



Distribution And Antibiotic Resistance Profiles Of Faecal Indicator Bacteria In The Ganga River At Kanpur, Uttar Pradesh

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ABSTRACT

The Ganga River is a vital freshwater resource supporting millions of people for domestic, agricultural, religious, and industrial purposes. However, increasing urbanization and inadequate wastewater management have led to severe microbial pollution, particularly in major cities such as Kanpur, Uttar Pradesh. The present study assessed faecal contamination and antibiotic resistance patterns among indicator bacteria isolated from the Ganga River along a 20 km urban stretch in Kanpur. Water samples were collected from five strategically selected sites representing varying degrees of anthropogenic influence, including upstream reference, bathing ghats, sewage discharge zones, industrial effluent-affected areas, and downstream locations. Faecal indicator bacteria, namely *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*, were isolated using membrane filtration and selective culture techniques. Antibiotic susceptibility testing was carried out using the disc diffusion method against commonly used antibiotics. All sampling sites showed the presence of faecal indicator bacteria, confirming widespread faecal contamination of the river. The highest bacterial densities were recorded at sites receiving direct municipal sewage discharge, while comparatively lower counts were observed at the upstream reference site. Antibiotic resistance was prevalent among all bacterial groups, with high resistance observed against ampicillin, amoxicillin, tetracycline, and cefotaxime. A substantial proportion of isolates exhibited resistance to multiple antibiotics, particularly those recovered from sewage- and industrially impacted sites. Lower resistance levels were recorded for ciprofloxacin and gentamicin. The findings indicate that the Ganga River at Kanpur acts as a reservoir and dissemination pathway for antibiotic-resistant bacteria. Continuous discharge of untreated sewage and antibiotic residues into the river poses significant risks to public health and aquatic ecosystems. The study emphasizes the need for improved wastewater treatment, rational antibiotic usage, and regular monitoring of microbial water quality to mitigate the spread of antibiotic resistance in riverine environments.

Keywords: Antibiotic resistance; faecal indicator bacteria; Ganga River; sewage pollution; surface water quality

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INTRODUCTION

Freshwater ecosystems are under increasing pressure worldwide due to intensified anthropogenic activities such as urbanization, industrialization, agricultural expansion, and population growth (UNESCO, 2020). In many developing countries, the pace of urban development often exceeds the capacity of sanitation and wastewater treatment infrastructure, resulting in the direct discharge of untreated or partially treated sewage into nearby rivers and streams (Kumar et al., 2019). Such practices significantly degrade water quality and transform rivers into reservoirs of pathogenic microorganisms and antibiotic-resistant bacteria, thereby posing serious threats to public health and aquatic biodiversity (WHO, 2017).

Rivers receiving continuous inputs of domestic sewage and industrial effluents provide favorable conditions for the survival, proliferation, and dissemination of faecal bacteria and associated resistance determinants (Marti et al., 2014). Antibiotics consumed by humans and animals are often excreted in unmetabolized or partially metabolized forms and enter aquatic environments through sewage, hospital wastewater, pharmaceutical manufacturing effluents, and agricultural runoff (Kümmerer, 2009). The presence of these antimicrobial residues in surface waters creates selective pressure that promotes the persistence and spread of antibiotic-resistant bacteria in natural ecosystems (Baquero, Martínez, & Cantón, 2008).

Faecal indicator bacteria such as *Escherichia coli*, *Enterococcus* spp., and *Klebsiella* spp. are commonly employed to assess microbial water quality and the extent of faecal pollution in freshwater systems (APHA, 2017). These bacteria originate primarily from the gastrointestinal tract of humans and other warm-blooded animals, and their detection in surface waters indicates contamination by human or animal excreta (Edberg et al., 2000). Beyond serving as indicators, many of these organisms are also opportunistic pathogens capable of causing gastrointestinal, urinary tract, and systemic infections, particularly in immunocompromised individuals (Leclerc, Mossel, Edberg, & Struijk, 2001).

In recent years, increasing attention has been directed toward antibiotic resistance among faecal indicator bacteria, as these organisms can act as reservoirs and vectors of resistance genes in aquatic environments (Rizzo et al., 2013). Resistance genes carried by indicator bacteria may be transferred to pathogenic bacteria through horizontal gene transfer mechanisms

such as conjugation, transformation, and transduction, thereby amplifying the public health risk associated with contaminated water bodies (Aminov, 2011). Studies have shown that surface waters impacted by urban sewage often harbor bacteria resistant to multiple classes of antibiotics, including β -lactams, tetracyclines, and fluoroquinolones (Blaak et al., 2015).

