

# Qualitative Risk Assessment of Microbial Indicators in the Lower Reaches of Meenachil River, Kerala, India

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**Abstract** Pristine natural freshwater resources being severely contaminated by microbiological pollutants is still a big issue in many parts of the world. In the present study, water samples taken from the upstream, midstream, and downstream reaches of the Meenachil River were analysed for the presence of microbial pollution indicators such as faecal coliforms (FC), faecal streptococci (FS), and *Escherichia coli*. The *E. coli* isolates were subjected to antimicrobial susceptibility tests and the multiple antibiotic resistance (MAR) indexes were calculated so as to delineate the potential risk posed by infections to humans and animals using the river for routine potable and recreational purposes. *E. coli* was consistently isolated from all the samples collected from the different zones of the river. Other faecal coliforms such as *Klebsiella oxytoca*, *Citrobacter koseri*, *Enterobacter*, *Proteus vulgaricus*, and *Shigella boydii* were also identified. Higher FC/FS ratios were noticed for the midstream and the downstream sites indicating the contamination from high-risk sources such as human and anthropogenic domestic wastes. The MAR index values of *E. coli* isolates ranged from 0.33 upstream, 0.416 for midstream and 0.583 for the downstream reaches. Given that *E. coli* is an ideal indicator of faecal pollution, their existence and extended survival of multiple antibiotic resistance natural freshwater pose a potential risk for the consumers. Rapid urbanization and resultant waste drainage in the downstream stretches of the river might have accelerated the deterioration of the water quality up to a certain extent. Furthermore, the riverine system as it flows downstream loses its self-purification capacity due to the loss of natural water drifts. This may be attributed to the periodic shutdown of the salinity barrier, Thanneermukkam Bund in the confluence zone of the river which results in a temporary stagnation of the running waters in the lower stretches.

**Keywords:** antibiotic resistance, aquatic environment, Faecal coliforms, sewage, pollutants

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## 1. Introduction

River waters play a significant role in the functioning of aquatic ecosystems and provide several essential services for human well-being [1]. The presence of antibiotics and antimicrobial-resistant genes is causing significant concerns in the aquatic environment due to the possibility of the development of pathogens that are antibiotic-resistant [2,3,4]. Since the rivers collect wastewater containing different contaminants from different sources, they are thought to be the potential repositories of developing contaminants (drugs, metals, and antibiotic-resistant genes (ARG) [5,6,7]. Several antibiotics given to people or animals are partially metabolized in the digestive system and released with faeces into hospital or community effluents, which end up in treated or untreated environmental water bodies [5].

Freshwater resources being severely contaminated by microbiological pollutants is still a big issue in many regions of the world [Haller 2009. Worldwide, antibiotic residues in natural river water have been identified [8], and their levels are fast rising, primarily because of the widespread use of antibiotics (AB) in human and veterinary medicine. In addition to negatively affecting native microbial populations, which are crucial for essential biological cycles, mechanisms, and processes and for the preservation of water quality, antibiotics in rivers may also compromise basic ecological processes [9]. However, because they are AR reservoirs, aquatic ecosystems are also thought to be a significant pathway for spreading resistance elements. [10,11].

Variable numbers of virulence genes have been found in various types of water worldwide. In Nigeria, India, and Australia, virulence genes were found in 91, 611, and 85% of the isolates from rivers and creeks, respectively [12,13,14]. Different Indian water sources

have been documented to have antimicrobial-resistant bacteria and their genes. High-end antibiotic-resistant genes have been reported from the Mutha River, which flows through Pune, India, with a concentration that is 30-times greater in the sediments close to the city and is derived from domestic and municipal sewage waste (<https://outbreakwatch.blogspot.com/2017/09/proahedr-antibioticresistance-08-india.html>). A study on the sewage treatment plans in South India documented *E. coli* isolates resistant to third-generation Cephalosporin when the input was household water alone at the rates of 25 %, domestic waste mixed with hospital effluent at 70 %, and hospital effluent alone was 95% [15].

