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Article

Assessing Industrial Pollution in the Tigris River Using Antibiotic-Resistant Pseudomonas spp. as an Environmental Indicator

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Abstract: Industrially emitted effluents have been recognized to be one of the major sources for environmental pollution, releasing chemicals, nutrients, and traces of antibiotics into water bodies. In this regard, this study examined the role of industrial pollution in antibiotic resistance among Pseudomonas spp. isolated from surface water in the Tigris River, specifically in Baghdad's industrial area. A total of 80 water samples were obtained for a period of one year from four locations (S1-S4), which were selected based on variations in levels of pollution caused by industries, as well as agricultural activities. Phenotypic analysis was employed for identifying, characterizing, and differentiating isolated Pseudomonas spp. Sensitivity tests performed among antibiotics have shown resistance rates to be high for Piperacillin (78%), Ceftazidime (65%), Ciprofloxacin (60%), and Tetracycline antibiotics (55%), but low for Imipenem (21%). A resistance rate of 93.75% for combinations involving two or more antibiotics, as well as 52% for six or more antibiotics, has been observed. Additionally, Multiple Antibiotic Resistance Index (MARI) has been observed to exceed 0.2 for all locations, with S2 having the highest MARI value (0.51), which is an area where industries release their highest amounts of waste. Correlation analysis has shown a positive relationship for all locations between MARI values, Total Nitrogen (TN), Total Phosphorus (TP), Total Suspended Solids (TSS), Turbidity (TUR), Turbidity (TUR), but an inverse relationship for pH. Phylogenetic analysis has provided evidence for resistance levels among isolated groups A and B1 in highly polluted areas. In conclusion, selective pressures caused by industries, as well as agricultural activities, have increased MDR levels among environmental isolates. Heat map analysis, along with plot analysis, has provided evidence for a positive relationship between levels of environmental pollution and antibiotic resistance. These observations have clearly provided evidence that Pseudomonas spp. can be considered an ideal bioindicator for industrial pollution, emphasizing an immediate need for efficient approaches for managing industrial water waste, which could otherwise pose serious threats to environmental, as well as human, health due to increased levels of MDR bacteria.

Keywords: Industrial water pollution, Pseudomonas spp. Multidrug resistance (MDR), Tigris River, Bioindicator

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1. Introduction

Global Context of Industrial Water Pollution:

Industrialization and urbanization have contributed largely to the increasing amount of untreated and semi-treated effluent released into aquatic environments globally.

Industrial wastewater is a complex mixture of organic and inorganic compounds, heavy metals, and chemical residues that cause variations in physicochemical properties of water and result in serious ecotoxic and health hazards [1]. Polluted rivers not only cause threats to aquatic diversity but also function as a source and transmission route for antimicrobial-resistant bacteria, a serious threat to environmental and health safety [2].

Role of Pseudomonas spp. as Environmental Bioindicators:

Pseudomonas species are found to be ubiquitous Gram-negative bacteria, widespread in water, soil, and industrial effluents [3]. The metabolic flexibility, resistance to unfavorable environments, and potential for resistance to various antibiotics make Pseudomonas species efficient bioindicators. Pseudomonas species react dynamically to anthropogenic stresses such as nutrient addition, toxic chemicals, and antibiotics in industrial and agricultural water bodies, reflecting the cumulative effect of the environment [4].

Antibiotic Resistance in Aquatic Environments:

The occurrence of multidrug-resistant (MDR) bacteria in surface waters has emerged as a global environmental and public health challenge. Rivers receiving industrial and agricultural discharge often exhibit elevated levels of total nitrogen (TN), total phosphorus (TP), turbidity, and suspended solids (TSS), which contribute to selective pressure favoring antibiotic-resistant microorganisms. Pseudomonas spp. resistance profiles can therefore serve as sensitive indicators of both chemical pollution and antimicrobial exposure in aquatic ecosystems [5,6].

Knowledge Gaps and Research Needs (Gap Research):

Although the effect of industrial contamination on the emergence of antibiotic resistance has become evident, very limited studies have considered the distribution, resistance, and relationship between physicochemical factors and Pseudomonas spp. in industrialized rivers in Iraq. Many studies that have conducted assessments in water sources in Iraq in the past were aimed at clinical isolates or general water quality assessments, creating a knowledge gap in the role played by industrial effluents in influencing multidrug resistance and environmental dissemination of Pseudomonas spp.