The Ganga River, one of the most important and culturally revered rivers in India, supports millions of people for drinking, bathing, irrigation, and industrial activities (Dwivedi et al., 2018). As the river flows through major urban centers, it receives enormous quantities of untreated domestic sewage and industrial effluents, leading to severe deterioration of its water quality (Central Pollution Control Board, 2021). Microbial pollution has been identified as one of the most critical challenges affecting the ecological and sanitary status of the Ganga, particularly in densely populated cities (Kumar et al., 2020).

Kanpur, a major industrial hub in Uttar Pradesh, is known for its extensive leather tanning industry, which discharges large volumes of wastewater along with domestic sewage into the Ganga River (Trivedi et al., 2010). The combined input of municipal sewage, tannery effluents, hospital waste, and urban runoff introduces not only faecal bacteria but also antibiotic residues and heavy metals into the river system (Singh, Rai, & Mishra, 2018). Continuous exposure of aquatic microbial communities to such contaminants may facilitate the selection and persistence of antibiotic-resistant strains (Zhang et al., 2015).

Despite the recognized importance of microbial contamination in the Ganga River, limited information is available on the antibiotic resistance profiles of faecal indicator bacteria in the Kanpur stretch. Understanding resistance patterns in these bacteria is essential for evaluating environmental health risks and developing effective water management strategies (Berendonk et al., 2015). Therefore, the present study aimed to isolate faecal indicator bacteria from the Ganga River at Kanpur and assess their resistance patterns against commonly used antibiotics, thereby contributing to the growing body of evidence on environmental antibiotic resistance.

MATERIALS AND METHODS

Study area and sampling sites The study was carried out along an approximately 20 km stretch of the River Ganga flowing through Kanpur city, Uttar Pradesh,

India. Kanpur is a densely populated urban and industrial center located on the southern bank of the river and is known for extensive domestic sewage discharge and industrial activities, particularly leather tanning. The selected river stretch represents a gradient of anthropogenic influence, ranging from relatively less disturbed upstream regions to highly polluted downstream sections.

Five sampling sites were selected based on the intensity of human activities, sewage inflow, and industrial effluent discharge. Site I was located upstream of the city and served as a reference point with comparatively lower anthropogenic impact. Site II was situated near a major urban bathing ghat frequently used for religious and recreational purposes. Site III was located at a point receiving direct municipal sewage discharge through open drains. Site IV was selected near an area affected by industrial effluents, primarily from tannery and small-scale industrial units. Site V was located downstream of the city, representing the cumulative impact of urban and industrial pollution. Geographic coordinates of each site were recorded, and sampling locations were kept consistent throughout the study period.

Sample collection: Water sampling was conducted during the pre-monsoon season to minimize dilution effects caused by rainfall and to capture peak pollution levels. At each site, triplicate surface water samples were collected in sterile 500 ml borosilicate glass bottles. Prior to sampling, bottles were rinsed with river water at the respective site to equilibrate temperature conditions, taking care to avoid contamination. Samples were collected approximately 30 cm below the water surface while facing upstream to prevent interference from disturbed sediments.

All samples were clearly labeled with site code, date, and time of collection. Immediately after collection, samples were stored in an insulated ice box maintained at approximately 4°C and transported to the laboratory. Microbiological analysis was initiated within 6 hours of collection to ensure sample integrity and to prevent changes in bacterial population density.

Isolation and identification of indicator bacteria: The membrane filtration technique was used for the isolation and enumeration of faecal indicator bacteria. Depending on the expected bacterial load, appropriate volumes of water samples (10–100 ml) were filtered through sterile

cellulose nitrate membrane filters with a pore size of 0.45 µm using a vacuum filtration unit. After filtration, membranes were aseptically transferred onto selective and differential culture media.

Eosin Methylene Blue agar was used for the isolation of *Escherichia coli*, where characteristic colonies showing a metallic green sheen were considered presumptive *E. coli*. m-Enterococcus agar was used for the selective isolation of *Enterococcus faecalis*, with typical red or maroon colonies recorded as presumptive enterococci. MacConkey agar was employed for the isolation of *Klebsiella pneumoniae*, identified by large, mucoid, lactose-fermenting pink colonies.