The microbiological quality of water sources is typically monitored using faecal indicator bacteria (FIB), such as *Escherichia coli* and *Enterococcus sp.*, found in the digestive tracts of warm-blooded animals. *E. coli* is frequently used as a sentinel for resistance to antimicrobials with Gram-negative activity in national “One Health” monitoring programs for antimicrobial-resistant enteric infections [16]. Commensal *E. coli* strains may share acquired resistance traits with pathogens like *Salmonella* or pathogenic *E. coli* through horizontal gene exchange, especially in environments like the gastrointestinal tract where enormous species diversity and density allow for the conjugation-mediated exchange of ARGs between bacterial populations [17].

In tropical regions, particularly South India, there are presently very few published studies on rivers as possible ARG reservoirs [18]. The physico-chemical properties of sediments receiving untreated hospital and urban discharges were the subject of a few studies [19,20]. There needs to be more information about the microbiological water quality in tropical rivers and the prevalence of clinically essential ARGs brought on by untreated urban and hospital effluents.

Meenachil river, the lifeline of central Kerala, is one of the most affected rivers of the Southern Western Ghats.

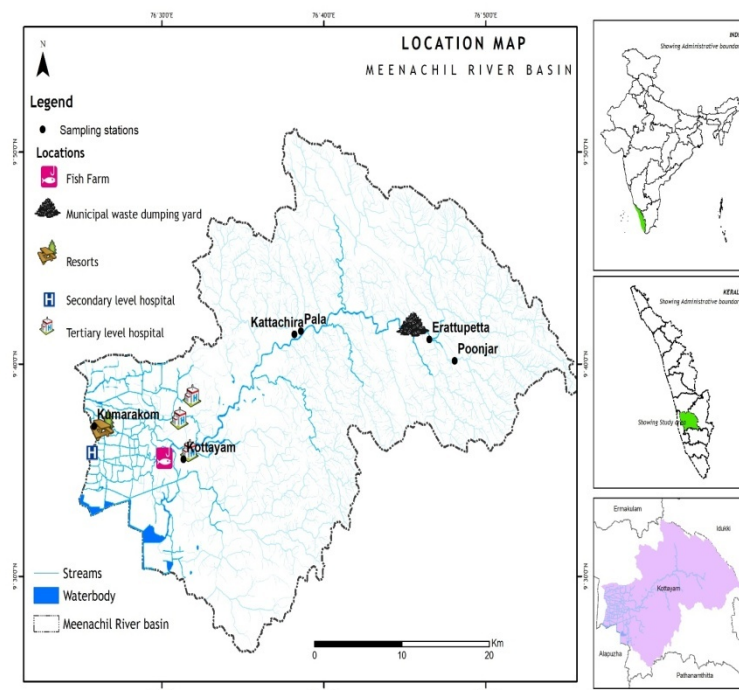
From its source to its confluence with Vembanadu Lake, it is the only river of the Western Ghats to have human settlement along its whole length. Apart from the rampant sand mining that occurred at many locations in the past, the biological and chemical pollution of the river, including the large-scale release of sewage and hospital effluents at different points along its course, is a critical issue causing the present deterioration of its water quality. A study was carried out by [21] to examine the prevalence of pathogenic microorganisms during the pre-monsoon and post-monsoon seasons in the Meenachil river 2015.

As the river is the primary water source for domestic and recreational purposes for the people of Central Kerala, continuous monitoring of river waters is essential to ensure water quality. The study's objective is to evaluate the present status of water contamination from the upstream, midstream, and downstream zones due to microbes and the antimicrobial property of the *E. coli* isolates from Meenachil River, Kerala, India.

## 2. Material and Methods

### 2.1. Study Area

Meenachil River originating from Southern Western Ghats with a length of 78 km, has a basin area of 1272 km<sup>2</sup>, and a watershed extends from a latitude of 9°25' to 9°55' and longitude of 76°20' to 76°55' (Figure 1). The major tributary originates from the northeastern part of the sub-watershed Anakunnumudi at an elevation of +922m above MSL [22]. On the banks of the Meenachil River, major towns and commercial centres of the Kottayam district have come up (Erattupetta, Pala, Ettumanor, and Kottayam) [23]. The river is the prime drinking water source for a large population of the Kottayam district of Kerala.