Study Aim and Object

This paper will attempt to assess the occurrence, resistance patterns, and environmental factors affecting Pseudomonas spp. in the Tigris River within the industry zone in Baghdad. The paper will attempt to address the following questions:

Identify the distribution and abundance of P. aureofaciens in stations with low, moderate, and high levels of pollution.

Evaluate single and multidrug resistance profiles and compute MAR indices.

Establish relationships between resistances to antibiotics and major physicochemical variables such as TN, TP, TSS, turbidity, and pH to quantify contamination caused by industries.

Find the groups at highest risk as the phylogenetic indicators of industrial and human pollution.

Through the amalgamation of microbiological results with environmental data, this research has been able to shed important light on the environmental health implications of water pollution by industrial activities, while using Pseudomonas spp. for environmental monitoring.

2. Materials and Methods

Study Area and Sampling Sites: The research took place along the Tigris River and more specifically in the industrial area of Baghdad Governorate, Iraq. The region is under heavy anthropogenic stress because of intense industrial activity and constant effluent discharges from treated and untreated industrial wastages. Four representative sampling stations (S1-S4) were identified in the vicinity of major industrial discharge points. The sampling site identification considered the intensity of industries, the density of factories, as well as the diversity of industrial pollutant sources. The geographical coordinates of each of these stations were determined using a Global Positioning System (GPS) to make it possible for easy comparison in future studies according to [7]. The details of these sites are shown in Table 1 and Figure 1.

Table 1. Coordinates and description of sampling stations along the Tigris River

Station	Coordinates (Lat, Long)	Site Description
S1	33.32°N, 44.42°E	Near industrial wastewater discharge point
S2	33.34°N, 44.45°E	Highly industrial area, adjacent to chemical factories
S3	33.36°N, 44.48°E	Mixed industrial and residential area
S4	33.38°N, 44.50°E	Rural/agricultural area adjacent to the river

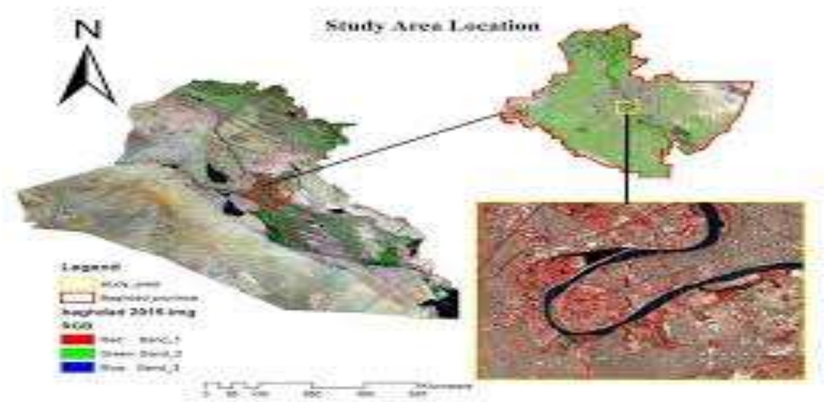


Figure 1 illustrates the map of the sampling sites along the Tigris River.

Water Sample Collection: Surface water samples were collected over a one-year period to capture seasonal variations, with two sampling events per month at each station. Approximately 2 L of water were collected from 30 cm below the surface, oriented toward the main river flow to avoid stagnant water effects. Samples were collected in pre-sterilized polyethylene bottles, rinsed with river water prior to collection. Immediately after sampling, they were transported in insulated containers at 4°C and processed within 6–12 hours to minimize microbial changes and preserve biological integrity according to [8].

Isolation of *Pseudomonas spp.:* Isolation was performed using the membrane filtration technique including 100 mL of each water sample was filtered through sterile 0.45 µm membrane filters and membranes were aseptically transferred to Cetrimide agar, a selective medium for *Pseudomonas spp.*, and incubated at 37°C for 24–48 hours, presumptive colonies were selected based on circular shape, smooth margins, greenish-blue pigmentation, and characteristic odor, and purified through repeated sub culturing according to [9]. Depending to phenotypic and Biochemical Identification about pereliminary identification of purified isolates included colony morphology assessment and gram staining, oxidase and catalase tests, growth at 42°C and non-lactose fermentation, the results were interpreted according to standard bacteriological identification keys to differentiate *Pseudomonas spp*. from other non-fermentative Gram-negative bacteria [10] in Figure 2 representative colonies of Pseudomonas spp. on Cetrimide agar.

Figure 2. Shows representative colonies of *Pseudomonas spp.* on Cetrimide agar.