All plates were incubated at 37°C for 24–48 hours. Representative colonies from each medium were sub-cultured to obtain pure cultures. Identification was further confirmed by Gram staining and standard biochemical tests, including indole production, methyl red, Voges–Proskauer, citrate utilization, catalase test, oxidase test, and sugar fermentation tests. Confirmed isolates were preserved on nutrient agar slants at 4°C for further analysis.

Antibiotic susceptibility testing: Antibiotic susceptibility of the confirmed isolates was determined using the Kirby–Bauer disc diffusion method. Pure cultures were grown in nutrient broth and adjusted to match the turbidity of 0.5 McFarland standard. Sterile cotton swabs were used to evenly spread the bacterial suspension over the surface of Mueller–Hinton agar plates to form a uniform bacterial lawn.

Commercially available antibiotic discs were placed aseptically on the inoculated plates using sterile forceps. The antibiotics tested included ampicillin (10 µg), amoxicillin (20 µg), tetracycline (30 µg), chloramphenicol (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), and gentamicin (10 µg). Plates were incubated at 37°C for 18–24 hours.

After incubation, the diameter of inhibition zones around each disc was measured in millimeters. The susceptibility or resistance of isolates was determined based on standard clinical laboratory interpretative criteria. Isolates showing resistance to three or more antibiotics were classified as multiple antibiotic resistant.

Statistical analysis: Bacterial counts were expressed as colony-forming units per 100 ml of water (CFU/100 ml). Mean values and standard deviations were

calculated from triplicate samples for each site. Antibiotic resistance was expressed as the percentage of resistant isolates for each antibiotic tested. Descriptive statistical analysis was performed to compare bacterial abundance and resistance patterns across different sampling sites and to identify spatial trends associated with pollution intensity along the river stretch.

Here is an expanded, well-structured Results section written in a clear scientific narrative suitable for a research journal. The data you provided have been integrated smoothly, and trends are explained without interpretation drifting into discussion.

RESULTS

Occurrence of faecal indicator bacteria: Faecal indicator bacteria were detected in all water samples collected from the five sampling sites along the Ganga River in Kanpur, indicating widespread faecal contamination throughout the studied river stretch. Among the three indicator organisms examined, *Escherichia coli* was the most frequently isolated species at all sites, followed by *Enterococcus faecalis* and *Klebsiella pneumoniae*. The abundance of these organisms varied considerably across sampling locations, reflecting differences in the intensity of anthropogenic inputs and sewage discharge.

The lowest bacterial counts were recorded at Site I, the upstream reference location, suggesting comparatively lower faecal pollution at this site. In contrast, the highest densities of all three indicator bacteria were observed at Site III, which receives direct municipal sewage input. Elevated bacterial counts were also observed at Site IV and Site V, indicating the cumulative impact of urban and industrial activities downstream of the city.

Mean bacterial counts at different sites: Mean bacterial counts, expressed as colony-forming units per 100 ml of water, showed a clear increasing trend from the upstream site toward sewage-impacted locations, followed by a slight decline at the downstream site.

At Site I, mean counts of *E. coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* were 180 CFU/100 ml, 95 CFU/100 ml, and 70 CFU/100 ml, respectively. At Site II, located near an urban bathing ghat, bacterial counts increased markedly, with *E. coli* reaching 420 CFU/100 ml, *Enterococcus faecalis* 260 CFU/100 ml, and *Klebsiella pneumoniae* 190 CFU/100 ml.

Site III exhibited the highest level of faecal contamination among all sampling locations. Mean counts at this site were 980 CFU/100 ml for *E. coli*, 620 CFU/100 ml for *Enterococcus faecalis*, and 510 CFU/100 ml for *Klebsiella pneumoniae*. At Site IV, which is influenced by industrial effluents, bacterial densities remained high, with *E. coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* recorded at 760 CFU/100 ml, 480 CFU/100 ml, and 390 CFU/100 ml, respectively.

At the downstream Site V, bacterial counts showed a moderate reduction compared to Site III and Site IV; however, levels remained substantially higher than those observed at the upstream site. Mean counts at Site V were 540 CFU/100 ml for *E. coli*, 310 CFU/100 ml for *Enterococcus faecalis*, and 260 CFU/100 ml for *Klebsiella pneumoniae*.