**Figure 1.** Map showing the sampling stations and the locations of the municipal waste dumping yard, health care stations, and aquaculture units within the immediate vicinity of Meenachil River

## 2.2. Sampling

Samples were collected from the upstream, midstream, and downstream zones of the Meenachil River (Figure 1) during 2019-2020. The upstream sampling stations included Teekoy and Erattupetta. Midstream included Pala, Bharananganam, and Cherpunkal, and the lower stations included Kottayam town and tourism station Kumarakom. Locations were given in the river map with specific mention of the major hospitals, the municipal waste dumping yard, the fish farms, tourist resorts, and pet trading markets nearby. A study was done to evaluate the contamination of water due to microbes and the antimicrobial property of the *E. coli* isolates. Samples were collected in sterile glass bottles and then transported to the lab in a cooler box for further analysis.

### 2.2.1. Processing of Water Samples

#### 2.2.1.1. Estimation of Most Probable Number (MPN) of Faecal Coliforms (FC) in Water Samples

Three dilutions and three-tube replication of lactose broth (Himedia) were used to determine the MPN of total coliforms. 10, 1 and 0.1 ml of appropriately diluted samples were inoculated into respective dilution tubes (10 ml sample in double strength medium of 10ml, 1 and 0.1 ml sample in single strength medium of 10ml each). The tubes were incubated at 44.5°C for 24-48 hrs and examined for growth and gas production. The MPN index was determined by checking the number of positive tubes in each set and comparing the values with the standard MPN table. The values were expressed as the MPN index of faecal coliforms per 100 ml of processing water.

#### 2.2.1.2. Estimation of Most Probable Number (MPN) of Faecal Streptococci (FS) in Water Samples

Three tube dilution method using Azide Dextrose Broth (ADB) (Himedia) as a medium was used to find out the MPN of faecal streptococci. From appropriately diluted samples, three 10ml samples, three 1ml samples three 0.1 ml were inoculated into ADB. 10 ml samples were inoculated into 10 ml double-strength broth, and 1 ml and 0.1 ml samples were inoculated into 10 ml single-strength ADB. The samples were incubated at 37°C for 24-48 hours and checked for turbidity. The tubes showing turbidity were considered as positive presumptive. The MPN index was determined by checking the number of positive tubes in each set and comparing the values with the standard MPN table. The values were expressed as the MPN index of faecal streptococci per 100 ml of processing water.

#### 2.2.1.3. Calculation of FC/FS Ratio

The ratio of faecal coliform (FC) to faecal streptococci (FS) concentrations has been conventionally used to differentiate human from non-human sources of faecal contamination. A ratio of four or greater is considered human faecal contamination, and a ratio of less than 0.7 suggests non-human sources such as duck (0.6), chicken and pig (0.4), and cow (0.2). The FC/FS ratio of the processing wastewater samples was calculated to identify the contamination source.

Representative samples of tubes showing positive results from presumptive MPN tests were streaked onto McConkey agar (HiMedia) and Eosin Methylene Blue (EMB) agar (HiMedia) and incubated at 37°C for 24-48 hours. After incubation, typical colonies (lactose fermenting pink coloured), colonies on Mac Conkey agar, and black colonies on EMB agar were selected to characterize the family Enterobacteriaceae. Typical colonies were isolated, purified, and subjected to IMViC tests.

### 2.2.1.4. Antibiotic Sensitivity Tests

The antibiotics susceptibility test for *Escherichia coli* isolates was done using the disc diffusion method per National Committee for Clinical Laboratory Standards, 1999 (ref). A lawn culture of the bacterial strains was prepared on sterile Muller-Hinton agar plates (Himedia), and antibiotic discs were placed on the surface of the agar. After incubation at 37°C for 24 hrs, the zone of clearance around each antibiotic disc was measured in millimetres. Based on the interpretations from the standard Kirby-Bauer's chart, the strains were classified as sensitive or resistant, with intermediate considered as resistant [24]. The strains were tested against the following antibiotics for in vitro susceptibility: Gentamycin (30 mcg), Vancomycin (30 mcg), Streptomycin (5 mcg), Erythromycin (15 mcg), Ifipenem (10 mcg), Ampicillin (30 mcg), Clindamycin (5 mcg), Tetracycline (30 mcg), Penicillin G (10 mcg) and Chloramphenicol (30 mcg), Cefepime (30 mcg) and Bacitracin (10 mcg).

