Sponsored Thesis Project Competition on

"RE-IMAGINING URBAN RIVERS"

Season- 3

Project Title : Comparison of Anti-microbial Resistance in Three River Ecosystems and Assessment for Behaviour Change Interventions

Creator : Sara Afzal, BSc. (Hons.), Department of Geology, A. M. U.

Contents

List of tables and Figures:

1. Introduction

1.1 Antimicrobial resistance (AMR): A global health threat

Antimicrobial resistance (AMR) is a growing public health concern worldwide. It refers to the ability of microorganisms, such as bacteria, viruses, fungi, and parasites, to resist the effects of antimicrobial drugs that were once effective in treating infections. The emergence and spread of AMR have been attributed to several factors, including the overuse and misuse of antibiotics in human and animal health, poor infection prevention and control practices, and inadequate sanitation and hygiene.[1]

AMR poses a significant threat to global health, as it can lead to increased morbidity and mortality, longer hospital stays, and higher healthcare costs. In addition, AMR can limit the effectiveness of medical treatments, such as chemotherapy and surgery, and compromise the ability to control infectious diseases. According to the World Health Organization (WHO), AMR is one of the top ten global public health threats facing humanity today.[2]

The emergence and spread of AMR have been driven by the selective pressure exerted by the use of antimicrobial drugs or antibiotics. The misuse and overuse of antibiotics in human and animal health and also agriculture have contributed to the development of resistance in bacteria, which can then spread to other bacterial strains and species. This is particularly concerning in the context of multidrug-resistant (MDR) bacteria, which are resistant to multiple classes of antibiotics and are increasingly prevalent in healthcare settings.[3]

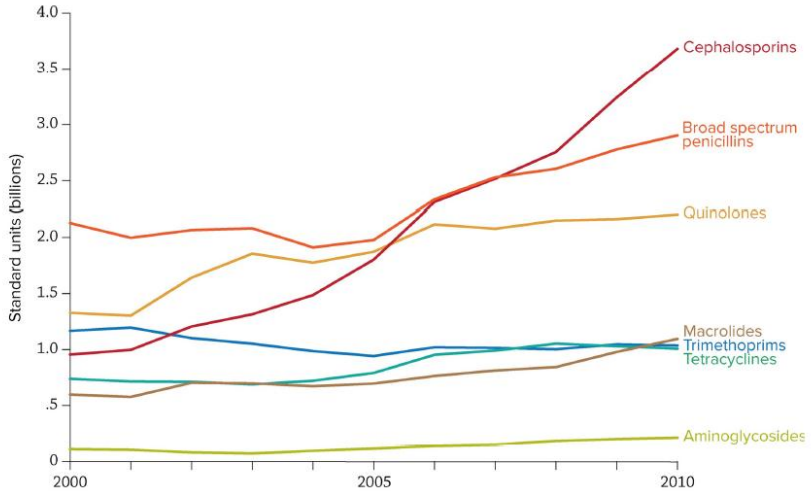


Figure 1: Trends in antibiotic consumption in India, 2000–2010.
(Source: Laxminarayan & Chaudhary, 2016)[4]

1.2 AMR in water bodies

The emergence and spread of AMR in water bodies has become a significant public health issue, with the potential to pose a threat to human health through the food chain or through direct contact with contaminated water. Water bodies, including rivers, lakes, and groundwater, have been identified as important reservoirs of AMR. The sources of AMR in water bodies are diverse, and include human and animal wastewater, agricultural runoff, and the disposal of antimicrobial drugs. Antibiotics are often used in human and veterinary medicine, and the widespread use of these drugs has contributed to the emergence of AMR in water bodies. Antibiotics and other antimicrobial agents are often not completely metabolized by the human or animal body, and as a result, they are excreted in feces and urine and can end up in wastewater. Antibiotics and antimicrobial agents may also be released into the environment through the disposal of unused or expired medications, which can enter water bodies through runoff or wastewater treatment plants.[5]

AMR in water bodies is widespread and has been found in both surface and groundwater. Studies have shown that AMR genes can persist in water for long periods of time, and can be transported over long distances by water currents. The distribution of AMR in water bodies is influenced by a variety of factors, including the presence of microbial communities, water flow, temperature, and pH.[6] Such an existence of AMR in water bodies can pose a risk to human health through the food chain or through direct contact with contaminated water. AMR genes can be transferred from bacteria in water to bacteria in food or to bacteria that cause disease in humans, making it more difficult to treat infections. Direct contact with contaminated water can also result in the transmission of AMR genes to humans, either through ingestion or through contact with contaminated skin.[7]

1.3 Pathways of AMR in rivers

Several pathways contribute to the dissemination of antimicrobial-resistant bacteria and genes in river ecosystems.

Wastewater Discharge*:* One of the primary sources of AMR in rivers is the discharge of untreated or inadequately treated wastewater from human settlements, hospitals, and industries. Wastewater contains antibiotic residues and resistant bacteria, contributing to the contamination of river water.[8]

Agricultural Runoff: The use of antimicrobial agents in agriculture, particularly in livestock farming and aquaculture, can lead to the accumulation of antibiotic residues in soil. These residues can then be carried into rivers through agricultural runoff, adding to the pool of antimicrobial resistance.[9,10]

Urban Runoff: Urban areas can release antimicrobial agents into rivers through various channels, such as improper disposal of unused medications and personal care products, which can further contribute to AMR development.[11]

Animal Carcasses: Improper disposal of animal carcasses, whether from farm animals or wildlife, can introduce resistant bacteria into rivers, exacerbating the spread of antimicrobial resistance.[12]

Biofilms and Sediment: Biofilms and sediments in riverbeds act as reservoirs for antimicrobial-resistant bacteria and genes. These reservoirs can persist over time and serve as potential sources of ongoing dissemination.[13]

Horizontal Gene Transfer: River environments facilitate horizontal gene transfer, a process that allows resistant genes to spread between different bacterial species. This mechanism plays a significant role in the evolution and dissemination of AMR in river ecosystems.[14]

Aquatic Wildlife: Aquatic wildlife, such as fish and other organisms, can serve as carriers of antibiotic-resistant bacteria, contributing to the spread of resistance through their movement within river systems.[15]

1.4 Antibiotics in Indian River ecosystems

India has a diverse range of river ecosystems, which are important for providing water for drinking, agriculture, and industry. These ecosystems are, however, under significant pressure due to pollution from various sources, including antibiotics. Antibiotic pollution in Indian River ecosystems has been found to be widespread, with a variety of antibiotics being detected in river water and sediment. Antibiotics are mainly introduced into the environment through wastewater discharge from hospitals, pharmaceutical industries, and households. Antibiotics are not completely metabolized in the body, and can be excreted unchanged or as metabolites in urine and feces. This means that wastewater can contain a range of antibiotics, as well as antibiotic-resistant bacteria.[16]

Antibiotic pollution in India's river ecosystems has the potential to cause significant impacts on human health and the environment. Antibiotic-resistant bacteria can be transmitted to humans through contaminated water or through the consumption of contaminated food. The presence of antibiotics in the environment can also contribute to the development and spread of antibiotic resistance, making it more difficult to treat infections in humans and animals. In addition, antibiotic pollution can have negative impacts on aquatic ecosystems, including the development of antibiotic-resistant microorganisms and the disruption of ecosystem processes.[17,18]

Researchers have clearly identified the threat of AMR in Indian rivers specifically in Ganga. An investigation Sood et. al. focused on the presence, distribution, and antibiotic resistance profile of staphylococci in River Ganga within the Uttarakhand region of India. A total of 128 staphylococcal strains were collected from 32 sites, encompassing three species: S. aureus, S. hominis, and S. aegilis. S. aureus was the predominant species, constituting 68.75% of the total staphylococcal population. The staphylococcal isolates were tested for sensitivity to twelve antibiotics, revealing that a significant proportion were resistant to erythromycin. Notably, 17.6% of S. aureus strains showed resistance to methicillin. Of particular concern, 93% of methicillin-resistant Staphylococcus aureus (MRSA) were found in the lower regions of River Ganga in Uttarakhand, which face intense anthropogenic activities. Considering that the water of River Ganga is widely utilized for drinking, religious bathing, and cleaning purposes, the presence of pathogenic,

multidrug-resistant staphylococci raises significant health risks associated with the direct consumption of untreated Ganga water and bathing in this particular stretch.[19]

Similarly, observation of diverse bacterial groups, including alpha, beta, gamma proteobacteria, and bacilli, was made by Niveshika et. al. covering various *ghats* at river Ganga in Varanasi. Specific bacterial strains such as Pseudomonas, Serratia, Enterobacter, and Proteus vulgaris were predominantly found at Dashashwamedh Ghat and Assi Ghat, exhibiting minimum inhibitory concentrations of 200–300 mg/L for copper, nickel, lead, and chromium. Comamonas sp., isolated mainly from Samne Ghat and Rajendra Prasad Ghat, demonstrated the ability to thrive even at high lead concentrations (400 mg/L). Moreover, some strains displayed multidrug resistance against ten different antibiotics, which is of utmost concern as these drugs are commonly used to combat various bacterial infections.[20]

Conducting a case study to see the status of pollution from tannery effluence in Ganga river in the stretch of Kanpur city, Khwaja, Singh and Tandon (1998)[21] examined the impact of waste on the physicochemical characteristics of Ganga water and sediments. Two sampling locations in Kanpur were selected, one upstream of tanneries and the other downstream. The same physicochemical parameters analyzed in the waste were monitored at these sites during two seasons. The findings indicate that most parameters show an increase as the river flows from the upstream to downstream sites. The elevated levels of parameters such as BOD, COD, Cl^-, and total solids may be attributed to both domestic and tannery wastes. While phenols and sulfides could originate from other sources, their likelihood of coming from tanneries is higher. On the other hand, chromium is primarily identified as originating from tanneries. The speciation analysis of sediment for chromium indicates significant leakage of chromium at the downstream site. Chromium levels at the second site are almost ten times higher than at the first site, with surface chromium primarily found in the residual fraction at the first site and in the Fe–Mn oxide fractions at the second site.

Presence of antibiotic resistance genes (ARGs) and Faecal Indicator Bacteria (FIB) is another AMR associated threat for Indian rivers. Naresh Devarajan et. al. (2015)[22] studied Cauvery River Basin for this purpose. In this research, quantitative PCR was employed to quantify the total bacterial load, abundance of FIB (E. coli and Enterococcus spp. (ENT)), Pseudomonas spp., and ARGs (blaTEM, blaCTX-M, blaSHV, blaNDM, and aadA) in the sediments collected from hospital outlet pipes (HOP) and the Cauvery River Basin (CRB) in Tiruchirappalli, Tamil Nadu, India. The results revealed significantly higher abundance of bacterial marker genes, specifically E. coli, Enterococcus spp., and Pseudomonas spp., at HOP compared to CRB, with fold increases of 120, 104, and 89, respectively. Among the ARGs, aadA and blaTEM were consistently detected in higher concentrations than other ARGs across all sampling sites. Furthermore, blaSHV and blaNDM were identified in CRB sediments contaminated by hospital and urban wastewaters. The abundance of ARGs exhibited a strong correlation ($r > 0.36$, $p < 0.05$, $n = 45$) with the total bacterial load and E. coli in the sediments, suggesting a common origin and ongoing contamination source. These findings indicate that tropical aquatic ecosystems receiving wastewaters may serve

as reservoirs of ARGs, which could potentially be transferred to susceptible bacterial pathogens at these locations.

1.6 Pharmaceutical effluent: Major contributor

The pharmaceutical industry is a significant player in the environmental sector, experiencing global growth due to the escalating demand for antibiotics, especially in low- and middle-income nations. Unfortunately, pharmaceutical effluents contain high concentrations of antibiotics and antibiotic resistance genes, turning these sites into hotspots for environmental contamination and the spread of antimicrobial resistance (AMR). The improper treatment and disposal of this effluent result in an unprecedented level of antibiotic pollution in the environment, leading to the persistent presence of antibiotics that profoundly alter bacterial genomes' expression, thus promoting the increase and spread of AMR. Despite the critical implications, many countries' National Action Plans on AMR lack sufficient interventions to address this issue. Surprisingly, there are no global regulations in place to control the level of antibiotic residues in pharmaceutical effluents, despite the industry's rapid expansion. To move forward, a risk-based approach focused on the environment and the establishment of relevant indicators are essential for countries involved in antibiotic manufacturing. Currently, efforts to tackle this problem are scattered and fragmented. To effectively address the issue, policymakers, regulators, manufacturers, researchers, civil society, and communities must come together in collaboration. This joint effort is crucial for ensuring sustainable antibiotic production and maintaining the effectiveness of these drugs in treating bacterial infections[23],[24],[25],[26].

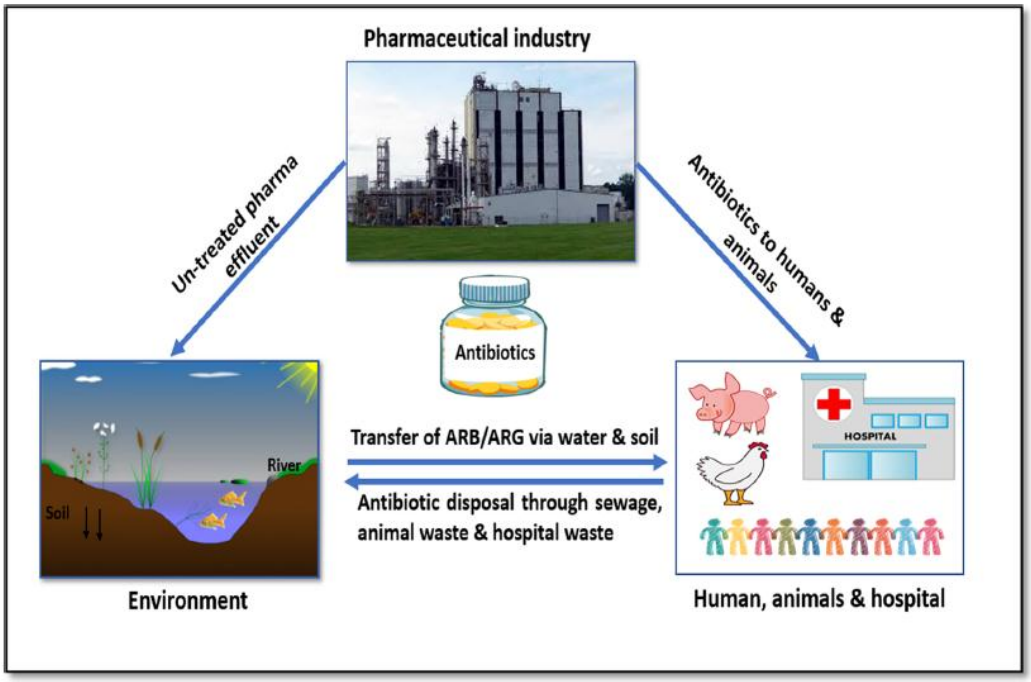


Figure 2: Interconnection of Pharma Industry, Water Bodies and Living Beings.
(Source: Kotwani, Joshi & Kaloni 2021)[27]

1.7 Extent of risk to public health due to river AMR

The extent of risk to public health due to AMR in rivers can be significant and multifaceted. AMR in river ecosystems poses various health risks to the general public, primarily through exposure to contaminated water sources and consumption of aquatic organisms carrying resistant bacteria. Several research studies have highlighted the potential consequences of river AMR on public health[28].

Waterborne Infections: Rivers contaminated with antimicrobial-resistant bacteria can serve as a reservoir for waterborne infections. Direct contact with or consumption of untreated water from AMR-contaminated rivers can lead to infections that are challenging to treat with standard antibiotics. This poses a considerable risk, especially in regions where the river water is used for drinking, bathing, and other domestic purposes[29].

Food Safety Concerns: Aquatic organisms in AMR-contaminated rivers can accumulate resistant bacteria and antibiotic residues, leading to food safety concerns. Consuming contaminated fish and other aquatic organisms can potentially transfer resistant bacteria to humans, further complicating the management of infections[30].