Antibiotic resistance pattern: A total of 120 bacterial isolates were subjected to antibiotic susceptibility testing, comprising 50 *Escherichia coli* isolates, 40 *Enterococcus faecalis* isolates, and 30 *Klebsiella pneumoniae* isolates. Resistance to one or more antibiotics was observed in a large proportion of isolates across all three bacterial groups. Overall, the highest levels of resistance were recorded against commonly used antibiotics, particularly ampicillin, amoxicillin, and tetracycline.

Resistance profiles differed slightly among bacterial species, but a consistent pattern of higher resistance to β -lactam antibiotics and tetracycline was evident. In contrast, relatively lower resistance was observed for ciprofloxacin and gentamicin across all isolates.

Percentage resistance of isolates to antibiotics:

Among *Escherichia coli* isolates, resistance to ampicillin was observed in 82% of isolates, followed by amoxicillin at 78% and tetracycline at 64%. Resistance to cefotaxime was recorded in 56% of isolates, while chloramphenicol resistance was observed in 38%. Lower resistance levels were noted for ciprofloxacin (22%) and gentamicin (18%).

Enterococcus faecalis isolates also showed high resistance to ampicillin (70%), amoxicillin (68%), and tetracycline (60%). Resistance to cefotaxime and chloramphenicol was observed in 42% and 35% of isolates, respectively. Ciprofloxacin and gentamicin resistance among enterococci was comparatively low, at 20% and 15%, respectively.

Klebsiella pneumoniae exhibited the highest resistance levels among the three indicator bacteria. Resistance to ampicillin and amoxicillin was recorded in 88% and 84% of isolates, respectively. Tetracycline resistance was observed in 66% of isolates, while resistance to cefotaxime and chloramphenicol was recorded in 62% and 40%, respectively. Ciprofloxacin and gentamicin resistance was detected in 25% and 20% of isolates (Table 1).

Multiple antibiotic resistance: Multiple antibiotic resistance was prevalent among faecal indicator bacteria

isolated from the Ganga River. Resistance to three or more antibiotics was detected in 68% of *Escherichia coli* isolates, 55% of *Enterococcus faecalis* isolates, and 72% of *Klebsiella pneumoniae* isolates. The highest frequency of multiple antibiotic resistance was observed in isolates recovered from Site III and Site IV, corresponding to areas receiving direct sewage and industrial effluent inputs. This spatial pattern indicates a strong association between pollution intensity and the occurrence of resistant bacterial populations in the river.

Table 1. The table showing dataset of faecal indicator bacterial abundance and antibiotic resistance profile in the Ganga River at Kanpur, Uttar Pradesh

Parameter	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i>	<i>Klebsiella pneumoniae</i>
Number of isolates tested (n)	50	40	30
Mean bacterial count (CFU/100 ml)			
Site I (Upstream reference)	180	95	70
Site II (Urban bathing ghat)	420	260	190
Site III (Sewage discharge zone)	980	620	510
Site IV (Industrial effluent area)	760	480	390
Site V (Downstream site)	540	310	260
Antibiotic resistance (%)			
Ampicillin (10 µg)	82	70	88
Amoxicillin (20 µg)	78	68	84
Tetracycline (30 µg)	64	60	66
Chloramphenicol (30 µg)	38	35	40
Cefotaxime (30 µg)	56	42	62
Ciprofloxacin (5 µg)	22	20	25
Gentamicin (10 µg)	18	15	20
Multiple antibiotic resistant isolates (%)	68	55	72

DISCUSSION

The present study demonstrates widespread faecal contamination of the Ganga River along the Kanpur stretch, as evidenced by the consistent detection and system (APHA, 2017; Kumar et al., 2019). The marked increase in bacterial density from the upstream reference site to sewage-impacted locations reflects the

high abundance of faecal indicator bacteria across all sampling sites. Elevated counts of *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*, particularly at sites receiving direct sewage discharge, clearly indicate continuous inputs of untreated or inadequately treated domestic wastewater into the river strong influence of urban activities on microbial water quality (Dwivedi et al., 2018).

