A possible reason for the low bacterial load in the drinking water of livestock farms is that these samples were taken early in the morning when fresh water was given to animals. There are quite high chances of greater bacterial load if samples were taken in the afternoon or the evening. Isolated bacterial populations may also be subject to seasonal variation.

All isolates were verified against at least four different classes of antibiotics. *Pseudomonas* isolate had shown the resistance to most classes of antibiotics followed by genus *Escherichia*. MAR Index less than 0.2 is considered as safe, conversely, greater MAR Index is a signal of fecal contamination [15]. All the genus identified and studied in our paper had a MAR Index greater than 0.40. Table 4 representing contamination from a source where antibiotics are in use frequently and gratuitously [16]. This is a confirmation of antibiotics use as growth promoter and as prophylactic in livestock and fish farming irrelevantly.

Isolated strains from the fish pond had the higher resistance against multiple antibiotics followed by livestock farm 1 and the least resistance was observed in livestock farm 2 samples. Similar kind of results has also been reported in various studies worldwide. A study from Japan has reported the presence of all 6 clinically important pathogens in waste water treatment plants and all these isolates were highly resistant to multiple antibiotics [17]. Similarly, another study from South Africa also reported the presence of a large number of ARBs from various environmental sources including irrigation water, waste water, surface water, and drinking water (also in food items and vegetables) [18]. Researchers from Saudi Arabia had reported the presence of ESKAPE pathogens in nearly half of the hospital-acquired bacterial infections. All

Table 4. Genus wise MAR Index

Genus	Average MAR Index
Pseudomonas	0.68
Aeromonas	0.62
Acenitobacter	0.50
Escherichia	0.75
Proteus	0.56
Serratia	0.56
Enterobacter	0.81
Klebsiella	0.56
Staphylococcus	0.63
Bacillus	0.68
Lactobacillus	0.50

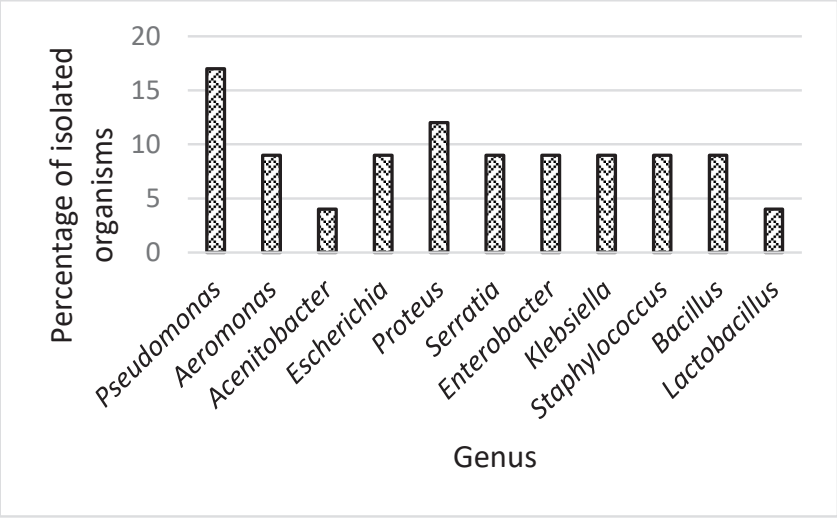

Fig. 3. Genus wise percentage of isolated strains

those pathogens were highly resistant to multiple antibiotics [19].

A study reported the presence of many distinguished MGEs in ESKAPE pathogens carrying resistance genes against Beta-lactam drugs and aminoglycosides [20]. This is a strong indicator of the spread of resistance against beta-lactam and other drugs in our as well as former studies by means of horizontal gene transfer mechanisms.

5. CONCLUSION

OA high burden of multidrug-resistant bacteria were isolated from all the water samples including bacteria from *Enterobacteriaceae, Pseudomonas, Aeromonas, Lactobacillus, Bacillus,* and *Staphylococcus*. Moreover, the MAR Index of all the isolated strains was greater than 0.4 indicating the unnecessary use and presence of antibiotics in selected environments. This means environmental water sources are playing a critical role in triggering the transmission of drug resistance through horizontal gene transfer mechanisms.

6. ACKNOWLEDGEMENTS

This manuscript is part of the Ph.D. thesis of author Hafiza Jawairia Hashmi. Authors are obliged to the University of the Punjab Lahore Pakistan for financial support.

7. CONFLICT OF INTEREST

Authors hereby declare no conflict of interest.