### 2.2.1.5. Calculation of Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotics resistance (MAR) index is a tool that reveals the spread of bacterial resistance in a given population. MAR index greater than 0.2 implies that the strains of such bacteria originate from environments where several antibiotics are used. MAR index when applied to an isolate is calculated by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics to which the isolate is exposed. This value is particularly useful in the risk assessment of the isolates. The Multiple Antibiotic Resistance (MAR) indices were calculated as follows [25].

*MAR index for isolate*

$$= \frac{\text{Number of antibiotics to which the isolate is resistant}}{\text{Total number of antibiotics tested}}$$

## 3. Results

In the present study, water samples taken from different sampling sites were analyzed for the presence of pollution indicator bacteria such as total coliforms. The FC/FS ratio was calculated in order to identify the source of contamination at different sites. The FC/FS ratio in MPN (Most Probable Number) of faecal coliforms is given in Table 1. Higher ratios were noticed for the midstream and the downstream sites, 3.20 and 4.80, respectively, indicating the presence of contamination from high-risk sources such as human and human-involved domestic wastes (Table 1). Bacterial isolates were biochemically

characterized, and the selected *E. coli* strains were subjected to an antimicrobial susceptibility test to ascertain the risk caused by the isolates in people who use the water for portable and recreational purposes. It was also noticed that the *E. coli* isolates from different stations showed varied antibiotic resistance patterns. Isolates showing resistance to three or more antibiotics were designated as multi-drug resistant (MDR). The multiple antibiotic resistance index of the upstream *E. coli* isolates was found to be 0.33, midstream 0.416, and downstream 0.583. (Figure 2). MAR index value higher than 0.2 is considered to have originated from a high-risk source of contamination. In the present study, isolates from the three river zones showed a MAR index greater than 0.2 indicating that the samples were from highly polluted sources. A higher MAR index of 0.583 was noticed from the downstream station.

**Table 1. Most probable number of Faecal Coliforms (FC) and Faecal Streptococci (FS) in water samples collected from Sampling sites**

| Sampling site | FC/FS ratio |
|---------------|-------------|
| Upstream      | 1.60        |
| Midstream     | 3.20        |
| Downstream    | 4.80        |

**Table 2. Biochemical characterization of isolates from the Sampling sites**

| Sampling site | Isolates                  |
|---------------|---------------------------|
| Midstream     | <i>Klebsiella oxytoca</i> |
| Midstream     | <i>Escherichia. coli</i>  |
| Upstream      | <i>Escherichia. coli</i>  |
| Downstream    | <i>Citrobacter koseri</i> |
| Upstream      | unidentified              |
| Upstream      | unidentified              |
| Midstream     | unidentified              |
| Downstream    | <i>Escherichia. coli</i>  |
| Downstream    | <i>Enterobacter</i>       |
| Downstream    | <i>Proteus vulgaricus</i> |
| Upstream      | <i>Shigella boydii</i>    |

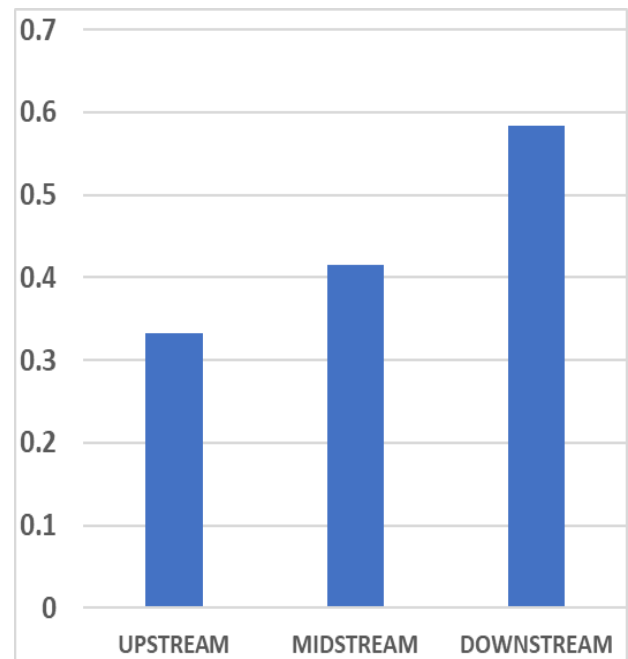
**Table 3. Antibiotic resistance pattern of *E. coli* strains isolated from the Sampling sites**

|                 | Upstream | Midstream | Downstream |
|-----------------|----------|-----------|------------|
| Penicillin      | R        | R         | R          |
| Ampicillin      | R        | R         | R          |
| Erythromycin    | R        | R         | S          |
| Chloramphenicol | S        | S         | S          |
| Cefepime        | R        | R         | R          |
| Clindamycin     | S        | R         | S          |
| Imipenem        | S        | S         | S          |
| Vancomycin      | S        | S         | R          |
| Tetracycline    | S        | S         | S          |
| Streptomycin    | S        | R         | S          |
| Gentamycin      | S        | S         | S          |
| Bacitracin      | R        | R         | S          |