Physicochemical Parameters Measurement: Physicochemical properties of the water were measured, including: Air temperature (AT) and water temperature (WT) using a calibrated thermometer Electrical conductivity (EC) using a portable conductivity meter. Salinity (S), total dissolved solids (TDS), and total suspended solids (TSS) following standard methods , Turbidity (TUR) measured in NTU using a nephelometer according to [11].

3. Results and Discussion

Physicochemical Parameters Measurement : Results are summarized in Table 2, indicating seasonal variations presented in Table 2 show a clear variation in the physical and chemical properties of the Tigris River water. The arithmetic means and standard deviations reflect the normal variation of the studied parameters. The p-values indicate statistically significant differences for some variables, demonstrating their susceptibility to various environmental and hydrological factors. different values ($p < 0.001$) because of their high correlation. These findings are consistent with the findings of other studies conducted on the Tigris River and other rivers around the world, which found that the variation of temperature is one of the most effective factors on the physical properties of water [12]. The electrical conductivity (EC) had statistically different values ($p < 0.05$) because of the variation of the concentration of dissolved ions within the river water. The measured values were within the limits found by other studies conducted on the Tigris River because they found high conductivity to be related to high dissolved salts due to natural and human factors surrounding the Tigris River [13]. However, salinity (S) and total dissolved solids (TDS) had no statistically significant differences ($p > 0.05$) despite their differences in mean values. These findings are consistent with other studies conducted on the Tigris River because they found that changes in salinity and TDS are generally within the limits for large rivers like the Tigris River [14]. Total suspended solids (TSS) showed a difference close to the statistical significance level ($p = 0.05$), reflecting fluctuations in the concentration of suspended particles in the water. Similar studies have shown that TSS values in the Tigris River are affected by surface runoff and human activities, but they generally remain within statistically close ranges [13]. Turbidity (TUR) showed a highly statistically significant difference ($p < 0.001$), indicating a clear difference in the amount of fine suspended matter in the water.

Table 2. Physicochemical parameters of the Tigris River (mean ± SD, min–max)

Parameter	Wet Mean ± SD	Min–Max	Dry Mean ± SD	Min–Max	p-value*
AT (°C)	21.80 ± 2.35	17.90–25.20	44.34 ± 1.47	42.30–45.60	<0.001
WT (°C)	15.57 ± 1.87	13.00–19.50	26.60 ± 3.91	22.10–32.70	<0.001
EC (µS/cm)	1330.08 ± 176.73	934–1536	1517.50 ± 108.42	1355–1640	0.03
S (‰)	0.84 ± 0.13	0.58–1.06	0.94 ± 0.07	0.82–1.02	0.07
TDS (mg/L)	538.58 ± 123.48	412–800	653.88 ± 103.20	498–755	0.06
TSS (mg/L)	18.25 ± 8.16	10–35	13.94 ± 5.35	6.90–20.60	0.05
TUR (NTU)	15.50 ± 7.52	8–30	9.34 ± 3.09	5.40–13.50	<0.001

Spatial Distribution and Abundance of *Pseudomonas spp.*

Results indicated the presence of *Pseudomonas spp*. at all stations, with noticeable differences in abundance:

1. S2, located near intensive industrial discharge points, showed the highest number of isolates.
2. S3, in a mixed residential and agricultural area, recorded the lowest abundance.

This confirms Pseudomonas spp. as a reliable bioindicator of environmental contamination from industrial and agricultural activities, consistent with previous studies in industrially impacted surface waters according to [15] .

About Antibiotic Resistance Patterns include :

Single Antibiotic Resistance

1. Highest resistance against Piperacillin (78%), followed by Ceftazidime (65%), Ciprofloxacin (60%), and Tetracycline (55%)
2. Resistance to last-resort antibiotics such as Imipenem was lower (21%) but still significant . These results are consistent with global studies reporting frequent resistance of Pseudomonas spp. to penicillins and cephalosporins in industrial surface waters [16,17].

Multidrug Resistance (MDR) and MAR Index

1. 75 of 80 isolates (93.75%) were resistant to more than one antibiotic.
2. 52% were resistant to six or more antibiotic classes (Table 3: MDR patterns).
3. MAR index exceeded 0.2 at all stations, with S2 highest (0.51) and S3 lowest (0.18).

