



Original Article

## Water Contamination by Commonly Used Antimicrobials Around Tertiary Care Centres in South Kerala, India: An Environmental Risk and Antimicrobial Resistance Perspective

Dr. Jitha Sushama<sup>1</sup>, Dr. Harikumar Nair<sup>2</sup>, Dr. Annapurna Yadavalli<sup>3</sup>, Dr. Scaria Thomas Pulikkunnel<sup>4</sup>, Dr. Pradeep Sadasivan Pillai<sup>5</sup>

<sup>1</sup>Professor (CAP), Department of Pharmacology, Government Medical College, Thiruvananthapuram-695011, email- [jitha.sus@gmail.com](mailto:jitha.sus@gmail.com)

<sup>2</sup>Professor, Department of Radiodiagnosis, Sree Gokulam Medical College, Thiruvananthapuram, Kerala, India

<sup>3</sup>Professor, Department of Pharmacology, Government Medical College, Thiruvananthapuram, Kerala, India

<sup>4</sup>Professor (CAP), Department of Pharmacology, Government Medical College, Kottayam, Kerala, India

<sup>5</sup>Professor, Department of Pharmacology, Dr Somervell Memorial CSI Medical College, Thiruvananthapuram, Kerala, India

 OPEN ACCESS

### Corresponding Author:

#### Dr. Jitha Sushama

Professor (CAP), Department of  
Pharmacology, Government  
Medical College,  
Thiruvananthapuram-695011,  
email-[jitha.sus@gmail.com](mailto:jitha.sus@gmail.com)

Received: 22-12-2025

Accepted: 15-01-2026

Available online: 28-01-2026

Copyright © International Journal of  
Medical and Pharmaceutical Research

### ABSTRACT

**Background-** Water contamination caused by antimicrobials in water sources and its consequences such as environmental risk and antimicrobial resistance is less explored in Kerala, India.

**Objectives-** The study aims to find the concentration of antimicrobials in water sources and to analyze the environmental risk and antimicrobial resistance risk due to the prevailing drug concentration in water sources.

**Methods-** Five commonly used antimicrobials in a tertiary care centre were identified from purchase records- Azithromycin, Cefotaxime, Ciprofloxacin, Meropenem and Metronidazole Thirty-one samples of surface and ground water including drinking water sources were identified. Water from these sources were preconditioned and passed through Liquid Chromatography Mass Spectrometry instrument to quantify the concentrations of antimicrobials.

**Results-** Measured environmental concentration (MEC) were- Azithromycin (2.93 µg/L), Cefotaxime (3.10 µg/L), Ciprofloxacin (0.80µg/L), Meropenem (3.42 µg/L and Metronidazole (2.35 µg/L). Using Predicted no effect concentration-environment (PNEC-ENV), environmental risk ( $R_{ENV}$ ) calculated for Azithromycin (97.67), Cefotaxime (25.83), Ciprofloxacin (1.76), Meropenem (2.28) and Metronidazole (78.0) are above the value of 1, which shows that they are at a level to cause high environmental risk. Highest MECs of the five antibiotics against PNEC-MIC (minimum inhibitory concentration) shows that risk of antimicrobial selection pressure ( $R_{AMR}$ ) is above 1.

**Conclusion-** The concentration of antimicrobials in the water sources are in the range of posing environmental risk and for selection of antimicrobial resistant organisms in the environment. Modification of policy regulations, proper and safe disposal of drugs and water purification methods have to be done to prevent pharmaceuticals from entering the water sources and to remove those present in them.

**Keywords:** water contamination, environmental risk, antimicrobial resistance, pharmaceuticals, predicted no effect concentration, water purification

### INTRODUCTION

Environmental contamination by pharmaceuticals is a major concern these days. Improper disposal of pharmaceuticals by manufacturers, hospitals and households reach our water sources. Presence of these pharmaceuticals in water sources and their long-term ingestion can lead to many health hazards to living organisms. Among the pharmaceuticals, antimicrobials

in the environment can cause serious public health hazard due to the emergence of antimicrobial resistance (Burnham et al. 2025 ). Overuse and misuse of antibiotics has led to the development of antimicrobial resistance in the community. It is estimated that by 2050, 10 million deaths per year are expected worldwide due to antimicrobial resistant infections according to Centers for Disease Control and Prevention. Sustained presence of antimicrobials in subtherapeutic concentrations is the cause of emergence and spread of antimicrobial resistant genes in promiscuous organisms (Barathe et al,2024 ). Antimicrobials reach water sources through drug manufacturing unit outlets and hospitals. They also reach the water sources from households through sewage and reach waste water treatment plants. Water purification process are insufficient to extract and remove many pharmaceuticals including antimicrobials. Thus, they reach the natural water sources from where they reach humans and other living organisms. The presence of these low concentrations of antimicrobials in the environment as well as in the body of living organisms cause the emergence of antimicrobial resistant genes in microorganisms and thus antimicrobial resistant infections in humans.