Hospital-Acquired Infections: AMR in rivers may contribute to the dissemination of resistant bacteria to nearby healthcare facilities. Patients in hospitals with weakened immune systems are at higher risk of acquiring infections caused by multidrug-resistant bacteria originating from the environment, including rivers[31].

Limited Treatment Options: As resistance to commonly used antibiotics increases in river environments, infections contracted from AMR-contaminated water sources may become more difficult to treat. Limited treatment options can result in longer hospital stays, higher healthcare costs, and increased morbidity and mortality rates[32].

Transmission of Resistance Genes: Rivers act as conduits for the horizontal transfer of resistance genes among different bacterial species. These genes can be transferred from environmental bacteria to potential human pathogens, increasing the potential for drug resistance to spread to clinically relevant strains[33].

It is essential to acknowledge that the extent of risk to public health varies depending on the level of AMR contamination in a particular river, the intensity of human exposure, and local healthcare capabilities. However, the overall global rise in AMR has raised concerns about the potential for public health crises if effective strategies for containment and control are not implemented.

1.8 Extent of risk to riverine ecology due to antibiotic contamination

The extent of risk to riverine ecology due to antibiotic contamination can be significant and far-reaching. The presence of antibiotics in river ecosystems can have various adverse effects on aquatic life, ecological balance, and overall ecosystem health. The natural microbial communities found in rivers play essential roles in ecosystem functions, including maintaining water quality through involvement in biogeochemical cycling and the degradation of organic contaminants. The

presence of antibiotics in rivers, originating from various sources, can impact the structure and functioning of these microbial communities in diverse ways. Antibiotics may have bactericidal or bacteriostatic effects, leading to the disappearance of certain microbial populations that are more susceptible, thereby affecting their ecological functioning. Additionally, the presence of antibiotics can promote the development of antibiotic resistance genes, making rivers significant pathways for the dissemination of resistance elements. Furthermore, specific microbial populations may develop genes related to antibiotic degradation, which is a crucial process, but it can be influenced by various biotic and abiotic factors[34].

Some of the factors that contribute to the extent of this risk have been specified below:

Biodiversity Loss: Antibiotics, even at low concentrations, can disrupt the natural microbial communities in rivers, leading to a reduction in biodiversity. Some bacteria may be more resistant to antibiotics, leading to a shift in the microbial composition, which can have cascading effects on other organisms in the food chain[35, 36].

Emergence of Resistant Bacteria: Antibiotics in river water can exert selective pressure on bacteria, favoring the survival of resistant strains. This can lead to the proliferation of antibiotic-resistant bacteria in the environment, contributing to the global problem of antimicrobial resistance[37].

Ecological Imbalance: The disruption of microbial communities can lead to ecological imbalances in river ecosystems. For example, certain bacteria play critical roles in nutrient cycling and decomposition of organic matter, and their disturbance can affect ecosystem functioning[38].

Toxicity to Aquatic Organisms: Some antibiotics can be toxic to aquatic organisms, particularly non-target species like fish, algae, and invertebrates. Exposure to antibiotics can impair their growth, reproduction, and survival, affecting the ecological integrity of the river[39].

Developmental and Reproductive Impacts: The presence of antibiotics in river water can have adverse effects on the developmental and reproductive processes of aquatic organisms, leading to population declines and reduced species diversity[40].

Bioaccumulation and Biomagnification: Antibiotics can bioaccumulate in aquatic organisms, meaning they can accumulate in the tissues of organisms over time. Additionally, through biomagnification, the concentration of antibiotics can increase as they move up the food chain, potentially affecting predators at higher trophic levels[41].

Resistance Gene Transfer: Antibiotic residues in rivers can facilitate the transfer of resistance genes between different bacterial species, further contributing to the spread of AMR in the environment[42].

1.9 Risk of AMR with Rivers Ganga, Yamuna and East Kali.

Of three rivers, River Ganga, holds tremendous cultural, religious, and ecological significance in India. Studies cited above, however, indicate that this river is also facing serious challenges concerning antimicrobial resistance (AMR)[43,44,45]. River Yamuna is another major rivers in India,

is facing significant challenges related to antimicrobial resistance (AMR). Similar to River Ganga, the Yamuna is subject to pollution from various sources, leading to the presence and spread of AMR in its waters[46,47,48,49,50]. However, East Kali River is a small river and a tributary to Ganga. It emerges from Muzaffarnagar of Western Uttar Pradesh and after touching many small cities and villages, it merges with Ganga in Kannauj. East Kali River doesn't receive heavy industrial effluence and also almost negligible human/anthropogenic interaction. It however receives effluence from villages and some small cities or towns. Although there are not much evidence of AMR available about this river, it is essential to understand that the presence of AMR in water bodies is a widespread and global concern, affecting various rivers and aquatic ecosystems. Considering the general scenario of AMR in river ecosystems, the East Kali River may also face similar challenges

AMR in Rivers Ganga, Yamuna and East Kali River is a growing concern due to various factors amongst which *polluted waters* is of greatest concern which comes to the rivers from various sources such as untreated sewage, industrial effluents, and agricultural runoff. These sources introduce antibiotics and resistant bacteria into the river, contributing to the development and spread of AMR[51]. Another major threat to these rivers is *Discharge of Pharmaceuticals.* Disposal of unused or expired antibiotics and other industrial pharmaceutical by products into the river can lead to the presence of antibiotic residues in the water, promoting AMR in bacterial populations[52],[53]. *Urbanization and Population Density* along the banks of Rivers Ganga and Yamuna results in increased antimicrobial usage and improper waste disposal practices, further exacerbating the problem of AMR[54],[55],[56]. *Religious Practices and Anthropogenic Activities* along the rivers such as religious bathing, and untreated waste from visitors can introduce antimicrobial agents and resistant bacteria into the water[57,58]. Likewise, *Animal and Agricultural Activities* involve usage of antibiotics which can find their way into the river through agricultural runoff, further contributing to AMR[59],[60].

The above sources of pollution in the three rivers consequently causes AMR in these water bodies which have serious implications. The most significant and crucial one is *Public Health Risk* that happens due to direct contact with or consumption of contaminated water and exposes individuals to antimicrobial-resistant bacteria, leading to infections that are challenging to treat with conventional antibiotics[61]. Another serious implication of AMR in these rivers is *Environmental Impact* which disrupts the balance of aquatic life, biodiversity, and the overall health of the river. Moreover, *Economic and Healthcare Burden* is another concerning implication. Treating infections caused by antimicrobial-resistant bacteria can be more expensive and time-consuming which strains healthcare resources[62],[63]. Finally, *Impact on Aquaculture* is another serious consequence of AMR presence in these rivers. Aquaculture practices are heavily affected due to the spread of resistant bacteria especially in cases of farmed fish and shrimp[64],[65],[66].

1.10 Ameliorative measures: A brief review

Addressing AMR in important rivers viz. Ganga, Yamuna and East Kali requires concerted efforts from multiple stakeholders, including government agencies, healthcare professionals, agricultural

industries, and the public. Implementing strict regulations on antibiotic usage, promoting responsible waste disposal practices, and raising awareness about the risks of AMR are essential steps to mitigate its impact on this revered river and its ecosystem[67]. One of the most warranted measures is Implement strict regulations on antibiotic usage in healthcare, agriculture, and veterinary sectors to minimize the release of antibiotics into the environment. Encourage the responsible use of antibiotics and promote alternatives, such as probiotics and vaccines, to reduce the selective pressure for AMR development[68,69]. Establishment of proper disposal systems for unused or expired pharmaceuticals to prevent their entry into rivers is another most important step that might curb the spread of AMR[70]. Another crucial step is to educate the public, healthcare professionals, and industries about the risks of AMR and the importance of responsible antibiotic use and waste management[71]. Furthermore, encouragement of sustainable and eco-friendly agricultural practices that minimize the use of antibiotics and promote biosecurity measures to prevent infections in livestock would also be fruitful[72]. Finally, strengthening the policies and regulations related to AMR management in river ecosystems sounds important[73]. Implementing strict penalties for non-compliance and reward adherence to best practices is already underway by the Indian Government[74,75,76].

1.11 Behaviour Change Approaches

Behaviour change interventions are strategies and techniques aimed at promoting positive changes in individuals' actions, habits, and behaviors. These interventions are commonly used in various fields, including healthcare, psychology, education, and public policy, to address issues such as health promotion, disease prevention, environmental conservation, and more. The overarching goal is to encourage individuals to adopt healthier, safer, and more sustainable behaviors[77],[78],[79].

Behaviour change interventions are designed based on various theoretical frameworks and models that help understand and predict human behavior. These theories provide a systematic understanding of how and why individuals adopt certain behaviors and guide the development of effective intervention strategies. Some of the key theories of behavior change employed with these interventions are Social Cognitive Theory, Trans-theoretical Model, **Health Belief Model**, Theory of Planned Behaviour, Self Determination Theory, **Social Ecological Model**, Cognitive Behaviour Theory and Social Norms Theory etc. These theoretical frameworks provide valuable insights into the factors influencing behavior change and serve as a foundation for designing effective interventions across various domains. Policy makers, public interventionists and behavior change practitioners essentially consider these theories and tailor their strategies to suit the specific context and target audience to maximize the success of their interventions[80,81,82,83].

In the context of present research we look for the most effective interventions that can positively influence public behavior regarding antimicrobial resistance awareness and the responsible use of antibiotics. This involves identifying interventions that work best in improving the public's understanding of antimicrobial resistance and encouraging them to use antibiotics prudently[84,85,86].

2. Methodology:

2.1 Objectives of the present research:

1. To understand the potential sources and drivers of antimicrobial resistance in river ecosystems.
2. To investigate the presence of antimicrobial resistant bacteria in river water samples from various locations of Ganga, Yamuna and East Kali.
3. To quantify the presence and concentrations of various antibiotics in river water samples using high-performance liquid chromatography (HPLC) as an analytical technique.
4. To conduct a detailed geochemical analysis of river water to determine the elemental composition and distribution patterns of major elements.
5. To conduct a detailed geochemical analysis of river sediments to determine the elemental composition and distribution patterns of major elements.
6. To assess AMR enhancing behavior of individuals who live around the rivers Ganga, Yamuna and East Kali.
7. To conduct rapid assessment of existing evidence related to behavior change interventions aimed at controlling the overuse of antibiotics in various settings.
8. To propose evidence-based strategies for mitigating the dissemination and proliferation of antimicrobial resistance in river ecosystems.

2.2 Procedure:

The current study is expected to provide a comprehensive understanding of the sources and pathways of antimicrobial resistance in river ecosystems, and contribute to the development of effective prevention and control strategies for AMR. Therefore, the study follows a tri-fold research strategy which involves comprehensive analysis of river water at microbiological and biochemical levels, synthesis of the river water and sedimentation at geochemical level and assessment of AMR behavior followed by detailing the intervention strategies that promote behavior change in different settings and reduce the spread of antimicrobial resistance. Thus, the methodology has been divided into three independent parts respectively.

2.2.1 Site description and sample collection

Although this methodology has been divided into three parts and the subsequent sub-sections will describe the analytical procedures accordingly, the sites of considered rivers are similar for all three parts and the collected samples of water and sediments are common to the first two parts.
The site of concern for this study is associated to riverine biodiversity and called *doab* (two waters) wherein river Ganga and river Yamuna are situated at the flanks of this region. Through studying the status of river waters, the present research tries to access the status of antimicrobial resistance in the corresponding area and its ultimate convergence to the regional river ecosystems. Therefore, besides including rivers Ganga and Yamuna in its sampling plan, the study also includes East Kali River that cuts across the *doab* region and receives major amount of effluence.

2.2.2 Sampling locations

Figure 3: Kali River, Gangiri Road
Coordinates: 27.853531, 78.401993

Figure 4: Kali River, Kasganj
Coordinates: 27.786880, 78.627852

Figure 5: Poiya Villege, Yamuna River
Coordinates: 27.249897, 78.028859

Figure 6: Agra City, Yamuna River
Coordinates: 27.190297, 78.029016

Figure 7: Anoopshahar, Ganga River
Coordinates: 28.365073, 78.278786

Figure 8: Kachhla, Ganga River
Coordinates: 27.930590, 78.856718

2.2.3 River-wise sampling locations on map:

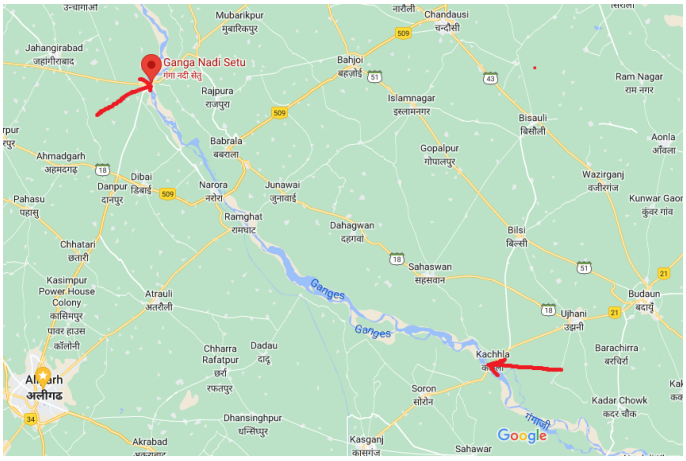


Figure 9: Ganga River Sampling sites

Figure 10: Yamuna River Sampling

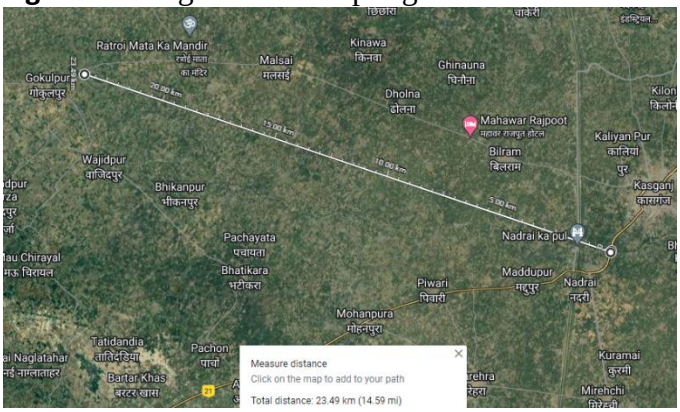


Figure 11: East Kali River Sampling Sites

Sampling sites were carefully chosen keeping in mind the areas where the rivers receive heavy effluence mainly laden with medical waste. For a comparative understanding of AMR due to human activity, a location where river flows through ravines with low human population was chosen. Sample of water from each location was extracted at three points of river breadth. Since all three rivers flew West to East at the sampling locations, the water sample were extracted from North Bank, Mid-Stream and South Bank on a straight imaginary line. River sediments were also extracted from similar locations but from banks only.

All samples were collected as per APHA, AWWA 2000 (Page No 6-2, 6010 B). Two types of samples were collected. Type 1was water only sample for microbiological and biochemical study for a direct understanding of AMR. Type 2 comprised of water as well as sedimentation; both were used to carry out geochemical study.

2.3 Level 1 Analysis: Microbiology and Biochemistry

The level-1 analysis was carried out at the Department of Microbiology, J. N. Medical College, A. M. U. under the supervision of supervisor-2 Dr. Fatima Khan, Associate Professor. Under this level a section of higher level analysis i.e. High-Performance Liquid Chromatography (HPLC)

were performed at the central laboratory of the Department of Botany, A.M.U. under the supervision of Professor Altaf Ahmad.

2.3.1 Samples for level 1 analysis:

Type 1 samples were collected in sterile vials which were kept in airtight iced plastic containers and were transported to the laboratory within 2–3 hrs of their collection. Collected samples were preserved at 4 °C until further analysis. Total 18 vials of samples were procured.