\*R: resistant, S: sensitive.

Biochemical characterization of isolates revealed the predominance of *E. coli* in all the study sites (Table 2). The antibiotic resistance pattern of selected *E. coli* strains isolated in the present study is given in Table 3, and the graphical representation of the calculated multiple antibiotic resistance index (MAR) is given in Figure 2.

The *E. coli* strains isolated downstream of the river revealed considerable resistance against the antibiotics tested in the present study. This is also evident from the high MAR index of 0.58 calculated for the isolates from the downstream area.



**Figure 2. Multiple Antibiotic Resistance Indexes of *E. coli* strains isolated from the Sampling sites**

## 4. Discussion

FC/FS ratio is a traditional method used as a mathematical expression to correlate the relationship between the number of faecal coliforms and the faecal streptococci, assessing the source of contamination. As can be observed from the data (Table 1), the midstream and downstream sampling stations provided the highest FC/FS ratio. This was anticipated because the rivers downstream typically harbor bacterial pathogens flushed out during monsoons from its upstream and midstream urbanized zones with human and animal inhabitation. However, running water may affect the temporal distribution of microorganisms. According to [26], when it rains, the microbial loads in running water can increase and quickly reach the reservoir bodies. According to the observation above, bacterial contamination rises from the upper to lower reaches, as revealed in the FC/FS ratios obtained in the present study. This might result from increased human activity at several locations throughout the lower reaches. Rapid township growth may have contributed to the increased runoff and, to some extent, accelerated the deterioration of the river's water quality in the area surrounding the lower reaches [21].

In the present study, biochemical characterization of the isolates revealed the predominance of *E. coli*. Other faecal coliforms such as *Klebsiella oxytoca*, *Citrobacter koseri*, *Enterobacter sp*, *Proteus vulgaricus*, and *Shigella boydii* were also identified (Table 1). As *E. coli* was consistently isolated from all the samples from the different zones of the river in the present study, given that *E. coli* is an ideal

indicator of faecal pollution. The faecal coliforms and streptococcal count obtained in the present study were in agreement with the study reports by [21]. Since *E. coli* are present in human faeces and the environment, they are used as an indicator to determine the faecal contamination of water (U.S.EPA 2012). Although they are not typically thought of as pathogens, their presence signals the possible presence of pathogens because high *E. coli* levels in recreational waters have been linked to an increased risk of gastrointestinal sickness linked to swimming [27,28]. Even though information about the serotypes of *E. coli* are lacking in the present study, more than 40 serotypes of *E. coli* include potential pathogens such as enterohaemorrhagic *E. coli* (ehec), enteropathogenic *E. coli* (epec), Enterotoxigenic *E. coli* (etec) and Uropathogenic *E. coli* (upec) have been reported [29] from Vembanad lake the confluence zone of Meenachil River. *E. coli* resistance to first-line antibiotics, such as extended-spectrum cephalosporins, is particularly concerning since it makes it more challenging to treat *E. coli* infections effectively (WHO 2014). According to WHO research, these AR *E. coli* are among the nine bacteria that are of global concern since they are one of the most frequent causes of infections in both community and hospital settings globally (WHO 2014) [30,31].