Widespread use of antimicrobials in animal husbandry, aquaculture and agriculture is a serious concern apart from its use in humans. Policy regulators and administrators should take meticulous steps to mitigate this devastating problem by imposing regulations on the safe and judicious use of antimicrobials as well as proper disposal of used, unused and left-over drugs including antimicrobials. Denmark model of antimicrobial usage is a set example to follow with respect to judicious use of antimicrobials. They have imposed policies on judicious prescription of antimicrobials by veterinarians and right usage practices by cattle and poultry farmers. Unavailability of the antimicrobials without prescription and also health education and awareness generation among farmers regarding the importance of right usage practices of antimicrobials stand a long way in preventing emergence of antimicrobial resistant infections. Nowadays zoonotic diseases are causing serious concerns as it spreads to humans. Covid caused by SARS- Cov virus and monkeypox disease caused by human metapneumo virus are recent examples of zoonotic diseases transmitted to humans causing epidemics and even pandemics. Virus, bacteria, protozoans and fungi can cause serious systemic infections in humans due to drug resistant strains.

Though studies are done in some parts of the world to find the concentration of antimicrobials in the environment, an exploratory research in Kerala, India is lacking. The presence of antimicrobials in water sources and the quantification of them helps to gather information on the potential risk of emergence of antimicrobial resistant organisms in the environment, environmental risk as well as the health hazards that may arise by the consumption of these subtherapeutic concentrations of antimicrobials for a long time. Long term consumption of water with these low concentrations of antimicrobials can alter the gut flora in humans which can predispose to a series of health consequences indirectly such as obesity, diabetes, metabolic dysfunctions and endocrine abnormalities. Recently much focus is given on the concept of gut- brain axis where gut is considered the second brain, as any alteration in the gut flora which can happen due to sublethal concentrations of antimicrobials can alter the release of neurotransmitters in the Central Nervous System and thus affect the higher mental functions like cognition which includes memory, intelligence, motivation, attention and concentration (Riordan et al. 2025)

#### **Materials and Methods-**

**Chemicals and reagents-** Antimicrobials such as Azithromycin, Cefotaxime, Ciprofloxacin, Meropenem, Metronidazole and formic used as mobile phase were of HPLC grade. Standard and mobile phases were prepared using methanol. All the chemicals were used without further processing them.

**Sampling-** Five commonly used antimicrobials in a tertiary care centre were identified from the data obtained from purchase records for a period of one year. To find the contamination of water sources due to antimicrobial agents, a two-kilometer radius area was identified in Thiruvananthapuram district of South Kerala, India where many tertiary care hospitals are situated. Thirty-one water sources were identified from this area using Google Earth software. One liter water was collected from these water sources in HDPE bottles. pH, Temperature, Total dissolved solutes (TDS) and Electrical Conductivity (EC) of the water samples were checked at the site of collection itself using TDS meter and pH meter. Water samples were transported in ice bags to the centre for analysis. The samples were stored at -20 degree Celsius at the centre before it was taken for analysis.

**Sample preparation-** The samples were preconcentrated using solid phase extraction technique before quantifying the drugs. For preconcentration of samples the water samples were passed through Whatman filter paper 41 and filtered under vacuum to remove sediments. The filtered water samples were extracted using Oasis HLB 600/ 5ml cartridge at the rate of 2ml/ minute after pre- conditioning them with 5mL methanol followed by 5mL milli-Q water. The cartridge was then eluted with 5ml of 100% methanol for drug extraction. This 5ml sample was taken for analysis using Shimadzu Liquid Chromatography Mass Spectrometry – Mass Spectrometry (LCMS- MS) instrument.

**LCMS/MS analysis-** Drug standards of LCMS grade were procured. The column used for the analysis of antimicrobials was C18 Shim-pack GIST 3µm C18 Material 3 x 150mm. Better ionization of the drugs were possible with 0.1% formic acid in water as aqueous phase and 0.1% formic acid in methanol as organic phase. The preconcentrated samples were run in the LCMS-MS instrument for 20 minutes at 2mL/min.