Sample Preparation: Aliquot of each water sample was filtered using a 0.22 µm pore size filter and transferred to a sterile petri dish containing nutrient agar and incubated for 24 hours at 37°C. After incubation, colonies were picked and sub-cultured on fresh nutrient agar plates. Pure cultures of bacteria were obtained by sub-culturing the isolates onto tryptic soy agar (TSA) and incubating them for 24 hours at 37°C.

Antibiotic Susceptibility Testing: The antibiotic susceptibility of each bacterial isolates were determined using the disk diffusion method. The bacterial isolates were inoculated onto Mueller Hinton agar plates and disks containing various antibiotics were placed on the surface of the agar. The plates were incubated at 37°C for 24 hours and the zone of inhibition around each disk were measured. The results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Pathogen Identification: The bacterial isolates were identified using standard microbiological methods, including Gram staining, biochemical tests, and molecular methods. PCR and sequencing analysis were performed to identify any pathogens present in the water samples.

Antibiotic Resistance Analysis: Overnight incubated, pure culture of pathogen strains which were develop through sterile techniques were streaked on the agar plate and antibiotic discs were placed onto the agar plates using sterile forceps. The discs were appropriately spaced apart to ensure that there is no overlapping. The plates were further incubated at the appropriate temperature and time. After incubation, the agar plates were examined for zones of inhibition around the antibiotic discs, the area where the bacterial growth is inhibited. The diameter of the zone of inhibition was measured using a caliper and recorded for each antibiotic disc. The size of the zone of inhibition was compared to the zone size interpretive standards for the specific organism strain being tested. If the zone of inhibition was greater than or equal to the interpretive standard, the bacterial strain was considered susceptible to the antibiotic. If the zone of inhibition was smaller than the interpretive standard, the bacterial strain was considered resistant to the antibiotic.

High-Performance Liquid Chromatography (HPLC): was conducted to separate, identify and quantify different antibiotics in our samples. The HPLC system we used is HITACHI LaChrom C18 (5 µm) 4.6 mm I.D.×150 mm L, installed in the Department of Botany, Aligarh Muslim University. Analyses were performed at a flow rate of 1.0 ml min−1 at the ambient temperature. A gradient flow programming with binary pumps was used, containing solvent A (0.1 % aqueous TFA) and solvent B (ACN) as mobile phase during the analysis. The details of flow programming were according to Mutiyar and Mittal, 2014[87]. The samples were filtered through 0.2 µm syringe

filter. The injection volume was fixed to 10 µl. All the compounds were eluted within 20 min, thus a chromatographic run was programmed for 30 min. The Diode Array Detector (DAD) was used for detection, and the chromatograms were extracted at different wavelengths. Different antibiotics in the samples interacted differently with the stationary phase and the mobile phase, resulting in their separation based on chemical properties. The retention time and peak areas of each antibiotic were compared with the reference standards to confirm their identity.

2.4 Level 2 Analysis: Geochemistry

The level-2 analysis was carried out at the Department of Geology, A. M. U., the parent department of the researcher, under the supervision of supervisor-1 Prof. M. Shamim Khan. Multiple lab facilities at the department were utilized.

Samples for level 2 analysis:

Groundwater Foundation Guidelines were adhered to collect samples for level 2 analysis that comprised hydrogeological as well as geochemical analysis. At this level of sampling, two types of samples were collected. A) water form sample: these samples were taken from the same sites from where the samples of level 1 analysis collected. Different sampling bottles with appropriate labeling were used and coded. Each sample was collected in 0.75 litre brown coloured sampling bottles which were previously sanitized. Before the collection, bottles were rinsed with the water being collected. The samples were taken to the hydrogeology lab in a cooled box. B) Sediment sampling: Water touching sediments were collected from each sampling site but only of North Bank and South Bank. Sediment collection from the central river bed was not done. Sediments were scraped with a cleaned stainless steel trowel and stored in pre-labelled, pre-coded plastic ziplock bags. Total 12 bags of samples were procured.

Spectrophotometer analysis: Following are the steps involved to analyze the targeted substances with this instrument.

Preparation of Calibration Standards: A series of standard solutions is prepared with known concentrations of the substance being analyzed. These calibration standards will serve as references for determining the concentration of the substance in the water sample.

Zeroing the Spectrophotometer: We start by setting the spectrophotometer to zero absorbance or transmittance using a blank solution. The blank solution must contain all the reagents and solvents except for the substance being analyzed. It helps to eliminate any background interference.

Wavelength Selection: An appropriate wavelength for the analysis based on the substance's absorbance is chosen.

Measurement of Calibration Standards: Absorbance or transmittance of each calibration standard is measured using the spectrophotometer. Readings are recorded for each standard.

Sample Preparation: Water sample is prepared through filtration, dilution, or other pre-treatment steps.

Measurement of Water Sample: Prepared water samples are placed into cuvette its absorbance or transmittance is measured using the spectrophotometer at the selected wavelength.

Calculation of Concentration: Through interpolation, extrapolation or regression analysis the calibration curve generated from the known concentrations of the calibration standards is used to determine the concentration of the substance in the water sample.

2.5 Level 3 Analysis: Behavioural Assessment and Policy Projection

The level-3 analysis was carried out at the Centre for Evidence-based Policy, Practice and Interventions (CEPPI) under the supervision of supervisor-3 Dr. Daud Salim Faruquie, Director CEPPI. Multiple lab facilities at the department were utilized.

2.5.1 Behavioural Assessment:

Development of a psychometric scale: In order to assess the extent of behavior in the populations that contributes to uncontrolled consumption of antibiotics, psychometric testing is required to be done one a sample of people preferably living around the rivers under study. A thorough review of literature revealed that a psychometric scale that can efficiently gauge the behavior behind the urge to over-use antibiotics by the individuals does not exist but needed for this study.

The rationale for developing a psychometric scale is to measure and assess psychological constructs, traits, or attributes in a standardized and reliable manner. Psychometric scales are used in psychology and other social sciences to quantify various psychological phenomena, such as personality traits, attitudes, beliefs, emotions, cognitive abilities, and behaviors. These scales provide a *standardized approach* to measure psychological constructs, ensuring consistency and comparability across different individuals and contexts. This allows researchers to make meaningful and valid comparisons between groups or individuals. These scales are designed to be *reliable*, meaning they yield consistent results over time and across different situations. High reliability ensures that the scale accurately measures the intended construct and minimizes measurement errors. These scales aim to have *high validity*, meaning they accurately measure the construct they intend to assess. Validity ensures that the scale is capturing the targeted psychological attribute and not measuring unrelated factors. The psychometric scales assign *numerical values* to psychological constructs, allowing researchers to quantify intangible attributes. This enables statistical analysis and hypothesis testing, leading to more rigorous and evidence-based research. By using psychometric scales, researchers can obtain data from a representative sample and draw conclusions that apply to larger populations, making research findings more generalizable.

Therefore, developing a psychometric scale for this study sounded essential for better understanding of the behavior behind antibiotic overuse. Thus, it was decided that **AMR Behaviour Scale** will be developed by a team of behavioural researchers in CEPPI. The researcher of this study was part of that team.

2.5.2 Development of AMR Behaviour Scale:

Initially the *concept of behavior* related to over consumption of antibiotics was developed and precise, comprehensive definition of the phenomenon was developed. It led the team to create a pool of potential *items* that represent different aspects of the construct. These items were in the form of statements which elicited rating responses. A panel of experts involving two psychologists one Professor of Pharmacology and one Professor of Biotechnology *reviewed the items* to ensure clarity, relevance, and alignment with the construct being measured. Later, the most appropriate and relevant items were selected from the initial pool based on expert feedback. The items were assessed for any issues with wording or interpretation. Items that didn't pass the criteria were either modified or deleted. The finalized set of 30 items covered the various aspects of the construct. This set of 30 items was administered with around 300 subjects and a dataset was developed for *statistical operations* such as item analysis and *principal component analysis*, both to be run with SPSS software. *Item Analysis* helped to evaluate the psychometric properties of the items, including item difficulty, discrimination, and variability. This process led the scale to be reduced to 20 items. This reduced item set was subjected to *principal component analysis* with *varimax rotation*. The rotated items were grouped into 4 subsets or *factors* according to their latent similarities. These 20 items were organized into a coherent and logical sequence and Likert Scale of responses with five ratings (from Strongly Disagree to Strongly Agree) was assigned to each item. Subsequently, the measure of *internal consistency* was measured Cronbach's alpha test. The high value of Alpha signifies that our scale should yield consistent results. For testing its validity, *content validity* measure was used by ensuring that the items cover all relevant aspects of the construct. In the coming days of data collection with this measure, norms and standard scores will be set up. These will provide context for interpreting individuals' scores in relation to the broader population.

The AMR Behaviour Scale was translated into Hindi language and verified. The purpose of translating the scale was to administer it with the people living around the river areas from where the water and sediment samples were collected for microbiological and geochemical analysis.

2.5.3 Assessment of AMR Behaviour:

Opportunity sampling or convenience sampling method was used to deploy the AMR Behaviour Scale around the areas of water and sediment sampling of rivers. Respondents were selected based on their availability and accessibility, rather than using random or systematic methods to choose participants from the entire population of interest. Six locations nearby the water and sediment sample collection were chosen. These were, *Anoopshahar*, *Kachhla*, *Gangiri Road*, *Kasganj*, *Poiya Village* and *Agra City*. A large number of contacted participants either denied to respond to the scale or left it in the midway. The number of responses were not representative of the population but provided a fair idea of the state of affairs. The collected data was analyzed with the help of SPSS software.

2.6 Rapid Assessment of Evidence (RAE) for intervention identification:

The Rapid Assessment of Evidence (RAE) is a methodology used in public health and evidence-based practice to quickly assess and synthesize the existing evidence on a specific topic or research question. The purpose of RAE is to provide timely and reliable information to inform decision-making, policy development, or program implementation in situations where a comprehensive systematic review is not feasible due to time constraints.

The assessment of AMR Behaviour provided an idea of overall, generalized high level of phenomenon regardless of the population differentiation. This indicates that we need to identify behavior change interventions to mitigate the problem of antibiotic overuse. Furthermore, previous studies have indicated two major risk factors. One of those being **irresponsible prescription behaviour** and **pharmaceutical industry effluence**. Researchers have particularly emphasized the role of later and it has also been pointed out in the current study because of overall heavy antibiotic contamination regardless of the water sampling locations. Therefore, the current research work carried out three parallel searches for RAE that looked for Behaviour Change Interventions for reducing antimicrobials consumption amongst individuals, Behaviour Change Interventions for clinicians and physicians controlled prescriptions and mitigating interventions for better waste management by the pharmaceutical industries.

RAE typically involves a more streamlined and accelerated approach compared to a full scale traditional systematic review. It begins with *formulating the research question* that clearly defines the specific question or topic that needs to be addressed. Next a focused and rapid search of the literature to *identify studies* that address the research question is done. Researchers set a specific *criteria for including or excluding* studies from the assessment based on relevance and quality. Data is then extracted from the selected studies to address the research question. *Critical appraisal* then helps to assess the quality of the included studies. Findings are summarized to highlight the key results. Ultimately the evidence is interpreted to arrive on the actionable recommendations. These recommendations are then shared with relevant stakeholders, decision-makers, or policymakers.

RAE is particularly valuable in situations where there is an urgent need for evidence-based information, such as during public health emergencies, outbreak responses, or policy development under time constraints. That is chiefly the reason it has been employed in the current study where some actionable policy recommendations were earnestly warranted.

On the basis of the previously established knowledge, the RAE method will be employed to identify most effective interventions that help to reduce **AMR Behaviour** in the individuals, **imprudent prescriptions** by health professionals and **pharmaceutical effluent** by the industries.

3. Results and Discussion:

This section presents the key findings and interpretation of our scientific research focused on investigating the presence of antibiotics in three rivers and resultant anti-microbial resistance alongwith the geochemistry of the riverine biodiversity. We present the empirical data obtained from multi-site sample collection of river water and sediments, as well as the outcomes of rigorous data analysis and statistical modeling. Additionally, this study explores the behavior responsible for spread of AMR and also looks for the successful interventions that have helped to reduce such problem through behavioural pathways. The discussion of these results revolves around understanding the complex relationships between AMR factors and biodiversity patterns and evaluating the implications of these findings for prevention and management strategies. Through this comprehensive analysis, we aim to contribute to the growing body of knowledge surrounding the vulnerability of riverine ecologies to antibiotics and AMR and provide insights that will aid in designing effective measures for preserving the vital river resources and their contribution to our environment.

3.1 Results of Level 1 Analysis: Microbiology and Biochemistry

Here we present the results of pathogen identification, their susceptibility, antimicrobial resistance, and High-Performance Liquid Chromatography (HPLC) analysis, aimed at gaining valuable insights into the microbial composition and resistance patterns of agents found in the river water samples. Moreover, the HPLC analysis provides a comprehensive examination of antimicrobial compounds, shedding light on their presence and concentrations in the samples. The integration of these analytical approaches offers a comprehensive and in-depth exploration of the complex dynamics between pathogens and antimicrobials, with potential implications for public health and riverine biodiversity.

Table: 1

Microbiology of water sample from Gangiri Road location of East Kali River

Sl No.	Antibiotics	Pathogens detected and their response	
		PSEUDOMONAS	E. COLI
1	Amikacin	**Resistant**	Susceptible
2	Gentamicin	Susceptible	Susceptible
3	Minocyclin	Susceptible	Susceptible
4	Cotrimoxazole	Susceptible	Susceptible
5	Levofloxacin	Susceptible	Susceptible
6	Meropenem	Susceptible	Susceptible
7	Cefepime	Susceptible	Susceptible
8	Polymyxin b	Susceptible	Susceptible
9	Chloramphenicol	Susceptible	Susceptible
10	Piperacillin	Susceptible	Susceptible
11	Tazobactum	**Resistant**	Susceptible
12	Cefoperazone	Susceptible	-
13	Sulbactum	Susceptible	-

Table: 2
Microbiology of water sample from Kasganj location of East Kali River

Sl No.	Antibiotics	Pathogens detected and their response	
		PSEUDOMONAS	E. COLI
1	Amikacin	Susceptible	Susceptible
2	Gentamicin	Susceptible	Susceptible
3	Minocyclin	Susceptible	Susceptible
4	Cotrimoxazole	Susceptible	Susceptible
5	Levofloxacin	Susceptible	Susceptible
6	Meropenem	Susceptible	Susceptible
7	Cefepime	Susceptible	Susceptible
8	Polymyxin b	Susceptible	Susceptible
9	Chloramphenicol	Susceptible	Susceptible
10	Piperacillin	Susceptible	Susceptible
11	Tazobactum	Susceptible	Susceptible
12	Cefoperazone	Susceptible	Susceptible
13	Sulbactum	Susceptible	Susceptible

Table: 3
Microbiology of water sample from Anoopshahar location of River Ganga

Sl No.	Antibiotics	Pathogens detected and their response
		PSEUDOMONAS
1	Amikacin	Susceptible
2	Gentamicin	Susceptible
3	Minocyclin	Susceptible
4	Cotrimoxazole	Susceptible
5	Levofloxacin	Susceptible
6	Meropenem	Susceptible
7	Cefepime	Susceptible
8	Polymyxin b	Susceptible
9	Chloramphenicol	Susceptible
10	Piperacillin	Susceptible
11	Tazobactum	**Resistant**
12	Cefoperazone	Susceptible
13	Sulbactum	Susceptible

Table: 4
Microbiology of water sample from Kachhla location of River Ganga

Sl No.	Antibiotics	Pathogens detected and their response		
		PSEUDOMONAS	E. COLI	CITROBACTUM
1	Amikacin	Susceptible	Susceptible	Susceptible
2	Gentamicin	Susceptible	Susceptible	Susceptible
3	Minocyclin	Susceptible	Susceptible	Susceptible
4	Cotrimoxazole	Susceptible	Susceptible	Susceptible
5	Levofloxacin	Susceptible	Susceptible	Susceptible
6	Meropenem	Susceptible	Susceptible	Susceptible
7	Cefepime	Susceptible	Susceptible	Susceptible
8	Polymyxin b	Susceptible	Susceptible	Susceptible
9	Chloramphenicol	Susceptible	Susceptible	Susceptible
10	Piperacillin	Susceptible	Susceptible	Susceptible
11	Tazobactum	Susceptible	Susceptible	Susceptible
12	Cefoperazone	Susceptible	Susceptible	Susceptible
13	Sulbactum	Susceptible	Susceptible	Susceptible