8. REFERENCES

1. M. Ye, J.Q. Su, X.L. An, and Y.G. Zhu. Silencing the silent pandemic: Eliminating antimicrobial resistance by using bacteriophages. *Science China. Life Sciences* 65: 1890-1893 (2022).
2. H. Carboné, M.S. Cotillas, E. Lacasa, P. Canizares, M.A. Rodrigo, and C. Sáez. Depletion of ARGs in antibiotic-resistance Klebsiella, Pseudomonas and Staphylococcus in hospital urines by electro and photo-electro disinfection. *Journal of Water Process Engineering* 49. 103035 (2022).
3. CDC. Antibiotic resistance threats in the United States, 2019. US Department of Health and Human Services, *Centres for Disease Control and Prevention, Atlanta* (2019).
4. C.J.L. Murray, K.S. Ikuta, F. Sharara, L. Swetschinski, G.R. Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, and E. Wool. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet* 399: 629-655 (2022).
5. M. Balasegaram. Learning from covid-19 to tackle antibiotic resistance. *ACS Infectious Diseases* 7: 693-694 (2021).
6. B.S. Lopes, A. Hanafiah, R. Nachimuthu, S. Muthupandian, Z.N.M. Nesran, and S. Patil. The role of antimicrobial peptides as antimicrobial and antibiofilm agents in tackling the silent pandemic of antimicrobial resistance. *Molecules* 27: 2995 (2022).
7. B.S. Lopes, N.J. Strachan, M. Ramjee, A. Thomson, M. MacRae, S. Shaw, and K.J. Forbes. Nationwide stepwise emergence and evolution of multidrug-resistant Campylobacter jejuni sequence type 5136, United Kingdom. *Emerging infectious diseases* 25: 1320 (2019).
8. J. Lu, and J. Guo. Disinfection spreads antimicrobial resistance. *Science* 371(6528): 474-474.
9. A.A.S. Sinegani, and N. Younessi. Antibiotic resistance of bacteria isolated from heavy metal-polluted soils with different land uses. *Journal of Global Antimicrobial Resistance.* 10: 247-255 (2017).
10. WHO. Glass whole-genome sequencing for surveillance of antimicrobial resistance. *World Health Organization, Geneva* (2020).
11. P.D. Patil, R.D. Sawant. Physico-chemical parameters for testing of water-a review. *International Journal of Environmental Sciences* 3: 1194 (2012).
12. M. Henderson, S.J. Ergas, K. Ghebremichael, A. Gross, and Z. Ronen. Occurrence of antibiotic-resistant genes and bacteria in household greywater treated in constructed wetlands. *Water* 14: 758 (2022).
13. H. Odoi, V.E. Boamah, Y.D. Boakye, and C. Agyare. Prevalence and phenotypic and genotypic resistance mechanisms of multidrug-resistant Pseudomonas aeruginosa strains isolated from clinical, environmental, and poultry litter samples from the Ashanti region of Ghana. *Journal of Environmental and Public Health* 2021: 9976064 (2021).
14. H. Tesfaye, H. Alemayehu, A.F. Desta, and T. Eguale. Antimicrobial susceptibility profile of selected Enterobacteriaceae in wastewater samples from health facilities, abattoir, downstream rivers and a

WWTP in Addis Ababa, Ethiopia. *Antimicrobial Resistance & Infection Control* 8: 1-11 (2019).

15. G.T. Gessew, A.F. Desta, and E. Adamu. High burden of multidrug resistant bacteria detected in little Akaki river. *Comparative immunology, microbiology and infectious diseases.* 80: 101723 (2022).
16. P.P. Amador, R.M. Fernandes, M.C. Prudêncio, M.P. Barreto, and I.M. Duarte. Antibiotic resistance in wastewater: Occurrence and fate of Enterobacteriaceae producers of class a and class c β-lactamases. *Journal of Environmental Science and Health, Part A* 50: 26-39 (2015).
17. M. Nishiyama, S. Praise, K. Tsurumaki, H. Baba, H. Kanamori, and T. Watanabe. Prevalence of antibiotic-resistant bacteria eskape among healthy people estimated by monitoring of municipal wastewater. *Antibiotics* 10: 495 (2021).
18. J. Denissen, B. Reyneke, M. Waso-Reyneke, B. Havenga, T. Barnard, S. Khan, and W. Khan. Prevalence of eskape pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. *International Journal of Hygiene and Environmental Health*. 244: 114006 (2022).
19. N.J. Ahmed, A. Haseeb, M.A. Hassali, E.E. Elsaid, and A.H. Khan. Antimicrobial resistance of eskape pathogens in a public hospital in Alkharj. *Latin American Journal of Pharmacy* 40: 442 (2021).
20. S. Das, S. Bombaywala, S. Srivastava, A. Kapley, R. Dhodapkar, and N.A. Dafale. Genome plasticity as a paradigm of antibiotic resistance spread in eskape pathogens. *Environmental Science and Pollution Research* 29: 40517-40529 (2022).

9. DECLARATION

Authors declare that:

(i) the results are original;
(ii) the same material is neither published nor under consideration elsewhere;
(iii) approval of all authors have been obtained; and
(iv) in case the article is accepted for publication, its copyright will be assigned to Pakistan Academy of Sciences.