Method validation- Quantification of the compounds seemed challenging due to the presence of contaminants. Therefore handling, storage and extraction were done with utmost care to minimize contamination. Calibration curves were plotted and the area of the curve was found to determine the concentration of the test drug. Calibration curve was plotted with five concentrations of a single drug and the concentration in the test samples were calculated. Limit of quantification (LOQ) and limit of detection (LOD) were calculated using the equation  $3.3 \sigma / S$  and  $10\sigma / S$  where  $\sigma$  is the standard deviation of the y- intercept, and S is the slope of the calibration curve. Values above the range of LOQ were used for analysis in the study.

Risk assessment-The environmental risk assessment of the drugs were calculated using the equation for Risk Quotient estimation provided by the European Commission's Technical Guideline Document using the equation  $RQ = \frac{MEC}{PNEC}$  and  $PNEC = EC_{50} \text{ or } LC_{50} \text{ or } NOEC / AF$ , where MEC is the measured environmental concentration, PNEC is the predicted no- effect concentration of the analyte and AF is the assessment factor.  $EC_{50}$  is the median effective concentration that produces a particular effect in 50% of the population,  $LC_{50}$  is the concentration that cause death in 50% of the population and NOEC is no observed effect concentration. Assessment factor (AF) is used as 1000 in situations to study chronic toxic effects. The AMR Industry Alliance has combined resources to set PNEC targets for antibiotics and to make the PNECs publicly available. It was used to find if the prevalent drug concentration in water sources is potential to cause environmental hazard. Similarly, PNEC-MIC, used specifically for substances like antibiotics to prevent the development of antimicrobial resistance (AMR), is calculated by taking the lowest Minimum Inhibitory Concentration (MIC) value of the antibiotic against relevant bacteria (often considering a distribution of sensitive and resistant strains) and then dividing this by a large, AF like 1000 to ensure the concentration to be low enough that it does not favour the selection of resistant strains in the environment. Technical Guidance Documents (TGD) of the European Union, which are foundational for many global risk assessments. define the use of the highest available exposure concentration of MEC to calculate the RQ. In ecological risk assessment (ERA) highest measured environmental concentration (MEC) is used for a worst-case scenario risk calculation. This is because the (Risk Quotient, RQ) calculation is designed to be a highly conservative, protective screening tool.

Data analysis- Data was entered in IBM SPSS Statistics version 29. Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equation  $3.3 \sigma / S$  and  $10\sigma / S$  where  $\sigma$  is the standard deviation of the y- intercept, and S is the slope of the calibration curve. Measured environmental concentration values above the range of LOQ were used for analysis in the study.

## Results

Limit of detection and limit of quantification of the drugs are depicted in Table 1.

Drugs	LOD ( $\mu\text{g/L}$ )	LOQ ( $\mu\text{g/L}$ )
Azithromycin	0.06	0.16
Cefotaxime	0.04	0.14
Meropenem	0.99	2.10
Metronidazole	0.00	0.01
Ciprofloxacin	0.02	0.12

Measured environmental concentration (MEC) of these antimicrobials in water sources were- Azithromycin (2.93  $\mu\text{g/L}$ ), Cefotaxime (3.10  $\mu\text{g/L}$ ), Ciprofloxacin (0.80  $\mu\text{g/L}$ ), Meropenem (3.42  $\mu\text{g/L}$  and Metronidazole (2.34  $\mu\text{g/L}$ ) (Figure 1).