Table: 5
Microbiology of water sample from Poiya Village location of Yamuna River

Sl No.	Antibiotics	Pathogens detected and their response	
		PSEUDOMONAS	E. COLI
1	Amikacin	**Resistant**	Susceptible
2	Gentamicin	Susceptible	Susceptible
3	Minocyclin	**Resistant**	**Resistant**
4	Cotrimoxazole	**Resistant**	**Resistant**
5	Levofloxacin	Susceptible	**Resistant**
6	Meropenem	Susceptible	Susceptible
7	Cefepime	**Resistant**	**Resistant**
8	Polymyxin b	Susceptible	Susceptible
9	Chloramphenicol	Susceptible	Susceptible
10	Piperacillin	Susceptible	Susceptible
11	Tazobactum	Susceptible	Susceptible
12	Cefoperazone	Susceptible	Susceptible
13	Sulbactum	Susceptible	Susceptible
14	CA3	**Resistant**	-
15	Ceftriaxone	-	**Resistant**
16	Cefexime	-	**Resistant**
17	Clavalunic Acid	-	**Resistant**
18	Amoxicillin	-	Susceptible

Table: 6
Microbiology of water sample from Agra City location of Yamuna River

Sl No.	Antibiotics	Pathogens detected and their response
		PSEUDOMONAS
1	Amikacin	Susceptible
2	Gentamicin	Susceptible
3	Minocyclin	Susceptible
4	Cotrimoxazole	**Resistant**
5	Levofloxacin	Susceptible
6	Meropenem	Susceptible
7	Cefepime	Susceptible
8	Polymyxin b	Susceptible
9	Chloramphenicol	Susceptible
10	Piperacillin	Susceptible
11	Tazobactum	Susceptible
12	Cefoperazone	Susceptible
13	Sulbactum	Susceptible
14	CA3	**Resistant**
15	Ceftriaxone	Susceptible
16	Cefexime	Susceptible
17	Clavalunic Acid	Susceptible
18	Amoxicillin	Susceptible

Culturing of these river samples indicated the growth of pathogens e.g. E Coli, Pseudomonas and Citrobactum and these pathogens are resistant to some of the mentioned antibiotics present in the sample. This is clear indication of the presence of anti-microbial resistance in our samples. The matter of concern is that the trend of contamination and AMR is abundant in the samples which we had assumed to be less polluted because of their geographical and civil locations. Amongst these samples, Yamuna river shows highest level of pollution and resultant AMR.

These samples were subjected to HPLC for better analysis of antibiotcs presence in the samples. The results follow here:

Table: 7
Profile of different antibiotics with HPLC analysis

River samples	**Antibiotics analyzed**				
	Ampicillin	Kanamycin	Cefexime	Levofloxacin	Amoxicillin
Gangiri Rd. (Kali River)	+	+	-	-	-
Kasganj (Kali River)	+	-	+	+	+
Anoopshahar (Ganga)	+	+	+	-	-
Kachhla (Ganga River)	+	+	+	+	+
Poiya Village (Yamuna)	+	+	+	-	-
Agra City (Yamuna)	+	+	+	-	+

Plots obtained from HPLC analysis

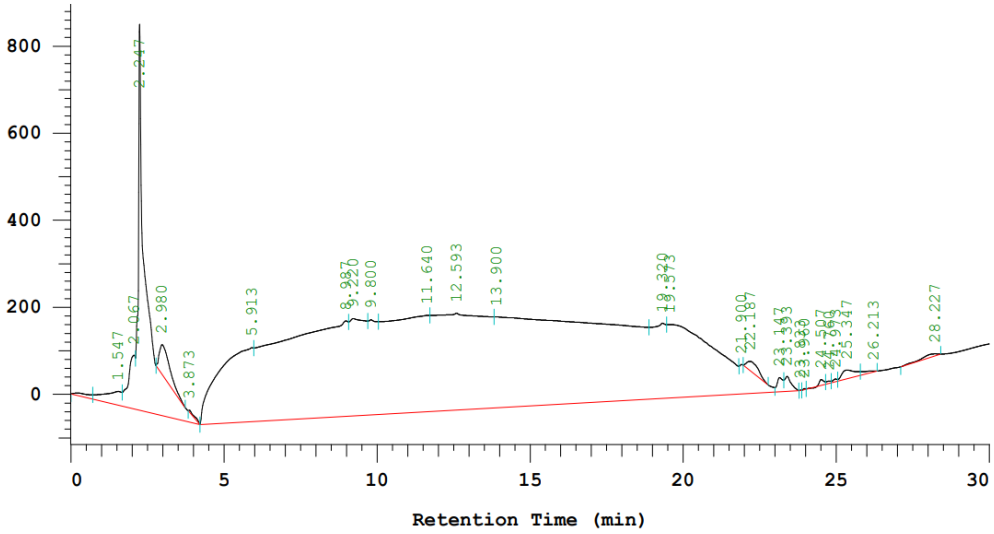


Figure 12: HPLC plot for standard of Ampicillin

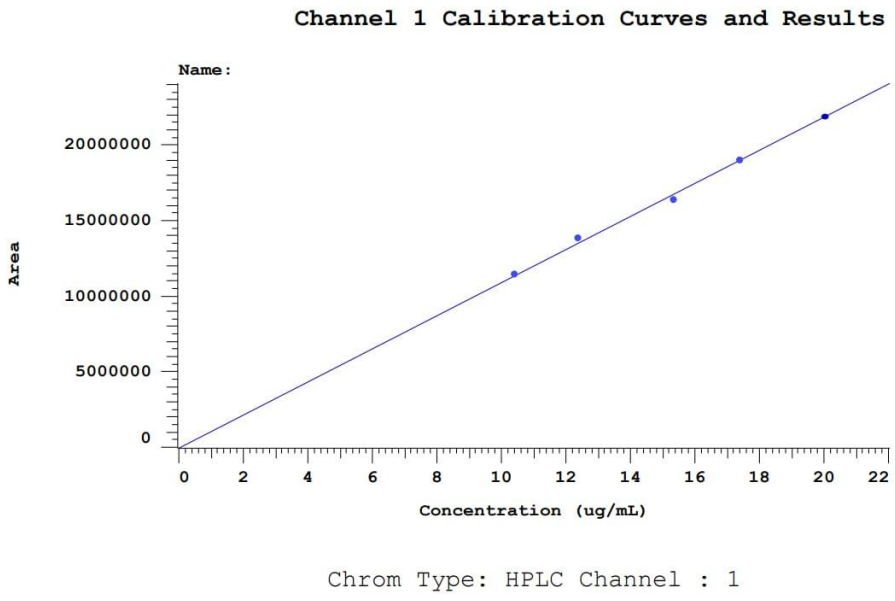


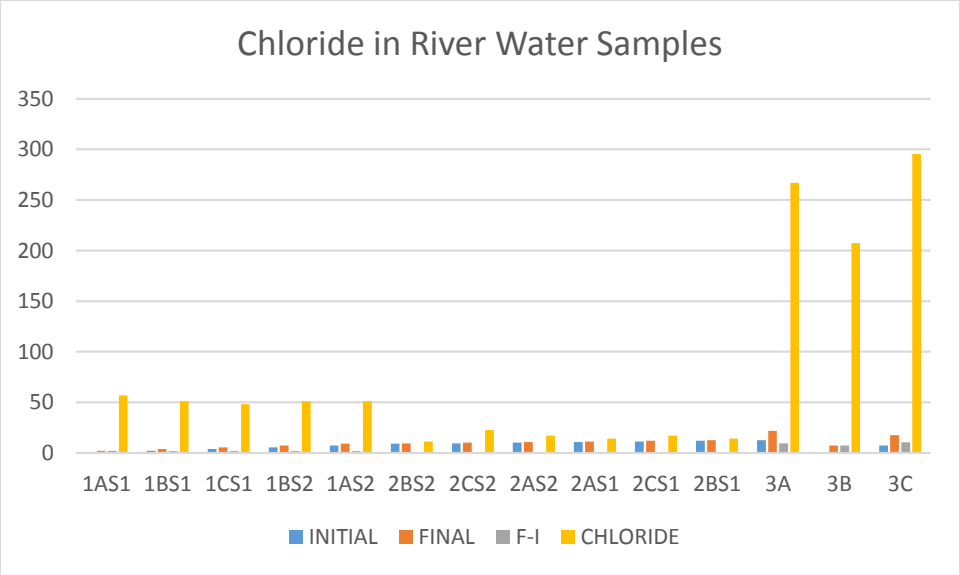
Figure 13: Calibration Curve of different concentrations of Ampicillin

3.2 Results of Level 2 Analysis: Geochemistry

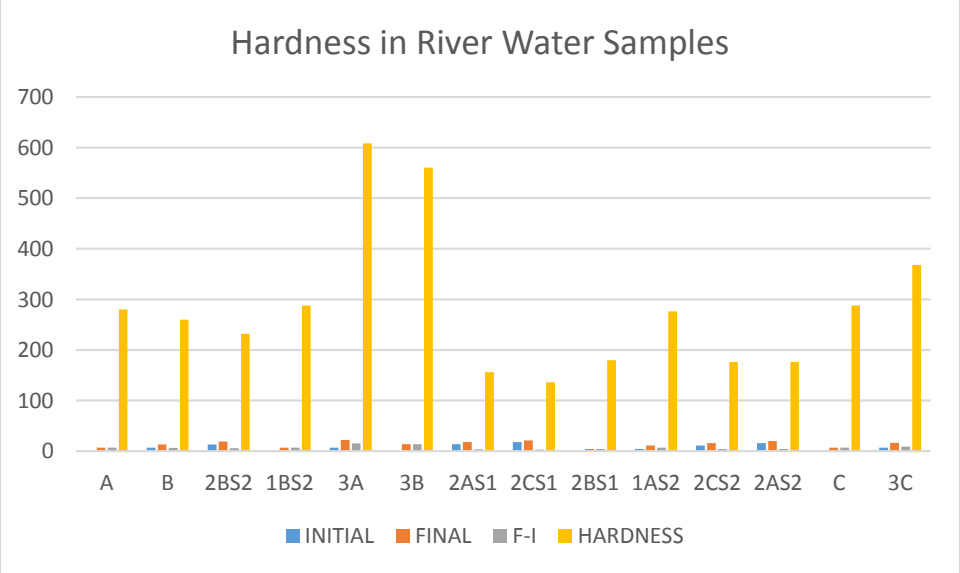
Geochemical analysis of river water and river sediments was carried out in eight sections for each. While the water was directly analyzed, sediments were first baked for two hours in the oven to achieve dryness and then dissolved in distilled water for 24 hours. After double filtration of this

sediment water, it was processed for analysis just like the sample water. The details of experimentation have been appended in **annexure-1.**

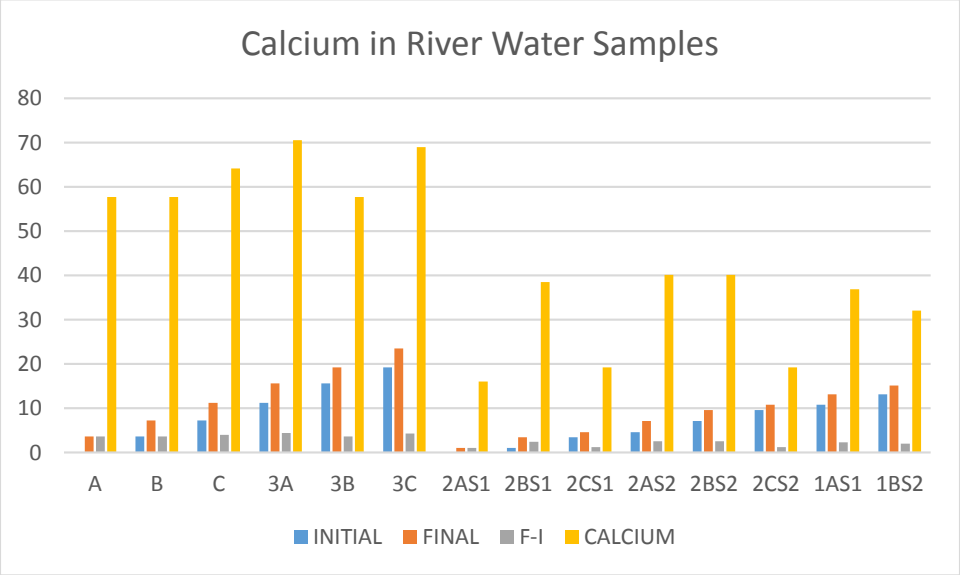
Results of analysis of Hardness, Chloride, Calcium and Bicarbonates that were performed with titration method have been given in the charts below:



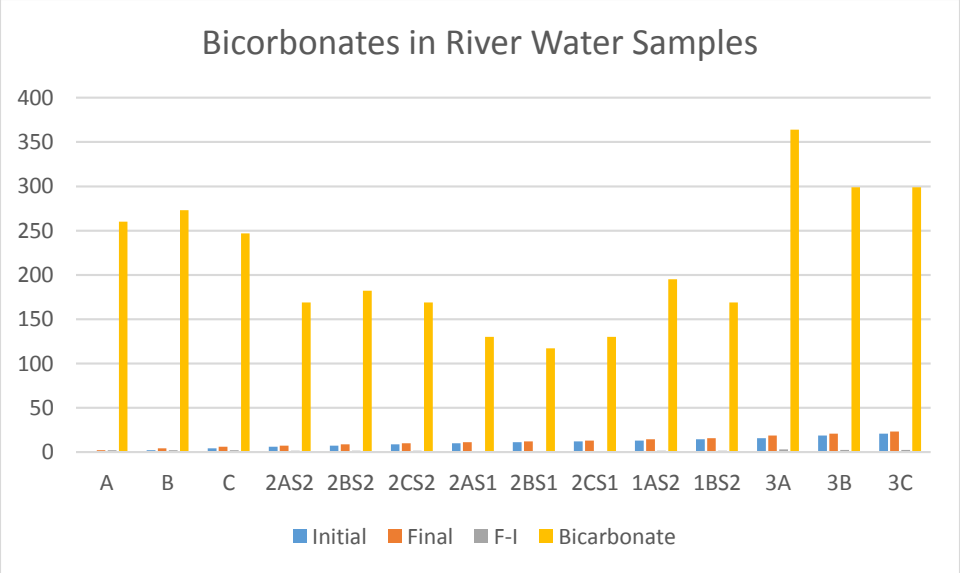
The graph of analysis given above indicates a very high contaminations of chloride in Yamuna river as compared to other two rivers.



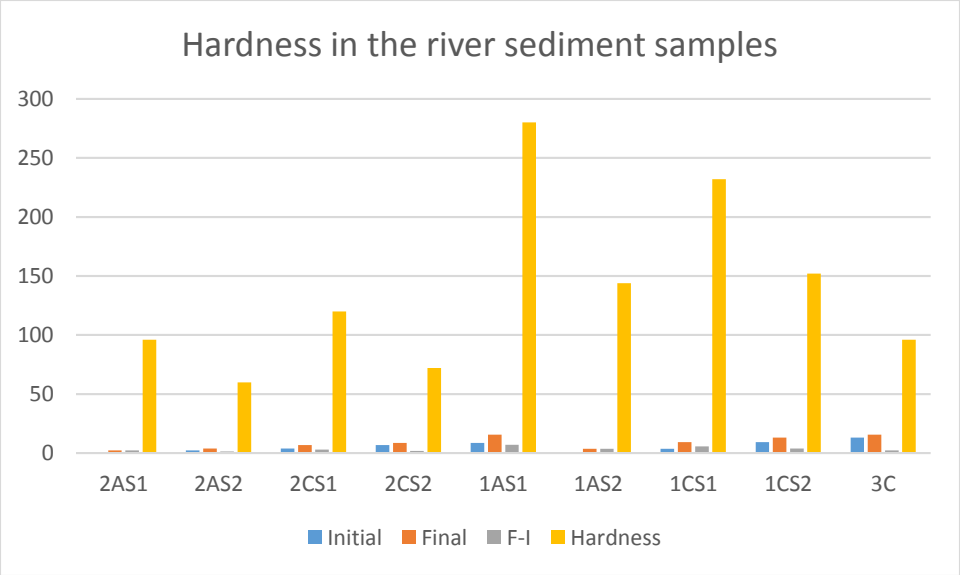
The above graph indicates high levels of hardness in Yamuna River, Kali river follows Yamuna on this measurement.