The Multiple Antibiotic Resistance indices obtained (Figure 2) in the present study indicated highly resistant strains of *E. coli* in the water samples analyzed from the lower stretches of Meenachil River, receiving the outlets of secondary and tertiary care hospitals. (Figure 1) shows the location of hospitals in the region. Isolates from the three zones of the river showed a MAR index greater than 0.2 indicating that the samples were from highly polluted sources. A higher MAR index of 0.583 was noticed from the downstream station. Hospitals and all other healthcare institutions are significant generators of antimicrobial waste, directly as wasted discarded medications or indirectly through patient secretions [32]. This may be due to the high concentration of antibiotics in hospital discharges, as reported in previous studies, or 'selection pressure' exerted by the antibiotics, which causes an increase in the existing number of resistant strains. Hospital effluent must undergo adequate treatment before being disposed of in order to successfully eliminate 80–85 percent of antibiotic residues [33]. Unfortunately, only about 45% of Indian healthcare facilities are equipped with efficient wastewater treatment systems.

The downstream of the river is the most affected and highly contaminated zone with tertiary and secondary care hospitals, aquaculture farms, and backwater tourism (Figure 1). Animal excretions can damage the environment indirectly or directly with antimicrobials or resistant organisms [34]. Aquaculture is a rapidly expanding business in India, and the careless use of antimicrobials to boost the output [35] can also serve as an emerging source of environmental AMR. The quantities of antimicrobials in the wastewater from Indian hospitals were so high that they altered bacterial strains and caused genotoxic changes [36]. The detection and isolation of *E. coli* and *Enterococcus* sp. from the lower stretches of the river indicate frequent dumping of sewage from the tourist resorts in the area, which contains pathogenic bacteria in higher concentrations which is detectable.

The temporary retention of water in the lower stretches of the Meenachil River is another serious ecological issue for the increasing microbes in the downstream confluence zone of the river. The shutdown of the salinity barrier, Thanneermukkam Bund in the confluence zone of the river causes a temporary stagnation of the running waters in the lower stretches. The barrage was built in the lake to block saltwater intrusion into the low-lying paddy fields between Vembanad Lake and the Arabian Sea [37]. Every year, the bund is closed for six months to prevent the rivers' water from easily flowing into the Arabian Sea. The transition of lotic to lentic waters interferes with the self-purifying capacity of the river [38] which favours the high prevalence of pathogenic bacteria in the lower stretches of the river with a high influx of sewage.

The consistently high load of *E. coli* and its isolation from the mid and downstream stations indicates that the lower stretches of the Meenachil River are undergoing severe sewage pollution. This is also reported in the studies conducted by [37,39]. The relatively high prevalence of pathogenic bacteria in the lower stretches of the river suggests the high influx of sewage and the excellent survival capabilities of the micro-organisms to the fluctuating hydrologic parameters of the estuarine zone of the river. During the last two decades, the rapid growth of the backwater tourism sector [40] in the downstream station has resulted in the massive discharge of domestic effluents to the river and lake from the houseboats, resorts, and hotels around the region. The persistence of this bacterium in the aquatic environment is a serious issue as most of the population in this region uses this water body for recreational and domestic purposes. The community living near the river banks relies primarily on the Meenachil River system for their drinking water. This is a severe problem since these dangerous bacteria can contaminate drinking water.

## 5. Conclusion

Humans live in the environment, and their health is inextricably linked to both the environment and the health of animals, as stated in the "One Health Approach" framework (One Health Commission, 2019; Walton, 2019). A growing concern for the public's health is the rise in antibiotic resistance in bacteria which are significant human pathogens, as well as the transfer of resistance outside of hospitals and into open populations. The threat of AMR could be efficiently controlled by reducing the discharge of antibiotic residues into the environment, particularly water bodies, as well as by improving surveillance methods and developing effective treatment, removal, and decomposition techniques. The public's participation in local management and disposal of antimicrobials is still a promising area for cooperation and policy development to address the AMR epidemic since it might raise awareness and foster a sense of responsibility.

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## Conflict of Interest

The author declares that they have no conflict of interest.