The highest bacterial loads recorded at the sewage discharge zone are consistent with observations from

other urban stretches of the Ganga and similar Indian rivers, where municipal wastewater has been identified as the primary source of faecal pollution (Central Pollution Control Board, 2021; Singh et al., 2018). The persistence of high bacterial counts at downstream sites further suggests limited self-purification capacity of the river in heavily urbanized areas, likely due to reduced flow velocity, organic load accumulation, and continuous pollutant input (Kumar et al., 2020). The presence of faecal indicator bacteria even at the upstream site indicates background contamination, possibly arising from diffuse sources such as open defecation, agricultural runoff, and upstream settlements (Edberg et al., 2000).

Beyond microbial abundance, the antibiotic resistance patterns observed among indicator bacteria raise serious environmental and public health concerns. High resistance to β -lactam antibiotics such as ampicillin and amoxicillin across all three bacterial groups suggests prolonged and widespread exposure of riverine bacteria to these commonly prescribed drugs (Baquero et al., 2008). These antibiotics are extensively used in human medicine in India and are frequently detected in municipal sewage and hospital effluents, which often enter surface waters without adequate treatment (Kümmerer, 2009; Rizzo et al., 2013). Resistance to tetracycline, another widely used antibiotic in both human therapy and animal husbandry, further supports the role of anthropogenic antibiotic inputs in shaping resistance profiles in aquatic environments (Zhang et al., 2015).

The detection of substantial resistance to third-generation cephalosporins such as cefotaxime among isolates is particularly concerning, as it may indicate the presence of extended-spectrum β -lactamase-producing bacteria in the river ecosystem (Blaak et al., 2015). Such resistance traits are often plasmid-mediated and can be rapidly disseminated among bacterial populations through horizontal gene transfer (Aminov, 2011). In contrast, relatively lower resistance levels to ciprofloxacin and gentamicin observed in the present study may reflect comparatively restricted environmental persistence, lower usage in the local setting, or higher bactericidal efficiency of these antibiotics in aquatic conditions (Berendonk et al., 2015).

The high prevalence of multiple antibiotic resistance among faecal indicator bacteria further underscores the role of the Ganga River as a reservoir and conduit for

resistance determinants. The greater frequency of multiple antibiotic-resistant isolates at sewage- and industrially impacted sites suggests a strong association between pollution intensity and the selection of resistant bacterial populations (Marti et al., 2014). Riverine environments receiving complex mixtures of antibiotics, disinfectants, heavy metals, and organic pollutants provide ideal conditions for co-selection of resistance traits, even in the absence of high antibiotic concentrations (Baquero et al., 2008; Zhang et al., 2015).

From a public health perspective, the presence of antibiotic-resistant faecal indicator bacteria in river water is of particular concern for communities that rely on the Ganga for bathing, irrigation, religious activities, and, in some cases, drinking water after minimal treatment (WHO, 2017). Direct human contact with contaminated water increases the risk of exposure to resistant bacteria, while irrigation with polluted river water may facilitate the transfer of resistance genes into agricultural soils and food crops (Berendonk et al., 2015). Thus, the findings of this study reinforce growing evidence that surface waters in urban regions play a critical role in the environmental dissemination of antibiotic resistance.

CONCLUSION

The present study clearly demonstrates that the Ganga River along the Kanpur stretch is heavily contaminated with faecal indicator bacteria, reflecting sustained inputs of untreated domestic sewage and urban effluents. The consistently high densities of *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*, particularly at sewage- and industrially impacted sites, indicate severe deterioration of microbial water quality and highlight the limited self-purification capacity of the river in densely populated urban settings.

In addition to microbial contamination, the widespread occurrence of antibiotic resistance among indicator bacteria presents a serious environmental and public health concern. High resistance to commonly used antibiotics and the substantial prevalence of multiple antibiotic resistance suggest that the river acts as a reservoir and dissemination pathway for resistance determinants. Such conditions increase the risk of human exposure to resistant bacteria through direct contact with river water and indirect pathways such as irrigation and food production.

These findings emphasize the urgent need for strengthening sewage treatment infrastructure, ensuring effective management of industrial and hospital effluents, and promoting rational antibiotic use to reduce environmental loading of antimicrobial agents. Regular surveillance of faecal indicator bacteria and their resistance profiles should be integrated into river water quality monitoring programs. Protecting the Ganga River from microbial and antibiotic pollution is essential not only for restoring ecological integrity but also for safeguarding public health and ensuring the sustainable use of this vital freshwater resource.

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