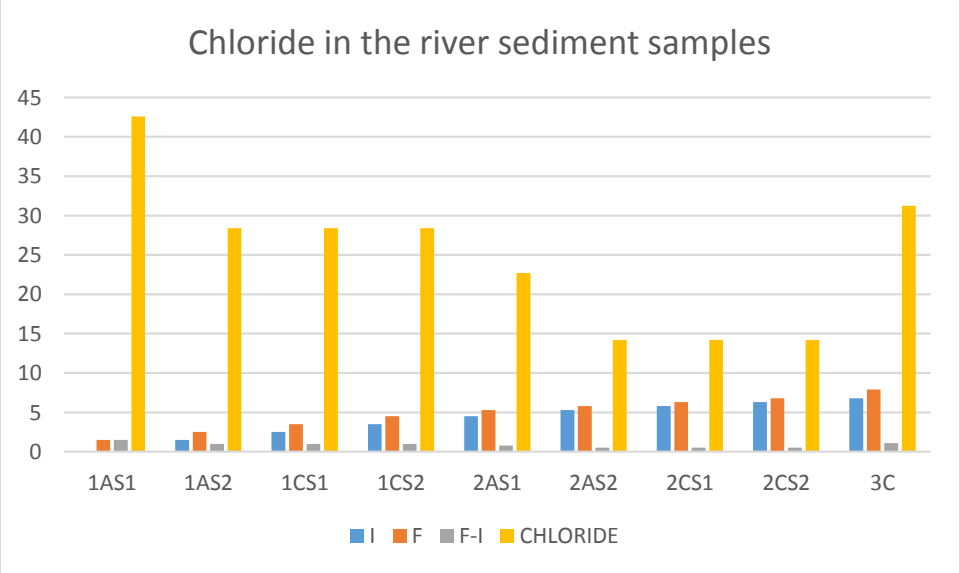
The above graph indicates an overall high levels of Calcium in Kali and Yamuna Rivers whereas Ganga River shows relatively less amount.



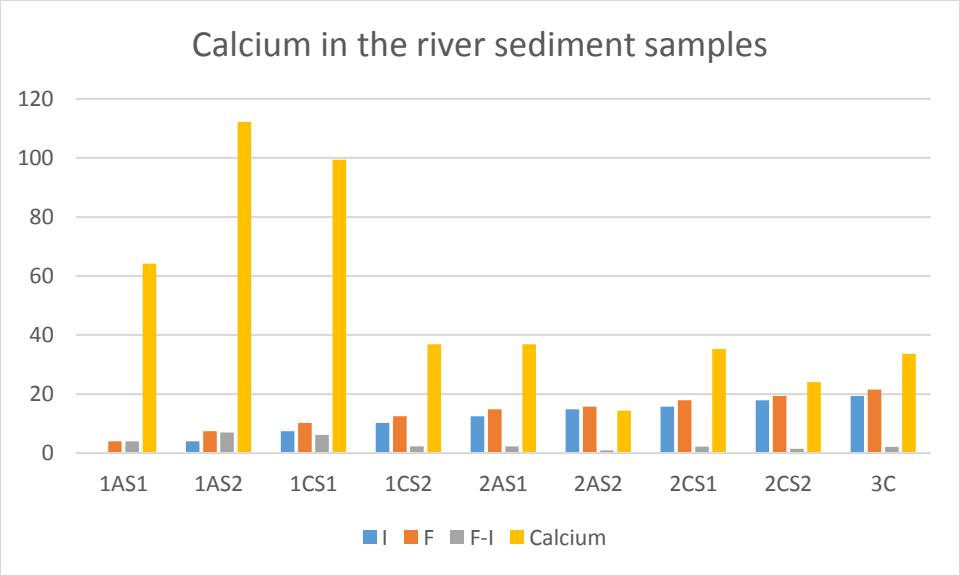
Above graph shows high levels of Bicorbonates in Yamuna and then Kali River whereas Ganga river shows a moderate level.



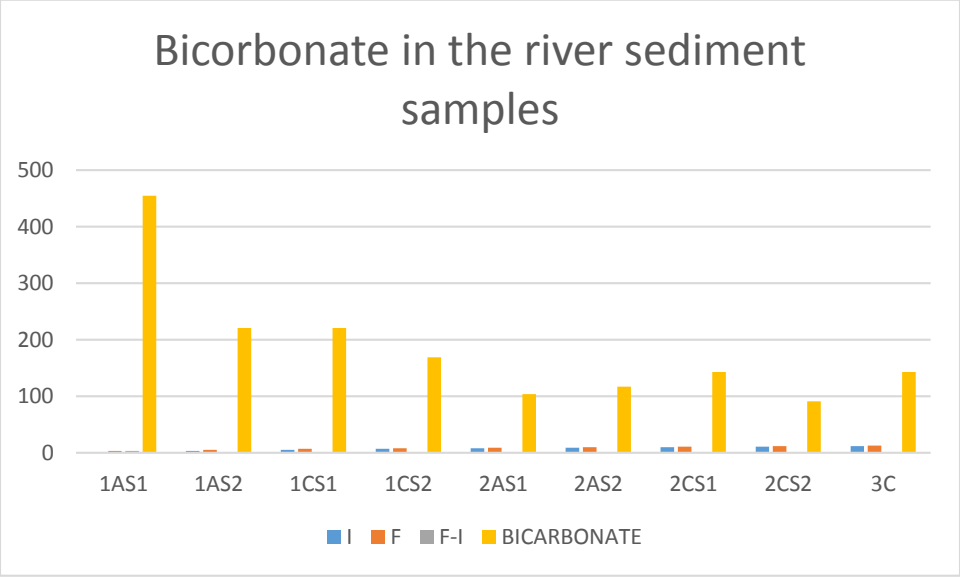
The above chart shows high level of hardness in the sediment samples of Yamuna and Kali rivers.



The above chart shows high level of Chloride in the sediment samples of Yamuna and Kali rivers whereas Ganga River shows moderate to low amounts.



The above chart shows high level of Calcium in the sediment samples of only Kali River whereas Ganga and Yamuna Rivers show moderate to low amounts.

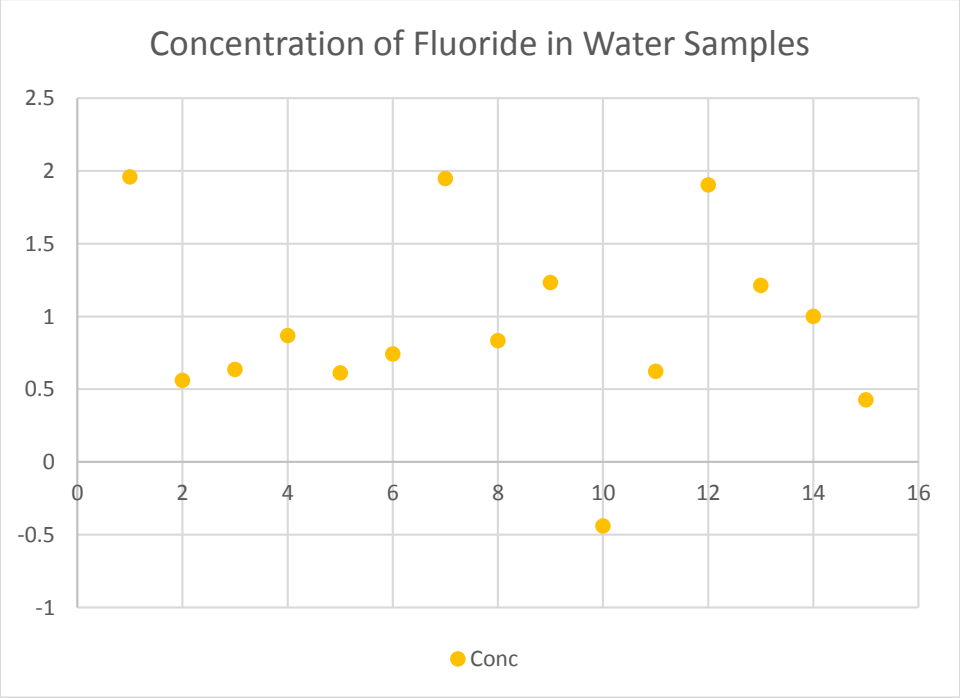


The above findings are indicative of high levels of bicarbonates only in Kali River.

Besides the above findings, Nitrate, Sulphate and Chloride in the river water as well in sediments were analyzed with the help of spectrophotometer. The results have been provided in the tables and charts here.

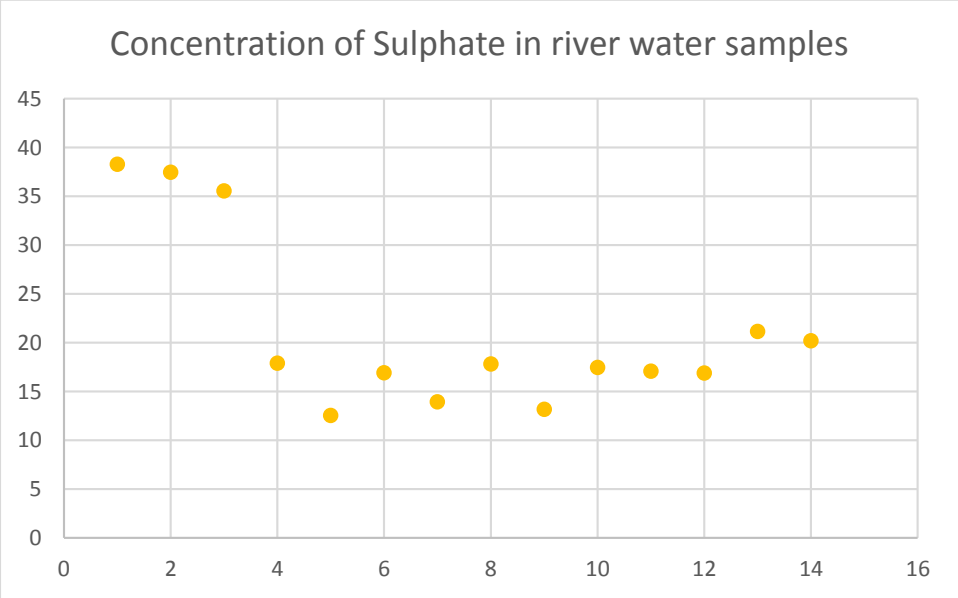
The first table is the output of spectrophotometer for concentration of Fluoride at the wavelength of 570.

Sample ID	Concentration	WL570.0
B	1.9594	0.8287
C	0.5617	0.9208
3A	0.6365	0.9159
3B	0.8692	0.9005
3C	0.6124	0.9174
2AS1	0.7426	0.9089
2BS1	1.9492	0.8293
2CS1	0.834	0.9028
2AS2	1.2338	0.8765
2BS2	-0.4391	0.9868
1AS2	0.6242	0.9167
1BS2	1.9039	0.8323
A	1.2142	0.8778
2CS2	1.0007	0.8919
2AS1s	0.4279	0.9296



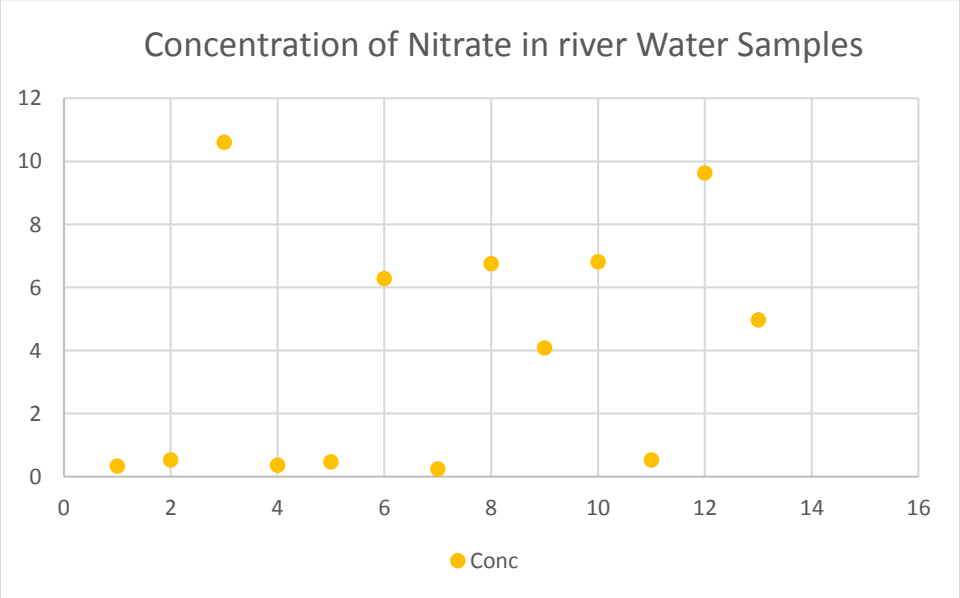
Concentration of Sulphate in River Water Samples has been analyzed at a wavelength of 420 and shown in the table and chart below:

Sample ID	Conc.	WL420.O
3A	38.2801	0.3565
3B	37.4537	0.3506
3C	35.5346	0.3369
2AS1	17.8818	0.2104
2AS2	12.5335	0.1721
2BS1	16.9169	0.2035
2BS2	13.9329	0.1821
2CS1	17.8009	0.2098
2CS2	13.1747	0.1767
A	17.4537	0.2073
B	17.0809	0.2047
C	16.8914	0.2033
IAS2	21.1534	0.2338
IBS2	20.1991	0.227



Concentration of Nitrate in River Water Samples has been analyzed at a wavelength of 410 and shown in the table and chart below:

Sample 1D	Conc	WL410.0
2AS1	0.3361	0.314
2BS1	0.5255	0.34
3A	10.6022	1.7246
3CS1	0.3597	0.3172
2BS2	0.4728	0.3328
A	6.2868	1.1317
2CS1	0.246	0.3016
B	6.7565	1.1962
3C	4.0843	0.829
1BS2	6.8181	1.2047
2AS2	0.5292	0.3405
1AS2	9.6345	1.5916
C	4.9746	0.9513



Above results taken from titration and spectrophotometer analyses provide us an overall understanding that these major ions and hardness in the waters of Kali River and Yamuna River are at higher side whereas the same are found in moderate or lower amounts in the Ganga River. This might be because Ganga being the most important river of the country is being treated for purity and such practices are helpful in keeping the river clean. On the other hand, river Yamuna that passes through Delhi and Agra and Pharma industry rich Himachal Pradesh received high amount of effluent. Likewise Kali river which passes through many towns also receives effluent

while it hardly gets any treatment. That sounds like a chief reason for high contamination of these rivers.

3.3 Behavioural assessment results:

Using AMR Behaviour Scale, the behavioural data was collected from the people who lived around (or nearest settlements) the sites from where the samples of water and sediments were taken from the three rivers. The sites were *Gangiri Road* (site at East Kali River assumed to be of low contamination), *Kasganj* (site at East Kali River assumed to be of high contamination), *Anoopshahar* (site at Ganga River assumed to be of low contamination), *Kachhla* (site at Ganga River assumed to be of high contamination), *Poiya Village* (site at Yamuna River assumed to be of low contamination) and *Agra City* (site at Yamuna River assumed to be of high contamination). People living around these sites were contacted and requested to complete the AMR Behaviour scale (please see **Annexure-2**). Since the sites of low contamination were assumed to be places where the rivers pass through unhabituated areas, the behavioural data collection was done from the localities which were found nearest to the location of water sampling. The data was organized and subjected to analysis of variance with the help of SPSS software.