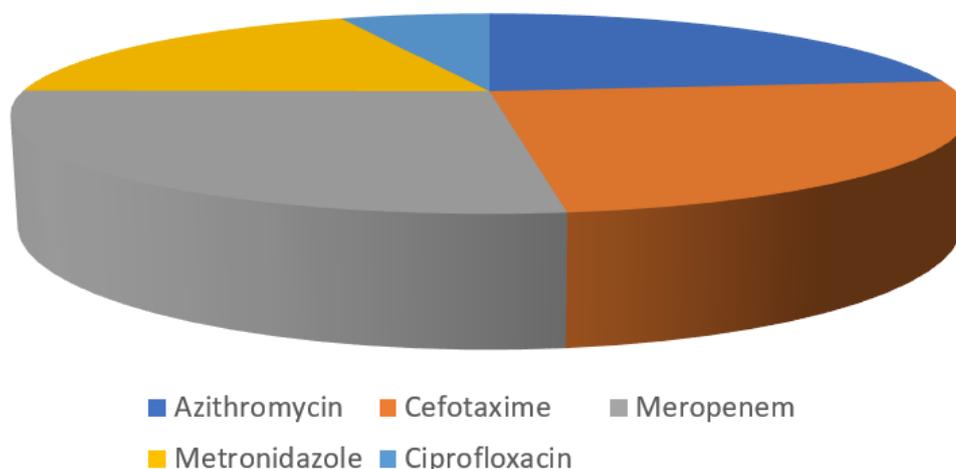


Figure 1- Measured Environmental Concentration of Antimicrobials

Using Predicted no effect concentration- Environment (PNEC-ENV), Environmental Risk ( $R_{ENV}$ ) calculated for Azithromycin (97.67), Cefotaxime (25.83), Ciprofloxacin (01.76), Meropenem (02.28) and Metronidazole (78.00) are above the value of 1, which shows that they are at a level to cause environmental risk (Figure 2).

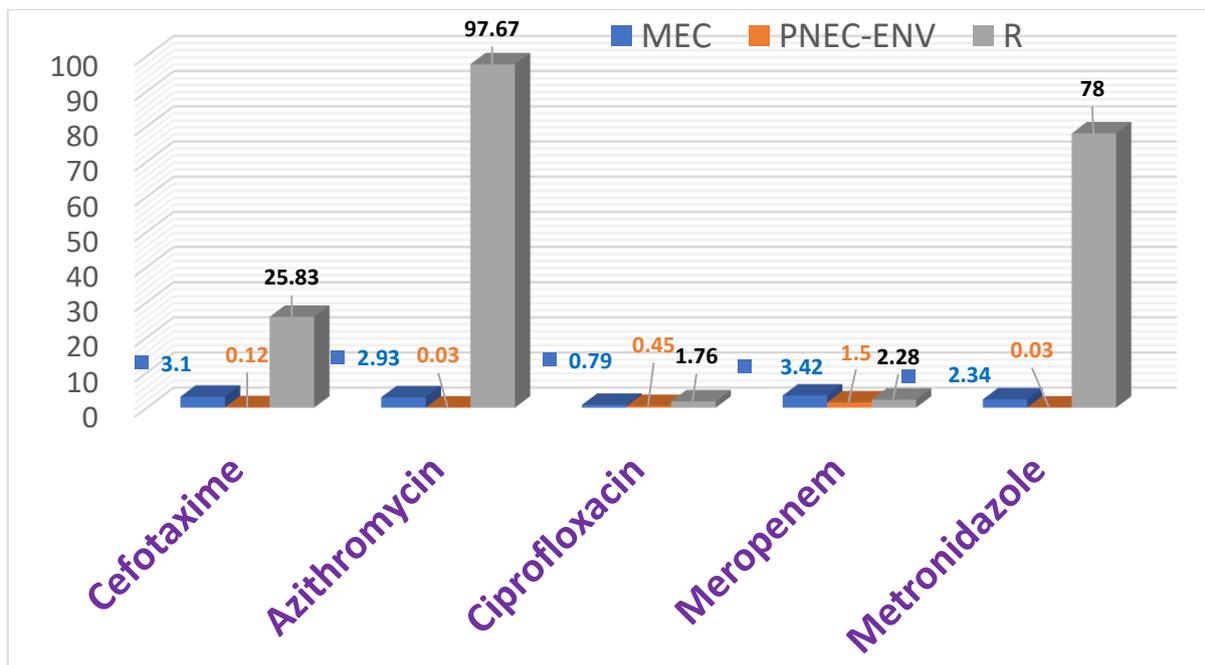


Figure 2- Environmental Risk ( $R_{ENV}$ ) caused by the antimicrobials

MECs of the five antimicrobials against PNEC-MIC (minimum inhibitory concentration)- Azithromycin (2.93, 0.25), Cefotaxime (3.10, 0.13), Ciprofloxacin (0.80,0.06), Meropenem (3.42, 0.06) and Metronidazole (2.35, 0.13) shows that MECs are above the PNEC- MIC values used for determining risk of developing antimicrobial resistance,  $R_{AMR}$  ( Figure 3).