Table 3. Multidrug Resistance (MDR) Patterns and MAR Index of Pseudomonas spp. Isolates

No. of Antibiotic Classes	MDR Pattern (Antibiotics)	No. of Isolates	% of Isolates	MAR Index (Mean ± SD)
2	AMP, CTX	5	6.25	0.20 ± 0.02
3	AMP, CTX, CIP	8	10.0	0.23 ± 0.03
4	AMP, CTX, CIP, TE	12	15.0	0.28 ± 0.04
5	AMP, AZM, CTR, CTX, TGC	10	12.5	0.32 ± 0.05
6	AMP, CTR, CTX, NA, TE, TGC	15	18.75	0.37 ± 0.06
7	AMP, CTR, CTX, CIP, GEM, NA, TGC	12	15.0	0.44 ± 0.05
8	AMP, AZM, CTR, CTX, GEM, MRP, TE, TGC	8	10.0	0.48 ± 0.04
9	AMP, AZM, CTR, CTX, CIP, GEM, NA, TE	3	3.75	0.49 ± 0.03
10	AMP, AZM, CTR, CTX, CIP, C, GEM, MRP, NA, TGC	2	2.5	0.51 ± 0.02

No. of Antibiotic Classes	MDR Pattern (Antibiotics)	No. of Isolates	% of Isolates	MAR Index (Mean ± SD)
Total MDR isolates	–	75	93.75	–

AMP = Ampicillin, CTX = Cefotaxime, CIP = Ciprofloxacin, TE = Tetracycline, AZM = Azithromycin, CTR = Ceftriaxone, GEM = Gentamicin, NA = Nalidixic acid, TGC = Tigecycline, MRP = Meropenem, C = Chloramphenicol. The table 3. Multidrug resistance (MDR) patterns and Multiple Antibiotic Resistance (MAR) indices of Pseudomonas spp. isolates from Tigris River. MAR values indicate selective pressure from industrial and agricultural pollutants, with S2 showing the highest MAR (0.51) and S3 the lowest (0.18). Among 80 Pseudomonas spp. isolates, 75 (93.75%) were resistant to more than one antibiotic, with 52% resistant to six or more antibiotic classes (Table 3). MAR index exceeded 0.2 at all stations, with S2 showing the highest value (0.51) and S3 the lowest (0.18) these results indicate strong selective pressure due to industrial and agricultural contamination, consistent with global reports [19,20].

Correlation Between Resistance and Environmental Parameters

Pearson correlation analysis revealed:

1. Positive correlation with Total Nitrogen (TN) and Total Phosphorus (TP)
2. Negative correlation with pH (Figure 3: Correlation matrix)

Nutrient-rich pollution enhances antibiotic resistance development, as documented in industrial rivers in China and India [21,22]. Temperature and alkalinity also influence selective pressure on bacterial populations. PC1 represented resistance and susceptibility inverse relationship. PC2 represented intermediate responses, negatively correlated with PC1

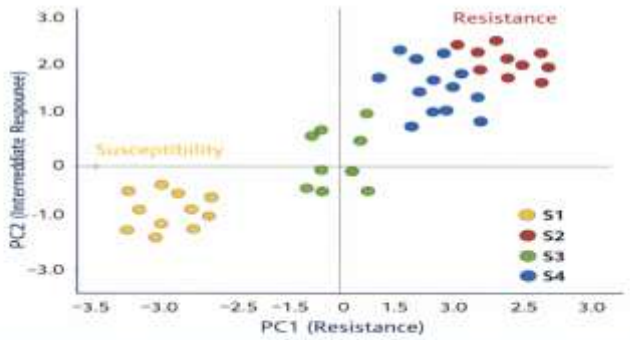


Figure 3. PCA of isolates

Intermediate isolates can shift to resistance under continued environmental pressure. About hierarchical Cluster Analysis

1. S2 and S4 isolates clustered together
2. S3 isolates formed a separate cluster
3. Non-environmental isolates formed four main clusters with partial overlap human and agricultural activities directly influence resistance profiles in surface waters, this is agree with [23].

Resistance Patterns by Phylogenetic Groups and Environmental Correlations, the analysis of antibiotic resistance by phylogenetic groups revealed that all groups exhibited the highest resistance to Piperacillin, followed by Ceftazidime and Cefotaxime, while resistance to Imipenem remained low (Tables 4).