## References

- [1] Rendón, O. R., Garbutt, A., Skov, M., Möller, I., Alexander, M., Ballinger, R., ... & Beaumont, N. “A framework linking ecosystem services and human well-being: Saltmarsh as a case study”. *People and nature*, 1(4), 486-496, 2019.
- [2] Ma, Y., Li, M., Wu, M., Li, Z., & Liu, X. “Occurrences and regional distributions of 20 antibiotics in water bodies during groundwater recharge.” *Science of the Total Environment* 518, 498-506, 2015.
- [3] Wang, J., Chu, L., Wojnárovits, L., & Takács, E. “Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: An overview.” *Science of the Total Environment* 744, 140997, 2020.
- [4] Zhuang, M., Achmon, Y., Cao, Y., Liang, X., Chen, L., Wang, H., ... & Leung, K. Y. “Distribution of antibiotic resistance genes in the environment.” *Environmental Pollution* 285, 117402, 2021.
- [5] Kümmerer, K. “Resistance in the environment.” *Journal of antimicrobial chemotherapy* 54, no. 2, 311-320, 2004.
- [6] Poté, J., Haller, L., Loizeau, J. L., Bravo, A. G., Sastre, V., & Wildi, W. “Effects of a sewage treatment plant outlet pipe extension on the distribution of contaminants in the sediments of the Bay of Vidy, Lake Geneva, Switzerland.” *Bioresource Technology* 99, no. 15, 7122-7131, 2008.
- [7] Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J., & Handelsman, J. “Call of the wild: antibiotic resistance genes in natural environments.” *Nature Reviews Microbiology* 8, no. 4, 251-259, 2010.
- [8] Charuau, L., Jardé, E., Jaffrézic, A., Thomas, M. F., & Le Bot, B. “Veterinary pharmaceutical residues from natural water to tap water: sales, occurrence and fate.” *Journal of hazardous materials* 361, 169-186, 2019.
- [9] Grenni, P., Ancona, V., & Caracciolo, A. B. “Ecological effects of antibiotics on natural ecosystems: A review.” *Microchemical Journal* 136, 25-39, 2018.
- [10] Marti, E., Variatza, E., & Balcazar, J. L. “The role of aquatic ecosystems as reservoirs of antibiotic resistance.” *Trends in microbiology* 22, no. 1, 36-41, 2014.
- [11] Nnadozie, C. F., & Odume, O. N. “Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes.” *Environmental Pollution* 254, 113067, 2019.
- [12] Ram, S., Vajpayee, P., Tripathi, U., Singh, R. L., Seth, P. K., & Shanker, R. Determination of antimicrobial resistance and virulence gene signatures in surface water isolates of *Escherichia coli*. *Journal of applied microbiology*, 105(6), 1899-1908 2008.
- [13] Sidhu, J. P., Ahmed, W., Hodggers, L., & Toze, S. “Occurrence of virulence genes associated with diarrheagenic pathotypes in *Escherichia coli* isolates from surface water.” *Applied and environmental microbiology* 79, no. 1, 328-335, 2013.
- [14] Titilawo, Y., Obi, L., & Okoh, A. “Occurrence of virulence gene signatures associated with diarrhoeagenic and non-diarrhoeagenic pathovars of *Escherichia coli* isolates from some selected rivers in South-Western Nigeria.” *BMC microbiology* 15, no. 1, 1-14, 2015.
- [15] Akiba, M., Senba, H., Otagiri, H., Prabhasankar, V. P., Taniyasu, S., Yamashita, N., ... & Guruge, K. S. “Impact of wastewater from different sources on the prevalence of antimicrobial-resistant *Escherichia coli* in sewage treatment plants in South India.” *Ecotoxicology and environmental safety* 115, 203-208, 2015.
- [16] Nyirabahizi, E., Tyson, G. H., Dessai, U., Zhao, S., Kabera, C., Crarey, E., ... & Tate, H. “Evaluation of *Escherichia coli* as an indicator for antimicrobial resistance in *Salmonella* recovered from the same food or animal ceca samples.” *Food Control* 115, 107280, 2020.
- [17] Huddleston, J. R. “Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes.” *Infection and drug resistance* 7, 167, 2014.
- [18] Skariyachan, S., Mahajanakatti, A. B., Grandhi, N. J., Prasanna, A., Sen, B., Sharma, N., ... & Narayanappa, R. “Environmental monitoring of bacterial contamination and antibiotic resistance patterns of the fecal coliforms isolated from Cauvery River, a major drinking water source in Karnataka, India.” *Environmental monitoring and assessment* 187, no. 5, 1-13, 2015.