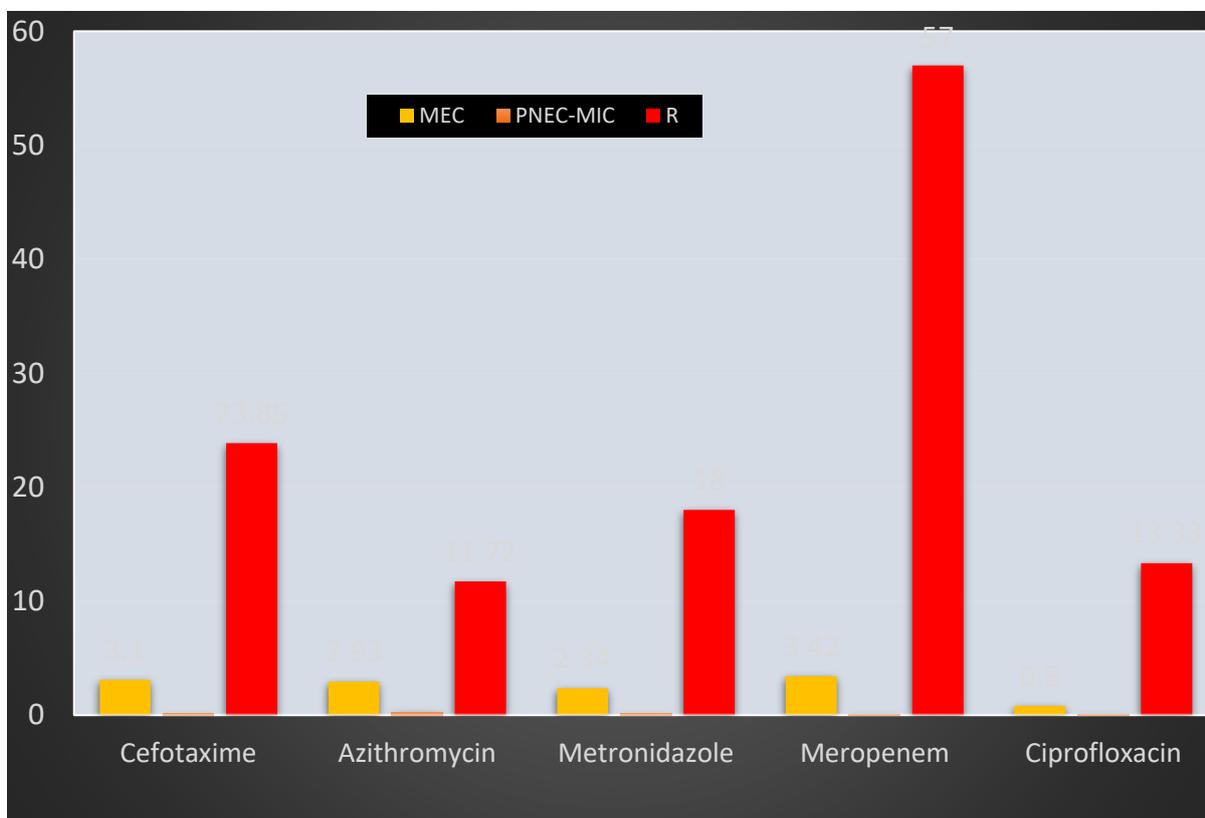


Figure 3- Risk of antimicrobial selection pressure ( $R_{AMR}$ ) caused by antimicrobials.

The measured environmental concentration of drugs, Environmental Risk  $R_{ENV}$  and Antimicrobial Resistance Risk  $R_{AMR}$  of the antimicrobials are summarized in Table 2.

Table 2- Environmental Risk  $R_{ENV}$  and Antimicrobial Resistance Risk  $R_{AMR}$  of the antimicrobials. Values given in brackets are the PNEC values used for assessing Risk Quotient.

Sl No:	Drugs	MEC $\mu\text{g/L}$	$R_{AMR}$ =MEC/PNEC <sub>MIC</sub>	$R_{ENV}$ = MEC/PNEC <sub>ENV</sub>
2	Azithromycin	2.93	11.72 (0.25)	97.67(0.03)
3	Cefotaxime	3.10	23.85 (0.13)	25.83 (0.12)
4	Ciprofloxacin	0.06	13.33 (0.06)	1.76 (0.45)
5	Meropenem	3.42	57 (0.06)	2.28 (1.5)
6	Metronidazole	2.35	18 (0.13)	78 (0.03)

## Discussion

The detection of the compounds confirms the entry of hospital-derived pharmaceuticals into the surrounding water matrix. These concentration levels are comparable to or higher than concentrations reported in global literature, such as macrolides (up to 3.85  $\mu\text{g/L}$ ) and quinolones (up to 0.66  $\mu\text{g/L}$ ) (Meidhli et al., 2022; Juarez et al., 2021). The ubiquitous detection of these compounds underscores the need to address pharmaceutical pollution as a critical component of the "One Health" approach.