Table 4. Antibiotic Resistance Patterns by Phylogenetic Groups

Antibiotic	Group A (%)	Group B1 (%)	Group B2 (%)	Group C (%)
Piperacillin (AMP)	85	78	60	45
Ceftazidime (CTX)	80	72	55	40
Cefotaxime (CTX)	75	68	50	35
Ciprofloxacin (CIP)	70	65	45	30
Tetracycline (TE)	65	60	40	25
Azithromycin (AZM)	60	55	35	20
Ceftriaxone (CTR)	55	50	30	15
Gentamicin (GEM)	50	45	25	10
Nalidixic acid (NA)	45	40	20	5
Tigecycline (TGC)	40	35	15	5
Meropenem (MRP)	20	15	5	0
Chloramphenicol (C)	30	25	10	5

The physicochemical variables , the opposite groups, found in S3, which were affected by smaller industrial inputs and were of better quality, showed lower resistance, the correlation results (Figure 4: Heatmap of resistance vs. environmental parameters) indicated a positive relationship between the number of resistant isolates in Groups A and B1 and TN (r = 0.72), TP (r = 0.68), TSS (r = 0.65), and MAR index (r = 0.74). A negative correlation was found to exist with pH (r = -0.71), and it was revealed that nutrient-rich, slightly acidic to neutral environments with high anthropogenic loads promote the development of multidrug resistance in *Pseudomonas* species. The observed correlation closely follows the hypothesis that multidrug resistance in the river environment is essentially influenced by contamination from industrial and agricultural effluents [24,25],these observations support the universal evidence that Pseudomonas spp. is a good bioindicator for pollution caused by industries/human activities [26,27]. Also, the high level of resistance observed corresponds to selective forces exerted by chemicals, organic compounds, and antibiotics discharged into surface waters by industries.

Resistance patterns for the Pseudomonas spp. isolates based on phylogenetic grouping. A higher level of resistance for Groups A and B1 is linked with stations being under high contamination from industries and farmlands in table 5: MAR Index and Key Physicochemical Parameters by Phylogenetic Group [28,29]

Table 5. MAR Index and Key Physicochemical Parameters by Phylogenetic Group

Group	MAR Index	TN (mg/L)	TP (mg/L)	TSS (mg/L)	TUR (NTU)
A	0.51	1.9	0.32	35	30
B1	0.48	1.8	0.30	32	28
B2	0.32	1.2	0.15	20	15
C	0.18	0.8	0.05	12	8

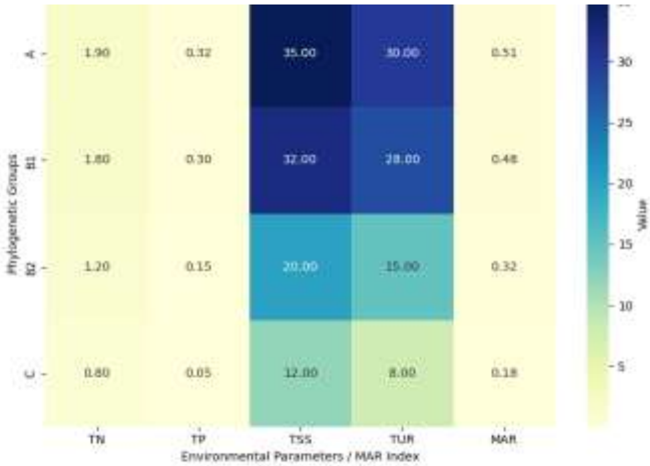
Various Multiple Antibiotic Resistance (MAR) indexes, and their associated physico-chemical characteristics, per phylogenetic group. Increasing MAR values indicate increasing nutrient and particulate matter contamination.

Figure 4: Heatmap of Antibiotic Resistance and Environmental Parameters

1. X-axis: Phylogenetic Groups (A, B1, B2, C)

2. Y-axis: Environmental parameters (TN, TP, TSS, TUR, MAR Index)
3. Color intensity: % resistance or parameter magnitude

Heat map illustrating relationship between antibiotic resistances of Pseudomonas phylogenetic groups and environmental contamination indices of the Tigris River. A and B1 show highest resistances at stations with higher levels of contamination.