Table - 8

Showing mean difference across different sampling sites on the counts of AMR Behaviour Scale

Sampling site	N	Mean	S. D.	F	p
Gangiri Rd.	22	70.45	16.091		
Kasganj	55	69.04	16.183		
Anoopshahar	17	73.29	17.456	1.304	0.263
Kachhla	26	74.65	15.268		
Poiya Village	39	67.26	12.502		
Agra City	69	72.71	13.035		
Total	228	70.94	14.719		

The above table shows that the difference amongst mean AMR Behaviour score across six sites is statistically non-significant. This signifies that AMR related behavior is found similar across different sites regardless of the locality. A careful observation of the means of all groups shows that **uniformly the AMR behavior is above average** which is an alarming revelation. This is vital finding and indicates that high levels of antibiotics universally found in the rivers might be linked to the overuse of antibiotics by the individuals and this behavioural manifestation is indicative of its link with anti-microbial resistance in the water bodies.

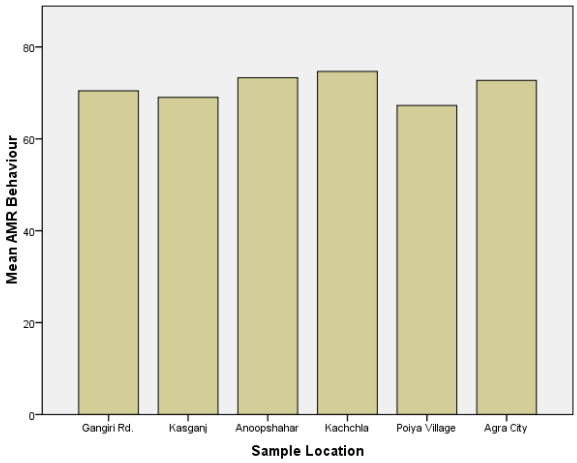


Figure 14: Bar diagram indicating similarity of AMR Behaviour across different sampling locations

The bar chart above indicates how AMR Behaviour is uniformly spread out across locations. It is worth mentioning here that the researcher also conducted pairwise comparisons by Bonferroni post-hoc statistics. No pair showed any significant difference thus verifying the ANOVA results.

Our behavioural data findings provide two important informations. One, AMR Behaviour is found universally regardless of the location. This signifies that how much the uncontrolled usage of antibiotics is found in the populations. Second, the level of AMR Behaviour is generally high. This information provides us a firm rationale of identifying behavior change interventions to deal with the problem.

3.4 Rapid Assessment of Evidence:

As per the methodology written earlier, inclusion and exclusion criterion, search and synthesis protocol for behavior change interventions aimed at reducing Antimicrobial Resistance (AMR) were set up separately for AMR behavior amongst individuals, imprudent prescription amongst health professionals and pharmaceutical effluents amongst industries. Target Population was patients or non-patient humans, including healthcare professionals, caregivers, or the general public. Interventions were considered that used theories of behavior change in order to bring a change in behavior. Outcomes focused were AMR reduction in the form of changes in antimicrobial prescribing patterns, adherence to treatment guidelines, awareness of AMR, or reductions in antimicrobial consumption. Study Designs considered were interventional studies that were randomized controlled trials (RCTs), quasi-experimental studies, controlled before-and-after studies, or well-designed observational studies. Studies published in English language were considered. Studies were excluded if they were showing irrelevant application of behavior change interventions, following poor methodology, non-peer reviewed or published in other languages than English.

An organized search on leading data bases yielded huge returns. The studies were filtered and sorted according to the objectives of the search which have been cited below:

Table -9
Recommended Interventions after Rapid Assessment of Evidence

Sr.	Reference	Type	Intervention	Location	Effectiveness
1.	Sumpradit et. al. 2012[88]	Individual and Prescription focused	Antibiotics Smart Use	Thailand	Theory of planned behavior used, outcomes achieved.
2.	Chaintarli et. al. 2016[89]	Individual and Prescription focused	Antibiotic Guardian Campaign	United Kingdom	increased from 30.7 % pre-campaign to 63.4 % post-campaign
3.	Ghafur et al 2013[90]	All three but mainly Pharma Effluents	The Chennai Declaration: Policy and Guidelines	India	Outcome mapping warranted

Rapid Evidence Assessment returned good amount of results but after careful observation of all studies, three representative interventions or mitigating approaches were suggested here as the way forward for this research.

4. Policy Projection:

This study picks first-hand information from the microbiological and geochemical data for a confirmation of presence of contamination and from behavioural data confirms the presence of higher level AMR behavior. Subsequently, evidence-based research provided the information about availability of behavior change interventions that could be used to mitigate the situation. Use of behavior change interventions to address AMR should prioritize a multi-faceted approach encompassing healthcare professionals, patients, caregivers, and the general public. Policymakers need to invest in comprehensive educational campaigns and training programs to raise awareness about AMR, appropriate antibiotics use. Additionally, policy frameworks should emphasize the importance of promoting responsible antibiotic prescribing, discouraging self-medication, and fostering patient adherence to treatment regimens. Public engagement through media campaigns, community outreach, and digital platforms can play a pivotal role in disseminating information and encouraging behavioral shifts. Another source of AMR with extreme importance is pharmacological effluent that seems to account for a major cause of AMR in rivers. Policy guidelines to curb such effluents is of prime importance. Collaborative efforts between government bodies, healthcare organizations, educational institutions, and civil society are vital to ensure effective implementation, monitoring, and evaluation of these interventions, ultimately mitigating the threat of AMR and safeguarding the biodiversity of rivers and water bodies.

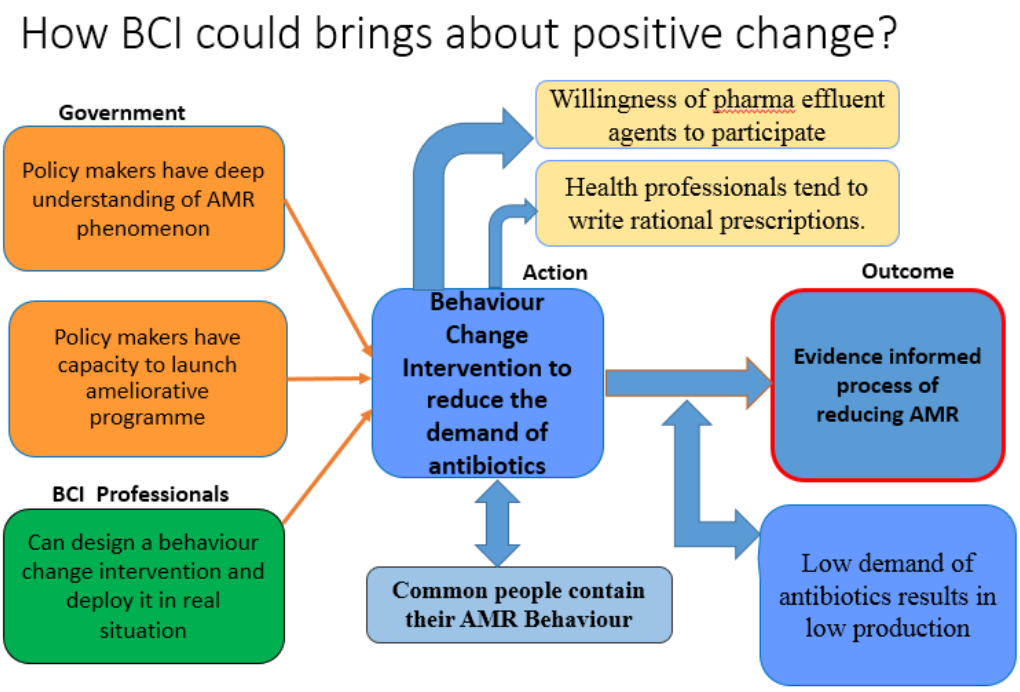


Figure 15: Model of Behaviour Change Interventions impacting AMR

4.1 Future Research Objectives:

Since every research is inconclusive and leads to further understanding of the phenomenon under study, this researcher felt limited at various points and envisaged some potential research ideas related this work. The ideas have been presented here in the form of research objectives.

- ✓ To assess the impact of anthropogenic activities, such as agricultural runoff, industrial discharge, and wastewater effluents, on the emergence and spread of AMR in river water.
- ✓ To identify key factors influencing the dissemination and persistence of AMR genes in the aquatic environment.
- ✓ To evaluate the risk of transmission of AMR from river water to humans and the broader environment through exposure pathways.
- ✓ To compare AMR profiles across different sampling sites to identify regions of high AMR prevalence and potential hotspots of resistance.
- ✓ To determine the correlation between environmental parameters (e.g., temperature, pH, nutrient levels) and the abundance of AMR genes/bacteria in river water.
- ✓ To explore the potential role of mobile genetic elements, such as plasmids and integrons, in facilitating the spread of AMR genes within the riverine microbial communities.

Annexure-1: Methods of Geochemistry (Titration)

pH of water

pH is a measure of hydrogen ion concentration or activity of an aqueous solution. The value of pH ranges from 0–14, with values below pH 7 exhibiting acidic properties and values above pH 7 exhibiting basic properties. pH 7 is the center of the measurement scale; it is neither acidic nor basic. The procedure for the determination of the pH of water is given below.

Take 60 ml water samples in thoroughly cleaned beakers with distilled water.

Calibrate the pH meter carefully.

Measure the pH of each sample with the help of a pH meter, and after measuring each sample rinse the pH meter with distilled water.

Electrical Conductivity (EC) and Total Dissolved Solids (TDS)

EC is the measure of the capability of the material to pass the flow of electric current.

TDS is the total of all the organic and inorganic compounds dissolved in water.

The procedure for the determination of EC and TDS

Take 60 ml of water sample in thoroughly cleaned beakers with distilled water.

Calibrate the EC and TDS meter carefully.

Measure the EC and TDS of each sample with the help of the EC and TDS meter, and after measuring each sample rinse the meter with distilled water.

Hardness of Water (TH)

Hardness is the property of water that prevents lather formation in soap. It has a higher concentration of cations, especially Ca^{2+} and Mg^{2+}.

Procedure for determination of hardness of water (EDTA method)

Preparation EDTA Solution

Dissolve 3.723 gm of disodium salt of EDTA in 1-liter distilled water.

Preparation of Buffer Solution

Take 16.9 gm of Ammonium Chloride (NH_4Cl) and dissolve it in 143 ml of Ammonium Hydroxide (NH_4OH).

In 50 ml of distilled water, dissolve 1.179 gm of the disodium salt of EDTA and 0.78 gm of $MgSO_4.7H_2O$.

Mix both solutions and dilute to 250 ml with distilled water.

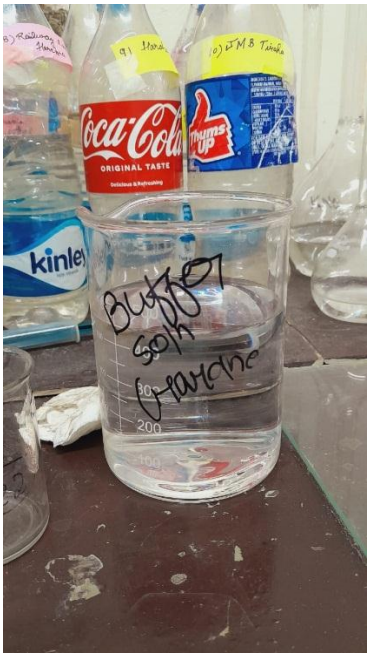
Steps for measuring hardness

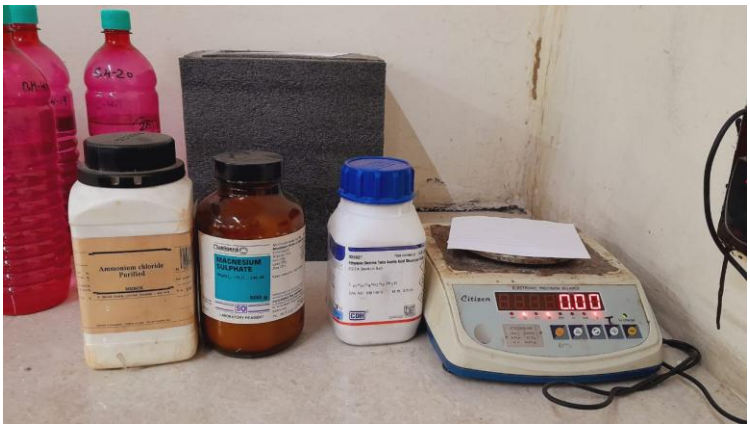
Take 25 ml water sample in thoroughly cleaned beakers with distilled water.

Add 0.5 ml of Buffer solution to each of the samples.

Add one pinch of EBT to each of the samples.

Titrate the prepared samples with EDTA solution and note different readings when the colour changed from wine red to blue.





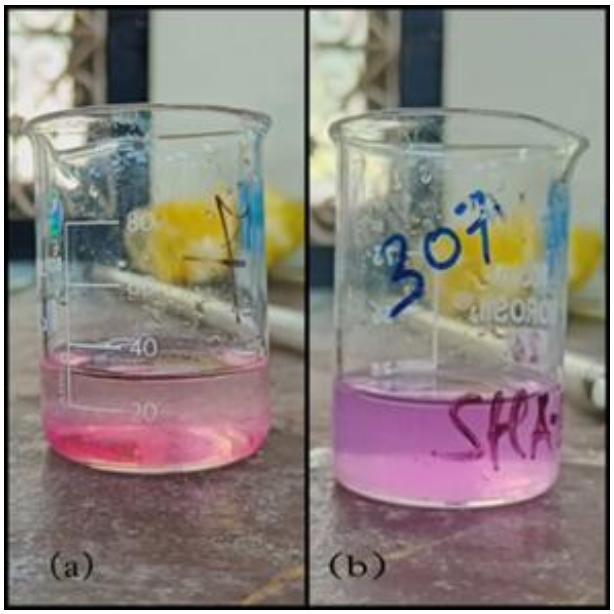
Calcium (Ca^{2+})

Preparation of NAOH solution

Take 40 gm of NaOH pellets into 1-Litre of distilled water.

Procedure

Take 25 ml water samples in thoroughly cleaned beakers with distilled water
Add 1 ml of NaOH solution and 1 pinch of murexide to each of the samples
Then the solution prepared is titrated against EDTA.
Note down the readings when the sample showed variation in colour from dark pink to purple, as shown in the figure below.



(a) Showing colour of the sample before titration of Ca^{2+}; **(b)** showing the variation of colour in the sample after titration of Ca^{2+}.

Carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-)

Preparation of Phenolphthalein

Take 50 ml of Ethanol and 50 ml of distilled water and add 0.5 gm of Phenolphthalein powder. It will indicate light pink colour and intense smell.

Preparation of Methyl Orange

Take 0.5 gm of Methyl water and 100 ml of distilled water.

Preparation of 0.1 N of HCl

Take 8.4 ml of HCl and dilute to 1000 ml with distilled water.

Procedure for determining CO_3^{-2}

Take 50 ml water samples in thoroughly cleaned beakers with distilled water.
Add 2 drops of Phenolphthalein in each of the samples. After waiting for few minutes, there is no change of colour, this indicates the absence of CO_3^{2-}.
If the sample shows a light pink colour this indicates the presence of CO_3^{2-}
Titration with 0.1 N HCl to determine the CO_3^{2-} concentrations till the colour disappears.

Procedure for determining HCO_3^-

Add 2 drops of Methyl Orange in each of the samples and titrate it against 0.1 N HCl then note down the readings till the color of the solution changes from yellowish to pink.