It is standard practice in ecological risk assessment (ERA) to use the highest measured environmental concentration (MEC) for a worst-case scenario risk calculation. This is because the (Risk Quotient, RQ) calculation is designed to be a highly conservative, protective screening tool. Academic and technical publications frequently cite the use of the maximum exposure concentration in the RQ formula (Khetan et al 2007, Vestel et al. 2016, 2022). The environmental risk quotient ( $R_{ENV}$ ) assesses the potential for toxicity to aquatic organisms by comparing the MEC to the PNEC- ENV. The calculated  $R_{ENV}$  values for all five antimicrobials are significantly above 1, confirming that they are present at levels that pose an environmental risk to the aquatic ecosystem. This poses a potential for toxicity to different trophic levels of aquatic organisms, including green algae, crustaceans, and fish. (Quadra et al., 2023; Vestel et al., 2016, 2022). The  $R_{AMR}$  values for all the five antimicrobials are above 1, indicating that their measured concentrations are sufficient to exert a selective pressure in favour of resistance mechanisms.

The presence of antimicrobials at these sub-inhibitory concentrations suggests that the aquatic environment is an active 'AMR bioreactor' where the selection and evolution of resistance are dynamically occurring. This environment facilitates Horizontal Gene Transfer (HGT), promoting the exchange of Antibiotic Resistance Genes (ARGs) between environmental bacteria and potentially clinically relevant pathogens, representing a direct public health threat (Rysz and Alonso, 2018). This confirms that the contaminated water sources serve as a critical bridge, transferring resistance determinants from the environment back to the human and animal populations.

The findings of this study confirm the pervasive contamination of surface and groundwater sources around hospitals and tertiary care centres in South Kerala by commonly used antimicrobials and underscore the critical environmental and public health risks associated with their presence.

The consistent detection and quantification of five high-usage antimicrobials in the sampling area validates that inadequate disposal and conventional wastewater treatment (WWT) methods create environmental hotspots (Gonzalez Plaza et al., 2019; Mheidli et al., 2022). The Measured Median Environmental Concentrations (MECs) are substantial, with Metronidazole (2.35  $\mu\text{g/L}$ ) and Cefotaxime (3.10  $\mu\text{g/L}$ ) being particularly high. These values exceed and are comparable to high-end concentrations reported in similar studies globally (Juarez et al., 2021; Meidhli et al., 2022). The presence of these compounds in both surface and groundwater emphasizes the mobility and persistence of pharmaceutical pollution within this local hydro-geological system.

Environmental Ecotoxicity Risk ( $R_{ENV}$ ) analysis revealed that all five antimicrobials are present at concentrations that pose a significant ecotoxicological risk ( $R_{ENV} > 1$ ). The extremely high quotients for Azithromycin ( $R_{ENV}=97.67$ ), Metronidazole ( $R_{ENV}=78.00$ ), and Cefotaxime ( $R_{ENV}=25.83$ ) are alarming. These elevated values indicate a severe potential for chronic, sub-lethal effects on non-target aquatic organisms, moving beyond the acute toxicity focus of traditional Environmental Risk Assessment (ERA) (Backhaus et al., 2018). Antimicrobials at these low  $\mu\text{g/L}$  levels can perturb key ecological functions such as nutrient cycling and interfere with the cellular processes of primary producers (Brain et al., 2004).

The most compelling finding relates to the potential for AMR selection, quantified via the  $R_{AMR}$ . With  $R_{AMR} > 1$  for all the five studied compounds, the concentrations are definitively capable of exerting a selective pressure on the environmental microbiome (Gullberg et al., 2011). The high  $R_{AMR}$  ratios for Meropenem ( $R_{AMR}=57$ ) and Cefotaxime ( $R_{AMR}=23.85$ ) are particularly critical. Meropenem is a carbapenem and a last-resort clinical antibiotic. Its presence at levels conducive to resistance selection suggests the environmental spread of selective mechanisms against critically important antimicrobial classes.