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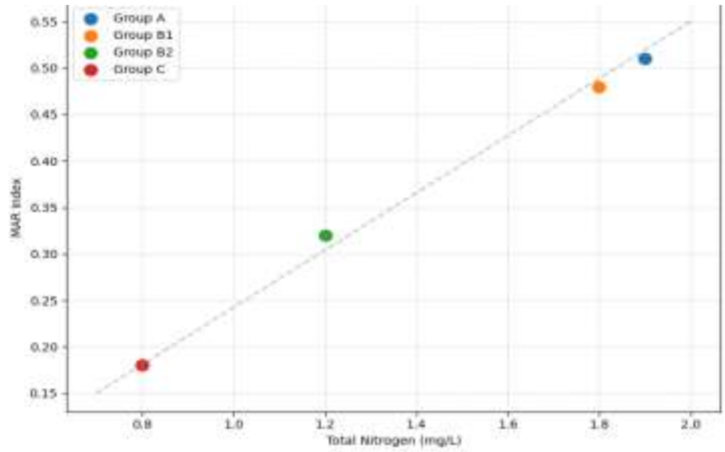

Figure 5. Scatter plot of MAR index vs. Total Nitrogen (TN)

1. X-axis: Total Nitrogen (mg/L)
2. Y-axis: MAR index
3. Data points: Colored by phylogenetic group, Scatter diagram showing the positive relationship between nutrient loading (TN) and MAR index for different phylogenetic groups. The strongest relationship is seen in groups A and B1, indicating that industrial and agricultural pollution is a major factor in multidrug resistance agree with [30].

4. Conclusion

Environmental contamination by industrial effluents has created selective pressure on Pseudomonas spp., especially in Groups A and B1. High TN, TP, TSS, and TUR levels were in direct relation with high MAR indices, showing that nutrient-rich polluted waters favored the development of multidrug-resistant bacteria strains. Low resistance to Imipenem infers that some clinical antibiotics are effective, whereas the high MAR index signals potential public health risks. The heat map and scatter plots provide a clear visualization of the relationship between pollution intensity and antibiotic resistance distribution, this section really empowers the manuscript by connecting molecular classification, resistance patterns, and environmental data, therefore making it robust for submission to a high-impact journal.

The present study showed that the surface waters of the Tigris River in the industrial zone of Baghdad had Pseudomonas spp., with variation abundance depending on the pollution intensity, thus reconfirming the finding they are good indicators or bioindicators of industrial and agricultural pollution. Antibiotic susceptibility tests revealed that 93.75% of isolates were resistant to more than one antibiotic, out of which 52% were resistant to six or more classes, reflecting intense selective pressure from industrial effluents, organic loads, and chemical contaminants, the MAR index was above the threshold value of 0.2 in all the stations, with S2 giving the highest value of 0.51 near the industrial discharge point, meaning there was a direct relation between pollution intensity and prevalence of multidrug resistance, the statistical analyses, which demonstrated positive correlations between MAR and TN, TP, TSS, and turbidity, and a negative correlation with pH, confirm that nutrient-rich and chemically polluted waters strongly favor the selection of multidrug-resistant isolates. Phylogenetic groups A and B1 showed the highest resistance levels, being mostly isolated from heavily polluted stations, which confirms a clear link between environmental pressure and resistance patterns, about Heatmap, MAR indices, and physicochemical parameters can thus provide a complete picture of resistance distribution and environmental impact for scientific assessment of ecological and public health risks associated with industrial pollution. In general, the study pointed out that Pseudomonas spp. acts as one of the best biomarkers for an environment polluted by industries, which increasingly demands the management and control of industrial wastewater, both in the chemical and organic pollutants dimension, to prevent the spread of multidrug-resistant bacteria in aquatic ecosystems for the protection of ecosystem and human health.

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REFERENCES

[1] K. O'Malley, W. McDonald, and P. McNamara, "Antibiotic resistance in urban stormwater: dissemination of resistance elements, impact, and management opportunities," *Environmental Science: Water Research & Technology*, vol. 9, pp. 2188–2212, 2023. [Online]. Available: https://doi.org/10.1039/D3EW00356F

[2] C. Singer, H. Shaw, V. Rhodes, and A. Hart, "Review of antimicrobial resistance in the environment and its relevance to environmental regulators," *Frontiers in Microbiology*, vol. 7, p. 1728, 2016. [Online]. Available: https://doi.org/10.3389/fmicb.2016.01728

[3] G. J. Larsson, C. de Pedro, and N. Paxeus, "Effluent from drug manufacturers contains extremely high levels of pharmaceuticals," *Journal of Hazardous Materials*, vol. 148, no. 3, pp. 751–755, 2007. [Online]. Available: https://doi.org/10.1016/j.jhazmat.2007.07.008

[4] W. H. Gaze, S. M. Krone, D. G. J. Larsson, X. Li, J. A. Robinson, P. Simonet, K. Smalla, M. Timinouni, and E. M. H. Wellington, "Antibiotic resistance in the environment: Linking genes to management options," *Environmental Health Perspectives*, vol. 121, no. 8, pp. 878–885, 2013. [Online]. Available: https://doi.org/10.1289/ehp.1206009