- [19] Devarajan N, Laffite A, Ngelikoto P, Elongo V, Prabakar K, Mubedi JI, et al. “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, 12941-12950, 2015.
- [20] Mubedi, J. I., Devarajan, N., Le Faucheur, S., Mputu, J. K., Atibu, E. K., Sivalingam, P., ... & Poté, J. “Effects of untreated hospital effluents on the accumulation of toxic metals in sediments of receiving system under tropical conditions: case of South India and Democratic Republic of Congo.” *Chemosphere* 93, no. 6, 1070-1076, 2013.
- [21] Vincy, M. V., Brilliant, R., & Pradeepkumar, A. P. Prevalence of indicator and pathogenic bacteria in a tropical river of Western Ghats, India. *Applied Water Science*, 7(2), 833-844, May 2017.
- [22] Kerala State Land Use Board [KSLUB], 1996, National resource data bank, Kottayam 2013.
- [23] Nair, I., Singh, K., Arumugam, M., & Clarson, D. Monitoring of trace metal pollution in Meenachil River at Kottayam, Kerala (India). *Journal of Chemistry*, 8, 10.1155/2011/952040, May 2011.
- [24] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.*; 45(4): 493-6. PMID: 5325707 1966 Apr.
- [25] Krumperman, P. H. “Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods.” *Applied and environmental microbiology* 46, no. 1, 165-170, 1983.
- [26] Kistemann, T., Claßen, T., Koch, C., Dangendorf, F., Fischeder, R., Gebel, J., ... & Exner, M. “Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff.” *Applied and environmental microbiology* 68, no. 5, 2188-2197, 2002.
- [27] Dufour, A. P. “Health effects criteria for fresh recreational waters.”, 1984.
- [28] Wade, T.J., Pai, N., Eisenberg, J.N.S. and Colford, J.M. Do US Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. *Environ Health Perspect* 111, 1102-1109, 2003.
- [29] Hatha, A. A., Chandran, A., & Rahiman, K. M. “Prevalence of diarrheagenic serotypes of *Escherichia coli* in the Cochin estuary, along west coast of India.”, 2004.
- [30] Fairbrother, J. M., & Nadeau, E. “*Escherichia coli*: on-farm contamination of animals.” *Rev Sci Tech* 25, no. 2, 555-69, 2006.
- [31] Somarelli, J. A., Makarewicz, J. C., Sia, R., & Simon, R. “Wildlife identified as major source of *Escherichia coli* in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints.” *Journal of environmental management* 82, no. 1, 60-65, 2007.
- [32] Mutiyar, P. K., & Mittal, A. K. “Risk assessment of antibiotic residues in different water matrices in India: key issues and challenges.” *Environmental Science and Pollution Research* 21, no. 12, 7723-7736, 2014.
- [33] Duong HA, Pham NH, Nguyen HT, Hoang TT, Pham HV, Pham VC, et al. Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam. *Chemosphere*; 72: 968-73 2008.
- [34] Wichmann, F., Udikovic-Kolic, N., Andrew, S., & Handelsman, J. “Diverse antibiotic resistance genes in dairy cow manure.” *MBio* 5, no. 2, e01017-13, 2014.
- [35] Taneja, N., & Sharma, M. “Antimicrobial resistance in the environment: The Indian scenario.” *Indian Journal of Medical Research* 149, no. 2, 119-128, 2019.
- [36] Diwan V, Tamhankar AJ, Khandal RK, Sen S, Aggarwal M, Marothi Y, et al. Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. *BMC Public Health*, 10: 414, 2010.

- [37] Padmakumar, K.G., Santhosh, N.K., Shanu, R., Rakesh, C.G., Bindusha, K.B. & Jasmine, B. A study on the fish catch from Vembanad lake, south of Thannermukkom barrage during the period from March 2011 to February 2012, *Natl. Sem., Biodiv. Farming Syst. Wetland ecology*, 22-23, 35-36, 2017.
- [38] Yustiani, Y. M., Nurkanti, M., Suliasih, N., & Novantri, A. Influencing parameter of self purification process in the urban area of Cikapundung River, Indonesia. *GEOMATE Journal*, 14(43), 50-54, 2018.
- [39] Cherian LP, Oommen M. Post-flood changes in the fish fauna of Meenachil river, Kerala South India. *Journal of Aquatic Biology & Fisheries*. Novembe, 8:53-61, 2020.
- [40] Remani, K. N., Jayakumar, P., & Jalaja, T. K. "Environmental problems and management aspects of Vembanad kol wetlands in South West coast of India." *Nature, Environment and Pollution Technology* 9, no. 2, 247-254, 2010.



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