The findings of this exploratory risk assessment in India emphasize the urgent need for robust environmental governance in public health and policy implications. The  $R_{AMR}$  results directly support the need to integrate environmental monitoring into One Health surveillance strategies. Beyond AMR, the long-term human ingestion of these trace antibiotic concentrations carries risks of altering the sensitive human gut microbiota, which is linked to metabolic dysfunction and alterations in the gut-brain axis (O’Riordan et al., 2025).

**Conclusion-** The findings confirm that pharmaceutical contamination in the study area's water sources presents both an immediate environmental hazard and a strong selection pressure for AMR, necessitating urgent policy intervention, including safe pharmaceutical disposal regulations and investment in advanced water purification technologies to effectively mitigate this public health and ecological threat. Water purification methods have to be developed to remove these drugs from water sources. Modification of policy regulations for proper and safe disposal of drugs have to be done and implemented to prevent pharmaceuticals from entering the water sources.

## REFERENCES

1. Alfonso-Muniozguren, P., P´erez-Elvira, R., Llorca, M., & Farr´e, M. (2021). Occurrence and removal of pharmaceutical compounds in wastewater treatment plants: A review. *Science of The Total Environment*, 783, 147137.
2. Backhaus, T., Karlsson, J., Wiberg, K., & Sumpter, J. P. (2018). Environmental risk assessment for pharmaceuticals: A paradigm shift needed. *Environmental Science & Technology*, 52 (14), 7545-7547.
3. Barathe, J. A., Singh, A. K., & Sharma, M. P. (2024). Sub-inhibitory concentrations of antibiotics accelerate the evolution of resistance: A review. *Environmental Pollution*, 342, 123048.
4. Brain, R A., Johnson, D.J., Richards, S. M., Hanson, M. L., Heberer T., & Williams, K. L(2004). Microcosm evaluation of the effects of an eight pharmaceutical mixture on aquatic communities. *Aquatic Toxicology*, 70(1), 23-40
5. Burnham, A., Thomas, R., & Gupta, M. (2025). Environmental pathways of antibiotic resistance: A global review. *Journal of Environmental Assessment and Monitoring*, 197 (1), 1–15.
6. Gonzalez Plaza, J. J., Heipieper, H. J., & Thomsen, T. M. (2019). High concentrations of pharmaceuticals in hospital effluents from India and their potential impact on antimicrobial resistance. *Environmental Science & Technology*, 53 (18), 10834–10842.
7. Gullberg, E., Cao, S., Berg, O. G., Ilbäck, C., Sandegren, L., Hughes, D., & Andersson, D. I. (2011). Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathogens*, 7 (7), e1002158.
8. Juarez, F., Guedes, I., & Santos, L. (2021). Occurrence and removal of antibiotics in Brazilian wastewater treatment plants: A review. *Science of The Total Environment*, 792, 148301.
9. Khetan, S. K., & Collins, T. J. (2007). The aquatic toxicity of pharmaceuticals and personal care products. *Science of The Total Environment*, 376 (1–3), 1–14.
10. Mheidli, B., Chebil, A., & Hadrich, B. (2022). High concentrations of antibiotics in effluents of wastewater treatment plants in Lebanon: A threat to the environment. *Environmental Monitoring and Assessment*, 194 (10), 654.
11. O’Riordan, T., Slevin, M., & Murphy, M. (2025). Antibiotics and the gut-brain axis: Implications for mental health and cognition. *Neuroscience & Biobehavioral Reviews*, 158, 105650.
12. Quadra, G. R., Dantas, A. A., & Aguiar, L. E. A. (2023). PNECMIC as a tool for environmental risk assessment of antibiotics. *Environmental Science & Technology*, 57 (38), 17094–17101.
13. Rysz, M., & Alonso, A. (2018). Environmental concentrations of antibiotics and their selection of resistance in the aquatic environment. *Water Research*, 137, 235–244.
14. Vestel, J., Lohmus, M., & Blüthgen, N. (2016). Predicted no-effect concentrations (PNEC) for pharmaceuticals in the aquatic environment. *Environmental Toxicology and Chemistry*, 35 (12), 3020–3029.
15. Vestel, J., Lohmus, M., & Blüthgen, N. (2022). Risk assessment of emerging contaminants: The role of the PNEC concept. *Science of The Total Environment*, 853, 158704.