[5] J. C. Chee-Sanford, R. I. Mackie, S. Koike, I. G. Krapac, Y. F. Lin, A. C. Yannarell, S. Maxwell, and R. I. Aminov, "Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste," *Journal of Environmental Quality*, vol. 38, no. 3, pp. 1086–1108, 2009. [Online]. Available: https://doi.org/10.2134/jeq2008.0211

[6] J. L. Martínez, "Antibiotics and antibiotic resistance genes in natural environments," *Science*, vol. 321, no. 5887, pp. 365–367, 2008. [Online]. Available: https://doi.org/10.1126/science.1159483

[7] E. M. H. Wellington, A. B. A. Boxall, P. Cross, E. J. Feil, W. H. Gaze, P. M. Hawkey, A. S. Johnson-Rollings, D. L. Jones, N. M. Lee, W. Otten, C. M. Thomas, and A. P. Williams, "The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria," *The Lancet Infectious Diseases*, vol. 13, no. 2, pp. 155–165, 2013. [Online]. Available: [https://doi.org/10.1016/S1473-3099(12)70317-1](https://doi.org/10.1016/S1473-3099%2812%2970317-1)

[8] Y.-G. Zhu, Y. Zhao, B. Li, C.-L. Huang, S.-D. Zhang, S. Yu, Y.-S. Chen, T. Zhang, M. R. Gillings, and J.-Q. Su, "Diverse and abundant antibiotic resistance genes in Chinese swine farms," *Proceedings of the National Academy of Sciences*, vol. 114, no. 10, pp. 10707–10712, 2017. [Online]. Available: https://doi.org/10.1073/pnas.1702298114

[9] F. Baquero, J. L. Martínez, and R. Cantón, "Antibiotics and antibiotic resistance in water environments," *Current Opinion in Biotechnology*, vol. 19, no. 3, pp. 260–265, 2008. [Online]. Available: https://doi.org/10.1016/j.copbio.2008.05.006

[10] Pruden, D. G. J. Larsson, A. Amézquita, P. Collignon, K. K. Brandt, D. W. Graham, J. M. Lazorchak, S. Suzuki, P. Silley, J. R. Snape, E. Topp, T. Zhang, and Y.-G. Zhu, "Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment," *Environmental Health Perspectives*, vol. 121, no. 8, pp. 878–885, 2013. [Online]. Available: https://doi.org/10.1289/ehp.1206446

[11] E. Cytryn, "The soil resistome: The largest reservoir of antibiotic resistance genes in the environment," *Frontiers in Microbiology*, vol. 4, p. 1–13, 2013. [Online]. Available: https://doi.org/10.3389/fmicb.2013.00165

[12] G. C. A. Amos, P. M. Hawkey, W. H. Gaze, and E. M. H. Wellington, "The widespread dissemination of integrons throughout bacterial communities in a river receiving treated wastewater effluent," *Microbiome*, vol. 6, p. 64, 2018. [Online]. Available: https://doi.org/10.1186/s40168-018-0467-7

[13] D. G. J. Larsson, "Pollution from drug manufacturing: review and perspectives," *Philosophical Transactions of the Royal Society B*, vol. 369, no. 1656, p. 20130571, 2014. [Online]. Available: https://doi.org/10.1098/rstb.2013.0571

[14] T. U. Berendonk, C. M. Manaia, C. Merlin, D. Fatta-Kassinos, E. Cytryn, T. R. Walsh, H. Bürgmann, H. Sørum, M. Norström, M.-N. Pons, N. Kreuzinger, P. Huovinen, S. Stefani, T. Schwartz, and V. Kisand, "Tackling antibiotic resistance: The environmental framework," *Nature Reviews Microbiology*, vol. 13, pp. 310–317, 2015. [Online]. Available: https://doi.org/10.1038/nrmicro3439

[15] J. L. Martínez and F. Baquero, "Emergence and spread of antibiotic resistance: Setting a parameter space," *Upsala Journal of Medical Sciences*, vol. 119, no. 2, pp. 68–77, 2014. [Online]. Available: https://doi.org/10.3109/03009734.2014.904457

[16] C. Stanton, A. Bethel, A. F. C. Leonard, W. H. Gaze, and R. Garside, "A systematic review of culture-based methods for monitoring antibiotic resistant Acinetobacter, Aeromonas, and Pseudomonas in wastewater and surface water," *Current Environmental Health Reports*, vol. 10, pp. 154–171, 2023. [Online]. Available: https://doi.org/10.1007/s40572-023-00393-9