Chloride (Cl^-)

Preparation of reagents:

For K_2CrO_4 solution: dissolve 5 g of K_2CrO_4 in 100ml of distilled water
For 0.02 N of $AgNO_3$: dissolve 3.4 g of dried $AgNO_3$ in 1-liter of distilled water and keep the solution in the dark.

Procedure for the determination of Cl^-

Take 25 ml of water samples in thoroughly cleaned beakers with distilled water.
Add 1 ml of Potassium chromate (K_2CrO_4) to each of the samples.
The prepared samples are titrated against 0.02 N Silver Nitrate ($AgNO_3$)
Note down the readings when the samples showed colour variation from yellowish to brick, as shown in figure below:

(a) showing colour of samples before titration of Cl^-; **(b)** showing variation of colour in samples after titration of Cl^-.

Sulphate (SO_4^{2-})

Preparation of buffer solution

Take 15 gm of $MgCl_2$ + 2.5 gm of Sodium Acetate (CH_3COONa) + 0.5 gm of KNO_3+ 10 ml of acetic acid glacial and fill it up to 500 ml of distilled water.

Preparation of standard

Take 0.07365 gm of Na_2SO_4 + 50 ml of distilled water.

From this standard solution, transfer 2.5, 5, 7.5, 10 and 12.5 ml in five conical flasks and fill it up to 50 ml.

Labelled the conical flask as 1s, 2s, 3s, 4s and 5s, respectively.

Extract 25 ml and fill it into a beaker add 5 ml of buffer solution and pinch of $BaCl_2$

Preparation of blank solution

Take 25 ml of distilled water and 5 ml of buffer solution.

The steps followed are

Take 12.5 ml of water samples in thoroughly cleaned beakers with distilled water.

Add 12.5 ml of distilled water + 5 ml of buffer solution and a pinch of $BaCl_2$ in each of the samples.

Leave the samples for 1 hour.

After 1 hour, the samples are analysed in the UV spectrophotometer for determination of the concentration of Sulphate (SO_4^{2-}).

Nitrate (NO_3^-)

Preparation of Standard

Add 10 ml of Zirconium solution in funnel and dilute to 250 ml distilled water.

Transfer 50 ml of the above solution into funnels named as

Standard 1 → 1 ml

Standard 2 → 2 ml

Standard 3 →3 ml

Standard 4 → 4 ml

Standard 5 → 5 ml

Add distilled water again in the following above funnels up to 50 ml.

Transfer 25 ml of the prepared standard solution into the beakers numbered as 1s, 2s, 3s, 4s and 5s.

Put the samples from 1s to 5s on the hot plate as the solution dried.

Steps followed are

Take 12.5 ml of water sample

Put them over a hot plate for 1 day till the precipitate form.

After about 24 hours, in all the dried samples and standards add 1 ml of Phenol disulphuric acid.

Take a beaker, name it as blank and add 1 ml of Phenol disulphuric acid.

Let the precipitation completely dissolve into it and add 24 ml distilled water into all beakers.

Add 3 ml of Ammonium solution into all beakers

If yellowish colour is obtained in the sample, it will indicate the presence of NO_3^-.

Observe all the samples, including standard and blank in UV spectrophotometer.

Fluoride (F^-)

•Preparation of Spand

Dissolve 479 mg of spand in distilled water and make it upto 250 ml.

•Preparation of Zirconyl Acid Reagent

Take 66.5 mg of zirconyl chloride octahydrate in 12.5 ml distilled water plus 175 ml of HCl and dilute it to 250 ml.

•Preparation of Reference Solution

Add 5 ml of spand and 50 ml of distilled water. To this add diluted HCl (3.5 ml concentrated HCl and 1.5 ml) distilled water.

•Preparation of Stock F^- Solution

Dissolve 0.110 gm of Sodium Fluoride (NaF) to 500 ml of distilled water

•Preparation of Standard Fluoride solution

Take 50 ml of Stock F^- solution and make it to 500 ml.

•Preparation of standards

1 ml of Standard Fluoride solution and 49 ml distilled water→ 0.2 ppm
2 ml of Standard Fluoride solution and 48 ml distilled water→ 0.4 ppm
3 ml of Standard Fluoride solution and 46 ml distilled water→ 0.6 ppm
4 ml of Standard Fluoride solution and 45 ml distilled water→ 0.8 ppm
5 ml of Standard Fluoride solution and 44 ml distilled water→ 1.0 ppm

• Preparation of Blank

Take 25 ml of distilled water and 5 ml of spand

Steps followed are

Mix in equal quantities acidic zirconyl and spand
Take 12.5 ml of water sample and add 2.5 ml of above mixture to each of the samples.
Observe all samples in UV spectrophotometer.

Annexure – 2: AMR BEHAVIOUR SCALE

Daud Salim Faruquie[1], Ilma Khowja[2], Sara Afzal[3], Asad U. Khan[4], S. Z. Rahman[5]

Scale Factors	
1	Self-justification
2	Pragmatism
3	Medical Skepticism
4	OTC Euphoria

Likert scale response rating and score				
Strongly Disagree (1)	Disagree (2)	Neutral (3)	Agree (4)	Strongly Agree (5)

Sr.	Items	Factor	Item - total (r)	Rotated values
1.	I always believe in curing any illness through homemade remedies.	4	.182	.626
2.	I prefer taking an anti-biotic whenever I catch cold.	1	.355	.600
3.	I believe in the idea of an anti-biotic consumption at every four weeks to prevent any oncoming disease.	1	.414	.688
4.	My fever cures only by an anti-biotic.	1	354	.663
5.	I don't believe in a doctor who prescribes just a paracetamol for cold and fever.	4	.246	604
6.	I don't seek a doctor's advice over usual infections.	3	.213	.457
7.	To avoid getting it serious, it is good to take an anti-biotic if fever starts.	1	383	.563
8.	I buy medicines by explaining symptoms to the pharmacist.	3	.352	557
9.	One should always keep certain anti-biotic medicines at home.	2	.251	.797

[1] Director, Centre for Evidence-based Policy, Practice and Interventions.
[2] Junior Research Fellow, Centre for Evidence-based Policy, Practice and Interventions.
[3] Student, BSc (Hons.) Geology, Aligarh Muslim University.
[4] Professor, Department of Biotechnology, Aligarh Muslim University.
[5] Professor, Department of Pharmacology, J.N. Medical College, Aligarh Muslim University.

10.	Anti-biotic should be easily available at every drug store.	2	.198	.748
11.	I refer to internet to find drugs for my illnesses.	1	.446	.532
12.	I don't like to continue with the full course of anti-biotics if the symptoms have subsided.	4	.279	428
13.	Everyone should keep a handy box of anti-biotic medicines for cold and fever.	2	.357	806
14.	I have no difficulty in buying medicines without a doctor's prescription.(3)	3	.325	.673
15.	A pharmacist gives effective medicine as he has an experience in pharmacy.	3	.284	.419
16	I believe in Ayurveda more than allopathy.	4	.266	.656
17	My immune system is safer with anti-biotic.	1	.241	.656
18	An anti-biotic is an answer to all my health problems.	1	.371	.701
19.	Anti-biotics are easily available at my nearby drugstore, that doesn't require prescription.	3	.220	.647
20.	Doctors just make money, so I avoid consulting them for usual fever and cold.	3	.236	.659

Hindi Translation:

1. मैं हमेशा घरेलू नुस्ख़ों से ही अपना इलाज कर लेता हूँ।
2. जब भी मुझे ज़ुखाम होता है तो मैं एंटी-बायोटिक्स लेना पसंद करता हूं।
3. मुझे कोई बीमारी न हो इस लिए हर 4 हफ्ते में एक बार एंटीबायोटिक ले लेता हूँ.
4. मेरा बुखार सिर्फ एंटीबायोटिक्स से ही सही होता है.
5. मैं उस डॉक्टर पर विश्वास नहीं करता जो सर्दी और बुखार के लिए सिर्फ पैरासिटामोल लिखता है।
6. मैं छोटी-छोटी बीमारी के लिए डॉक्टर की सलाह नहीं लेता हूँ.
7. बुखार शुरू होने पर इसे गंभीर होने से बचाने के लिए एंटी-बायोटिक लेना अच्छा होता है
8. मैं फार्मासिस्ट को ही लक्षण बताकर दवाएं खरीद लेता हूं.
9. घर में हमेशा कुछ एंटी-बायोटिक दवाइयां रखनी चाहिए.
10. हर दवा की दुकान पर एंटी बायोटिक आसानी से उपलब्ध होना चाहिए.
11. मैं अपनी बीमारियों के लिए दवाएँ खोजने के लिए इंटरनेट का सहारा लेता हूँ
12. बीमारी के लक्षण कम होने पर मैं एंटीबायोटिक दवाओं का कोर्स पूरा नहीं करता।

13. सर्दी और बुखार की एंटीबायोटिक का एक डिब्बा सबको अपने पास रखना चाहिए.
14. मैं डॉक्टर के प्रिस्क्रिप्शन के बिना ही बड़े आराम से दवाएं खरीद लेता हूँ।
15. एक फार्मासिस्ट प्रभावी दवा दे देता है क्योंकि उसके पास फार्मेसी का अनुभव होता है
16. . मैं एलोपैथी से ज्यादा आयुर्वेद पर विश्वास करता हूं.
17. मेरे शरीर की रोग प्रतिरोधकता एंटीबायोटिक्स के कारण सुरक्षित रहती है.
18. एक एंटी-बायोटिक मेरी सभी स्वास्थ्य समस्याओं का जवाब है
19. एंटी-बायोटिक्स मेरे नजदीकी दवा की दुकान पर आसानी से उपलब्ध हैं, जिसके लिए डॉक्टर के पर्चे की आवश्यकता नहीं होती है।
20. डॉक्टर सिर्फ पैसा कमाते हैं, इसलिए मैं सामान्य बुखार और सर्दी के लिए उनको दिखाने से बचता हूं

5. References:

[1] World Health Organization. Antimicrobial resistance. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

[2] Centers for Disease Control and Prevention. Antibiotic/antimicrobial resistance (AR/AMR). Available from: https://www.cdc.gov/drugresistance/about.html.

[3] O'Neill J. Review on Antimicrobial Resistance: Tackling drug-resistant infections globally. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.

[4] Laxminarayan, R. and Chaudhury, R.R., 2016. Antibiotic resistance in India: drivers and opportunities for action. *PLoS medicine*, *13*(3), p.e1001974.

[5] Baquero F, Martinez JL, Canton R. Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol. 2008;19(3):260-265. doi:10.1016/j.copbio.2008.05.006.

[6] Czekalski N, Berthold T, Caucci S, et al. Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into Lake Geneva, Switzerland. Front Microbiol. 2012;3:106. doi:10.3389/fmicb.2012.00106.

[7] Zhang XX, Zhang T. Occurrence, abundance, and diversity of tetracycline resistance genes in 15 sewage treatment plants across China and other global locations. Environ Sci Technol. 2011;45(7):2598-2604. doi:10.1021/es103309t.

[8] Martinez JL. Environmental pollution by antibiotics and by antibiotic resistance determinants. Environmental pollution. 2009 Nov 1;157(11):2893-902.

[9] Mukherjee M, Gentry T, Mjelde H, Brooks JP, Harmel D, Gregory L, Wagner K. Escherichia coli antimicrobial resistance variability in water runoff and soil from a remnant native prairie, an improved pasture, and a cultivated agricultural watershed. Water. 2020 Apr 28;12(5):1251.

[10] Baquero, F., & Tedim, A. P. (2013). Antibiotic resistance sponges: Obligate bacterial mutualists modelling antibiotic resistance acquisition and spread. Environmental Microbiology, 15(5), 1331-1343.

[11] Almakki A, Jumas-Bilak E, Marchandin H, Licznar-Fajardo P. Antibiotic resistance in urban runoff. Science of the Total Environment. 2019 Jun 1;667:64-76.

[12] Goel, S., Singh, A., Gupta, M., & Kumar, V. (2018). Antibiotic pollution in the environment: A review. Clean Techn Environ Policy, 20(6), 1305-1320. doi: 10.1007/s10098-018-1528-8.

[13] Tucker K, Mageiros L, Carstens A, Bröcker L, Archer E, Smith K, Mourkas E, Pascoe B, Nel D, Meric G, Sheppard SK. Spatiotemporal investigation of antibiotic resistance in the urban water cycle influenced by environmental and anthropogenic activity. Microbiology Spectrum. 2022 Oct 26;10(5):e02473-22.

[14] Vinayamohan PG, Pellissery AJ, Venkitanarayanan K. Role of horizontal gene transfer in the dissemination of antimicrobial resistance in food animal production. Current Opinion in Food Science. 2022 Oct 1;47:100882.

[15] Grilo ML, Sousa-Santos C, Robalo J, Oliveira M. The potential of Aeromonas spp. from wildlife as antimicrobial resistance indicators in aquatic environments. Ecological Indicators. 2020 Aug 1;115:106396.

[16] Kumar A, Kumar S. Occurrence and distribution of antibiotics in the Ganges River, India: a review. Chemosphere. 2019;215:674-686. doi: 10.1016/j.chemosphere.2018.11.165.

[17] Mohapatra DP, Brar SK, Tyagi RD, Surampalli RY. Environmental implications of antibiotic disposal from the Indian perspective. Environ Int. 2010;36(5):417-431. doi: 10.1016/j.envint.2010.03.002.

[18] Goel S, Singh A, Gupta M, Kumar V. Antibiotic pollution in the environment: A review. Clean Techn Environ Policy. 2018;20(6):1305-1320. doi: 10.1007/s10098-018-1528-8.

[19] Sood A, Pandey P, Bisht S, Sharma S. Anthropogenic activities as a source of high prevalence of antibiotic resistant Staphylococcus aureus in the River Ganga. Appl Ecol Environ Res. 2014 Jan 1;12(1):33-48.

[20] Niveshika, Singh S, Verma E, Mishra AK. Isolation, characterization and molecular phylogeny of multiple metal tolerant and antibiotics resistant bacterial isolates from river Ganga, Varanasi, India. Cogent Environmental Science. 2016 Dec 31;2(1):1273750.

[21] Khwaja, A.R., Singh, R. and Tandon, S.N., 1998. Treatment of leather board effluents by a coagulation/flocculation process. *Environmental technology*, *19*(8), pp.857-860.

[22] Devarajan, N., Laffite, A., Ngelikoto, P., Elongo, V., Prabakar, K., Mubedi, J.I., Piana, P.T., Wildi, W. and Poté, J., 2015. Hospital and urban effluent waters as a source of accumulation of toxic metals in the sediment receiving system of the Cauvery River, Tiruchirappalli, Tamil Nadu, India. *Environmental science and pollution research*, *22*, pp.12941-12950.

[23] Kotwani, A., Joshi, J. and Kaloni, D., 2021. Pharmaceutical effluent: a critical link in the interconnected ecosystem promoting antimicrobial resistance. *Environmental Science and Pollution Research*, *28*(25), pp.32111-32124.

[24] World Health Organization, 2011. Local production and access to medicines in low-and middle-income countries: a literature review and critical analysis.

[25] Babatunde, A.I., Bamgbola, E.P. and Oyelola, O.T., 2014. The effect of pharmaceutical effluents on the quality of groundwater: A case study of Ikeja industrial area of Lagos, Nigeria. *Int J Med Res Health Sci*, *4*.

[26] Fouz, N., Pangesti, K.N., Yasir, M., Al-Malki, A.L., Azhar, E.I., Hill-Cawthorne, G.A. and Abd El Ghany, M., 2020. The contribution of wastewater to the transmission of antimicrobial resistance in the environment: implications of mass gathering settings. *Tropical medicine and infectious disease*, *5*(1), p.33.

[27] Kotwani, A., Joshi, J. and Kaloni, D., 2021. Pharmaceutical effluent: a critical link in the interconnected ecosystem promoting antimicrobial resistance. *Environmental Science and Pollution Research*, *28*(25), pp.32111-32124.