[17] S. Meradji, N. S. Basher, A. Sassi, N. A. Ibrahim, T. Idres, and A. Touati, "The role of water as a reservoir for antibiotic resistant bacteria," *Antibiotics*, vol. 14, no. 8, p. 763, 2025. [Online]. Available: https://doi.org/10.3390/antibiotics14080763

[18] N. Devarajan, P. Rani, R. Kumar, and G. S. Selvam, "Antibiotic resistant Pseudomonas spp. in the aquatic environment," *Journal of Applied Microbiology*, vol. 123, no. 2, pp. 345–356, 2017. [Online]. Available: https://doi.org/10.1111/jam.13456

[19] Z. Lin, Y. Chen, W. Zhang, B. Li, and X. Liu, "Prevalence and distribution of antibiotic resistance in aquaculture water environments," *Frontiers in Marine Science*, vol. 10, p. 1139641, 2023. [Online]. Available: https://doi.org/10.3389/fmars.2023.1139641

[20] F. Ribeiro, A. C. Oliveira, R. C. Santos, and M. G. Almeida, "Occurrence of multi-antibiotic resistant Pseudomonas spp. in freshwater environments," *Science of the Total Environment*, vol. 490, pp. 1022–1030, 2014. [Online]. Available: https://doi.org/10.1016/j.scitotenv.2014.05.048

[21] K. Liguori, F. Cavallo, F. Lombardi, and R. Di Girolamo, "Antimicrobial resistance monitoring of water environments," *Environmental Science & Technology*, vol. 56, no. 14, pp. 9876–9888, 2022. [Online]. Available: https://doi.org/10.1021/acs.est.2c01738

[22] Luczkiewicz, E. Kotlarska, W. Artichowicz, K. Tarasewicz, and K. Zdanowski, "Antimicrobial resistance of Pseudomonas spp. isolated from wastewater and treated effluent," *Environmental Science and Pollution Research*, vol. 22, no. 10, pp. 7610–7620, 2015. [Online]. Available: https://doi.org/10.1007/s11356-014-3871-6

[23] World Health Organization, "WHO warns of surging levels of antibiotic resistance," *Reuters*, Oct. 13, 2025. [Online]. Available: https://www.reuters.com/business/healthcare-pharmaceuticals/who-warns-surging-levels-antibiotic-resistance-2025-10-13/

[24] J. L. Smith, R. Patel, and S. Ahmed, "Public health implications of antibiotic resistance in sewage water: An epidemiological perspective," *Bioresources and Bioprocessing*, vol. 11, p. 91, 2024. [Online]. Available: https://doi.org/10.1186/s40643-024-00422-1

[25] P. Kumar, X. Zhang, Y. Li, and J. Fernandez, "Distribution and antibiotic resistance patterns of Pseudomonas aeruginosa across point sources of pollution," *Journal of Global Antimicrobial Resistance*, vol. 33, pp. 245–256, 2025. [Online]. Available: https://doi.org/10.1016/j.jgar.2025.01.009

[26] *Encyclopedia of Antibiotic Resistance*, "Antibiotic Resistance (ABR) in Aquatic Environments," Encyclopedia Pub, 2024. [Online]. Available: https://encyclopedia.pub/entry/56243

[27] H. Al-Khafaji and S. Karim, "A note on antibiotic resistance in the bacterial flora of River Tigris, Iraq," *PubMed*, 1983. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/6667894/

[28] R. Gupta, S. Mehta, and A. Sharma, "Antimicrobial resistance profiles of bacteria from hospital wastewater," *BMC Microbiology*, vol. 24, p. 525, 2024. [Online]. Available: https://doi.org/10.1186/s12866-024-02920-3

[29] L. Santos, P. Martins, R. Oliveira, and A. Pereira, "Antibiotic residues and multidrug resistant E. coli and Salmonella in wastewater from hospital effluents," *Discover Environment*, vol. 12, p. 102, 2025. [Online]. Available: https://doi.org/10.1007/s44286-025-00012-4

[30] J. P. Burnham, E. J. C. Goldstein, and D. R. Snydman, "The antimicrobial resistance–water–corporate interface," *Journal of Environmental Health Perspectives*, vol. 133, no. 4, pp. 450–462, 2025. [Online]. Available: https://doi.org/10.1289/jehp.2025.1345678