[28] Reinthaler FF, Posch J, Feierl G, Wüst G, Haas D, Ruckenbauer G, Mascher F, Marth E. Antibiotic resistance of E. coli in sewage and sludge. Water research. 2003 Apr 1;37(8):1685-90.

[29] Hayward C, Ross KE, Brown MH, Whiley H. Water as a source of antimicrobial resistance and healthcare-associated infections. Pathogens. 2020 Aug 18;9(8):667.

[30] Founou LL, Founou RC, Essack SY. Antimicrobial resistance in the farm-to-plate continuum: More than a food safety issue. Future science OA. 2021 Jun;7(5):FSO692.

[31] Hernando-Amado S, Coque TM, Baquero F, Martínez JL. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nature microbiology. 2019 Sep;4(9):1432-42.

[32] Taviani E, Pedro O. Impact of the aquatic pathobiome in low-income and middle-income countries (LMICs) quest for safe water and sanitation practices. Current Opinion in Biotechnology. 2022 Feb 1;73:220-4.

[33] Devarajan N, Laffite A, Mulaji CK, Otamonga JP, Mpiana PT, Mubedi JI, Prabakar K, Ibelings BW, Poté J. Occurrence of antibiotic resistance genes and bacterial markers in a tropical river receiving hospital and urban wastewaters. PloS one. 2016 Feb 24;11(2):e0149211.

[34] Grenni P. Antimicrobial resistance in rivers: a review of the genes detected and new challenges. Environmental Toxicology and Chemistry. 2022 Mar;41(3):687-714.

[35] Grenni P, Ancona V, Caracciolo AB. Ecological effects of antibiotics on natural ecosystems: A review. Microchemical Journal. 2018 Jan 1;136:25-39.

[36] Eckert EM, Quero GM, Di Cesare A, Manfredini G, Mapelli F, Borin S, Fontaneto D, Luna GM, Corno G. Antibiotic disturbance affects aquatic microbial community composition and food web interactions but not community resilience. Molecular ecology. 2019 Mar;28(5):1170-82.

[37] Tello A, Austin B, Telfer TC. Selective pressure of antibiotic pollution on bacteria of importance to public health. Environmental health perspectives. 2012 Aug;120(8):1100-6.

[38] Palit K, Rath S, Chatterjee S, Das S. Microbial diversity and ecological interactions of microorganisms in the mangrove ecosystem: Threats, vulnerability, and adaptations. Environmental Science and Pollution Research. 2022 May;29(22):32467-512.

[39] Park S, Choi K. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. Ecotoxicology. 2008 Aug;17:526-38.

[40] Nilsen E, Smalling KL, Ahrens L, Gros M, Miglioranza KS, Picó Y, Schoenfuss HL. Critical review: grand challenges in assessing the adverse effects of contaminants of emerging concern on aquatic food webs. Environmental Toxicology and Chemistry. 2019 Jan;38(1):46-60.

[41] Zenker A, Cicero MR, Prestinaci F, Bottoni P, Carere M. Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment. Journal of environmental management. 2014 Jan 15;133:378-87.

[42] Kunhikannan S, Thomas CJ, Franks AE, Mahadevaiah S, Kumar S, Petrovski S. Environmental hotspots for antibiotic resistance genes. Microbiologyopen. 2021 Jun;10(3):e1197.

[43] Biswas, K., Paul, D. and Sinha, S.N., 2015. Prevalence of multiple antibiotic-resistant coliform bacteria in the water of river Ganga. *Frontiers in Environmental Microbiology*, *1*(3), pp.44-46.

[44] Niveshika, Singh, S., Verma, E. and Mishra, A.K., 2016. Isolation, characterization and molecular phylogeny of multiple metal tolerant and antibiotics resistant bacterial isolates from river Ganga, Varanasi, India. *Cogent Environmental Science*, *2*(1), p.1273750.

[45] Srivastava, A. and Verma, D., 2023. Urbanization led to the abundance of Gram-negative, chemo-organo-heterotrophs, and antibiotic resistance genes in the downstream regions of the Ganga River water of India. *Environmental Science and Pollution Research*, pp.1-22.

[46] Mittal, P., Prasoodanan PK, V., Dhakan, D.B., Kumar, S. and Sharma, V.K., 2019. Metagenome of a polluted river reveals a reservoir of metabolic and antibiotic resistance genes. *Environmental microbiome*, *14*, pp.1-12.

[47] Lamba, M., Sreekrishnan, T.R. and Ahammad, S.Z., 2020. Sewage mediated transfer of antibiotic resistance to River Yamuna in Delhi, India. *Journal of Environmental Chemical Engineering*, *8*(1), p.102088.

[48] Das, B.K., Behera, B.K., Chakraborty, H.J., Paria, P., Gangopadhyay, A., Rout, A.K., Nayak, K.K., Parida, P.K. and Rai, A., 2020. Metagenomic study focusing on antibiotic resistance genes from the sediments of River Yamuna. *Gene*, *758*, p.144951.

[49] Mutiyar, P.K. and Mittal, A.K., 2014. Occurrences and fate of selected human antibiotics in influents and effluents of sewage treatment plant and effluent-receiving river Yamuna in Delhi (India). *Environmental Monitoring and Assessment*, *186*, pp.541-557.

[50] Zeyaullah, M., Islam, B. and Ali, A., 2010. Isolation, identification and PCR amplification of merA gene from highly mercury polluted Yamuna River. *African Journal of Biotechnology*, *9*(24), pp.3510-3514.

[51] Reddy, S., Kaur, K., Barathe, P., Shriram, V., Govarthanan, M. and Kumar, V., 2022. Antimicrobial resistance in urban river ecosystems. *Microbiological Research*, *263*, p.127135.

[52] Jassal, P.S., Kaur, D., Kaur, M. and Sharma, D., 2023. Level of antibiotic contamination in the major river systems: A review on South Asian countries perspective. *Journal of Applied Pharmaceutical Science*, *13*(6), pp.010-017.

[53] Sinha, R.K. and Loganathan, B.G., 2015. Ganges river contamination: a review. *Water Challenges and Solutions on a Global Scale*, pp.129-159.

[54] Misra, A.K., 2011. Impact of urbanization on the hydrology of Ganga Basin (India). *Water resources management*, *25*, pp.705-719.

[55] Kumar, M., Sharif, M. and Ahmed, S., 2020. Impact of urbanization on the river Yamuna basin. *International Journal of River Basin Management*, *18*(4), pp.461-475.

[56] Lokhande, S. and Tare, V., 2021. Spatio-temporal trends in the flow and water quality: response of river Yamuna to urbanization. *Environmental Monitoring and Assessment*, *193*, pp.1-14.

[57] Dwivedi, A.C., Mayank, P. and Tiwari, A., 2016. The River as transformed by human activities: the rise of the invader potential of Cyprinus carpio and Oreochromis niloticus from the Yamuna River, India. *Journal of Earth Science & Climatic Change*, *7*(7), p.361.

[58] Chakarvorty, M., Dwivedi, A.K., Shukla, A.D., Kumar, S., Niyogi, A., Usmani, M. and Pati, J.K., 2015. Geochemistry and magnetic measurements of suspended sediment in urban sewage water vis-à-vis quantification of heavy metal pollution in Ganga and Yamuna Rivers, India. *Environmental Monitoring and Assessment*, *187*, pp.1-17.

[59] Khan, A.S., Anavkar, A., Ali, A., Patel, N. and Alim, H., 2021. A review on current status of riverine pollution in India. *Biosciences Biotechnology Research Asia*, *18*(1), pp.9-22.

[60] Khan, A.S., Anavkar, A., Ali, A., Patel, N. and Alim, H., 2021. A review on current status of riverine pollution in India. *Biosciences Biotechnology Research Asia*, *18*(1), pp.9-22.

[61] Alaoui Mdarhri, H., Benmessaoud, R., Yacoubi, H., Seffar, L., Guennouni Assimi, H., Hamam, M., Boussettine, R., Filali-Ansari, N., Lahlou, F.A., Diawara, I. and Ennaji, M.M., 2022. Alternatives therapeutic approaches to conventional antibiotics: advantages, limitations and potential application in medicine. *Antibiotics*, *11*(12), p.1826.

[62] Do, Q.T., Joshi, S. and Stolper, S., 2018. Can environmental policy reduce infant mortality? Evidence from the Ganga Pollution Cases. *Journal of Development Economics*, *133*, pp.306-325.

[63] Muthaiyah, N.P., 2020. Rejuvenating Yamuna River by wastewater treatment and management. *International Journal of Energy and Environmental Science*, *5*(1), pp.14-29.

[64] Taneja, N. and Sharma, M., 2019. Antimicrobial resistance in the environment: The Indian scenario. *The Indian journal of medical research*, *149*(2), p.119.

[65] Ray, P., 1998. *Ecological imbalance of the Ganga river system: its impact on aquaculture*. Daya Books.

[66] Rathore, G., Lekshmi, N. and Swaminathan, T.R., 2022. Antimicrobial resistance in aquaculture and fisheries-Status and way forward. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, *43*(1), pp.58-65.

[67] Pei, M., Zhang, B., He, Y., Su, J., Gin, K., Lev, O., Shen, G. and Hu, S., 2019. State of the art of tertiary treatment technologies for controlling antibiotic resistance in wastewater treatment plants. *Environment international*, *131*, p.105026.

[68] Hinchliffe, S., Butcher, A. and Rahman, M.M., 2018. The AMR problem: demanding economies, biological margins, and co-producing alternative strategies. *Palgrave Communications*, *4*(1).

[69] Jani, K., Srivastava, V., Sharma, P., Vir, A. and Sharma, A., 2021. Easy access to antibiotics; spread of antimicrobial resistance and implementation of one health approach in India. *Journal of Epidemiology and Global Health*, *11*(4), pp.444-452.

[70] Kotwani, A., Joshi, J. and Kaloni, D., 2021. Pharmaceutical effluent: a critical link in the interconnected ecosystem promoting antimicrobial resistance. *Environmental Science and Pollution Research*, *28*(25), pp.32111-32124.

[71] Adebisi, Y.A., 2023. Balancing the risks and benefits of antibiotic use in a globalized world: the ethics of antimicrobial resistance. *Globalization and Health*, *19*(1), p.27.

[72] Thakur, N., Kaur, S., Tomar, P., Thakur, S. and Yadav, A.N., 2020. Microbial biopesticides: current status and advancement for sustainable agriculture and environment. In *New and future developments in microbial biotechnology and bioengineering* (pp. 243-282). Elsevier.

[73] WEI, H.M., 2010. Studies of ecological environment management in the New Countryside construction progress. *Advanced Materials Research*, *113*, pp.1143-1146.

[74] Priyadarshini, K. and Gupta, O.K., 2003. Compliance to environmental regulations: The Indian context. *International Journal of Business and Economics*, *2*(1), p.9.

[75] Trombadore, O., Nandi, I. and Shah, K., 2020. Effective data convergence, mapping, and pollution categorization of ghats at Ganga River Front in Varanasi. *Environmental Science and Pollution Research*, *27*, pp.15912-15924.

[76] Gronwall, J. and Jonsson, A.C., 2017. Regulating effluents from India's textile sector: new commands and compliance monitoring for zero liquid discharge. *Law Env't & Dev. J.*, *13*, p.13.

[77] Wood, W. and Neal, D.T., 2016. Healthy through habit: Interventions for initiating & maintaining health behavior change. *Behavioral Science & Policy*, *2*(1), pp.71-83.

[78] Hardeman, W., Griffin, S., Johnston, M., Kinmonth, A.L. and Wareham, N.J., 2000. Interventions to prevent weight gain: a systematic review of psychological models and behaviour change methods. *International journal of obesity*, *24*(2), pp.131-143.

[79] Yardley, L., Morrison, L., Bradbury, K. and Muller, I., 2015. The person-based approach to intervention development: application to digital health-related behavior change interventions. *Journal of medical Internet research*, *17*(1), p.e4055.

[80] Glanz, K., 1997. *Theory at a glance: A guide for health promotion practice* (No. 97). US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute.

[81] Rothman, A.J., 2000. Toward a theory-based analysis of behavioral maintenance. *Health Psychology*, *19*(1S), p.64.

[82] Oinas-Kukkonen, H., 2013. A foundation for the study of behavior change support systems. *Personal and ubiquitous computing*, *17*, pp.1223-1235.

[83] Michie, S. and Abraham, C., 2004. Interventions to change health behaviours: evidence-based or evidence-inspired?. *Psychology & Health*, *19*(1), pp.29-49.

[84] Parveen, S., Garzon-Orjuela, N., Amin, D., McHugh, P. and Vellinga, A., 2022. Public health interventions to improve antimicrobial resistance awareness and behavioural change associated with antimicrobial use: a systematic review exploring the use of social media. *Antibiotics*, *11*(5), p.669.

[85] Edgar, T., Boyd, S.D. and Palamé, M.J., 2009. Sustainability for behaviour change in the fight against antibiotic resistance: a social marketing framework. *Journal of Antimicrobial Chemotherapy*, *63*(2), pp.230-237.

[86] Stålsby Lundborg, C. and Tamhankar, A.J., 2014. Understanding and changing human behaviour—antibiotic mainstreaming as an approach to facilitate modification of provider and consumer behaviour. *Upsala journal of medical sciences*, *119*(2), pp.125-133.

[87] Mutiyar, P.K. and Mittal, A.K., 2014. Occurrences and fate of selected human antibiotics in influents and effluents of sewage treatment plant and effluent-receiving river Yamuna in Delhi (India). *Environmental Monitoring and Assessment*, *186*, pp.541-557.

[88]Sumpradit, N., Chongtrakul, P., Anuwong, K., Pumtong, S., Kongsomboon, K., Butdeemee, P., Khonglormyati, J., Chomyong, S., Tongyoung, P., Losiriwat, S. and Seesuk, P., 2012. Antibiotics Smart Use: a workable model for promoting the rational use of medicines in Thailand. *Bulletin of the World Health Organization*, *90*, pp.905-913.

[89] Chaintarli, K., Ingle, S.M., Bhattacharya, A., Ashiru-Oredope, D., Oliver, I. and Gobin, M., 2016. Impact of a United Kingdom-wide campaign to tackle antimicrobial resistance on self-reported knowledge and behaviour change. *BMC Public Health*, *16*(1), pp.1-9.

[90] Ghafur, A., Mathai, D., Muruganathan, A., Jayalal, J.A., Kant, R., Chaudhary, D., Prabhash, K., Abraham, O.C., Gopalakrishnan, R., Ramasubramanian, V. and Shah, S.N., 2013. The Chennai Declaration: a roadmap to tackle the challenge of antimicrobial resistance. *Indian Journal of Cancer*, *50*(1), pp.71-73.

CERTIFICATE OF COMPLETION

This is to certify that this thesis project titled **"Comparison of anti-microbial resistance in three river ecosystems and assessment for behaviour change interventions"** was carried out by **Miss Sara Afzal**, a student of **BSc. (Hons.) Geology**, at the **Department of Geology, Aligarh Muslim University**. The research for this project was undertaken under the guidance of the afore-mentioned institute and completed during the period of **January 2023** to **July 2023.**

This project was shortlisted under the *Sponsored Thesis Project Competition on "RE-IMAGINING URBAN RIVERS" (Season- 3)* hosted by the National Institute of Urban Affairs (NIUA) and the National Mission for Clean Ganga (NMCG).

This report has been submitted by the student as a final deliverable under the competition. All parts of this research can used by any of the undersigning parties.

1. **Student**
   Name - **Sara Afzal**
   Signature -

2. **Institute**
   Name - Aligarh Muslim University, India
   Department - Department of Geology
   Authorized Representative - **Professor M. Shamim Khan** (Supervisor)
   Signature -

3. **Sponsors**
   Name - National Institute of Urban Affairs
   Authorized Representative - Hitesh Vaidya, Director
   Signature -

   Name - National Mission for Clean Ganga
   Authorized Representative - G Asok Kumar, Director General
   Signature -