

Study of Microbial Contamination of River Yamuna in District Baghpat and Auraiya (UP)

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www.jrasb.com || Vol. 3 No. 1 (2024): February Issue

Received: 07-02-2024

Revised: 20-02-2024

Accepted: 27-02-2024

ABSTRACT

Microbial contamination of River Yamunain Baghpat and Auraiya district, Uttar Pradesh, India, presents a significant public health challenge due to fecal pollution from untreated sewage and agricultural runoff. This study evaluated water quality across five stations—Kurdi, KakorKalan, Katha, ShergarhGhat, and Veejalpur (Near Ayana)—using Colilert® technology and the Most Probable Number (MPN) method to detect and quantify coliforms and *Escherichia coli* (*E. coli*) as fecal contamination indicators. Sampling stations were selected to represent a gradient of anthropogenic impacts in these rural and peri-urban areas. Water samples (100 mL) were collected in sterile conditions, maintained at 4°C, and analyzed within 6 hours per standardized guidelines. Colilert® results confirmed coliform presence at all stations, with *E. coli* detected at Kurdi, KakorKalan, ShergarhGhat, and Veejalpur, but absent at Katha, indicating site-specific fecal inputs. MPN analysis revealed total coliforms ranging from 1200 (Katha) to 2000 (ShergarhGhat) MPN/100 mL, and *E. coli* from 0 (Katha) to 1100 (ShergarhGhat) MPN/100 mL. ShergarhGhat exceeded the severe risk threshold (>1000 MPN/100 mL *E. coli*), classifying it as very high risk, while Kurdi and KakorKalan were high risk (900–1000 MPN/100 mL *E. coli*), Veejalpur moderate-to-high, and Katha moderate. These findings align with prior observations linking elevated microbial loads to sewage and runoff, with Katha's *E. coli* absence suggesting natural attenuation. Recommendations include routine Colilert® and MPN monitoring, pollution source control through engineered solutions, enhanced sanitation infrastructure with decentralized treatment systems, and investigation of Katha's attenuation factors for potential replication. Immediate interventions targeting high-risk stations, alongside sustainable practices such as eco-friendly agriculture and proper waste disposal, and regulatory enforcement, are essential to mitigate health risks and improve water quality in these districts, safeguarding public health and aquatic ecosystems.

Keywords- Microbial Contamination, Water Quality, Fecal Pollution, *E. coli*.

I. INTRODUCTION

Water quality degradation due to microbial contamination is a pressing global challenge, threatening human health, ecological stability, and economic sustainability (Bain *et al.*, 2014). Worldwide, waterborne diseases such as diarrhea, typhoid, and cholera claim millions of lives annually, with an estimated 1.8 million deaths linked to unsafe water in low- and middle-income countries alone (World Health Organization [WHO], 2017). Central to this issue is fecal pollution, which introduces pathogenic bacteria, viruses, and protozoa into water bodies through untreated sewage, agricultural runoff, and urban stormwater discharge (Ashbolt *et al.*,

2001). Among the most reliable indicators of such contamination are coliform bacteria and *Escherichia coli* (*E. coli*), whose presence signals potential health risks due to their association with fecal matter and enteric pathogens (Leclerc *et al.*, 2001).

Coliforms, a group of gram-negative, rod-shaped bacteria, thrive in diverse environments, including soil, water, and the gastrointestinal tracts of warm-blooded animals, making them broad markers of environmental pollution (Tallonet *et al.*, 2005). *E. coli*, a thermotolerant subset of coliforms, is more specific, predominantly originating from human and animal feces and rarely persisting outside these hosts (Fujioka, 2001). Their detection in water is a critical step in assessing

sanitary quality, as elevated levels often correlate with the presence of pathogens like *Salmonella* and *Shigella* (Cabral, 2010). In regions with limited sanitation infrastructure, such as rural or peri-urban areas, water bodies frequently serve as sinks for fecal waste, amplifying health risks for communities reliant on them for drinking, irrigation, or recreation (Payment *et al.*, 2000).

This study evaluates microbial contamination across five sampling stations—Kurdi, KakorKalan, Katha, ShergarhGhat, and Veejalpur (Near Ayana)—located along of River Yamuna potentially impacted by varying pollution sources. These sites were selected to represent a gradient of anthropogenic influence, from agricultural runoff to urban sewage inputs, reflecting common contamination scenarios in developing regions (Solo-Gabriele *et al.*, 2000). To achieve a comprehensive assessment, we employed Colilert® technology, a rapid and sensitive method for detecting coliforms and *E. coli* through enzymatic reactions, and the Most Probable Number (MPN) method, a statistically robust approach for quantifying bacterial density (Frickeret *al.*, 1997; Garthright& Blodgett, 2003). The primary objectives are threefold: (1) to confirm the presence of coliforms and *E. coli*, (2) to quantify their concentrations across the stations, and (3) to evaluate pollution levels and sanitary risks to inform water management strategies.

Given the escalating pressure on freshwater resources due to population growth, industrialization, and climate variability, understanding microbial pollution dynamics is more urgent than ever (Rose *et al.*, 2001). This research aims to provide actionable data to mitigate health risks, protect aquatic ecosystems, and support sustainable water use in contaminated regions. By integrating advanced diagnostic tools with field-based analysis, the study contributes to the growing body of evidence guiding water quality interventions worldwide.

II. REVIEW OF LITERATURE

The application of coliform bacteria and *Escherichia coli* (*E. coli*) as indicators of water quality is a cornerstone of environmental microbiology, grounded in their strong association with fecal contamination and potential pathogenic organisms (Tallonet *al.*, 2005). Total coliforms, a heterogeneous group of gram-negative bacteria, serve as broad indicators of environmental pollution, encompassing species found in soil, water, and the gastrointestinal tracts of warm-blooded animals (Leclerc *et al.*, 2001). In contrast, *E. coli* is a more precise marker of recent fecal input, as its survival outside mammalian hosts is limited due to its sensitivity to environmental stressors like UV radiation and desiccation (Fujioka, 2001). This specificity makes *E. coli* a critical proxy for assessing health risks, as its presence often correlates with pathogens such as *Vibrio*

cholerae, *Salmonella spp.*, and enteric viruses, which are implicated in diseases like gastroenteritis, cholera, and typhoid fever (Cabral, 2010).

Advancements in detection technologies have bolstered the reliability of these indicators. The Colilert® system, a defined substrate technology developed by IDEXX Laboratories, enables rapid and simultaneous detection of total coliforms and *E. coli* within 24 hours through enzymatic hydrolysis of chromogenic and fluorogenic substrates (Frickeret *al.*, 1997). Specifically, ortho-nitrophenyl-β-D-galactopyranoside (ONPG) produces a yellow color change in the presence of coliforms, while 4-methylumbelliferyl-β-D-glucuronide (MUG) fluoresces under UV light to confirm *E. coli* (Edberg *et al.*, 1990). Validation studies across diverse water matrices—freshwater, marine, and treated effluents—demonstrate its high sensitivity and specificity, making it a preferred tool for field and laboratory applications (Chao *et al.*, 2004). Complementing this, the Most Probable Number (MPN) method, based on multiple-tube fermentation, provides a statistical estimate of bacterial density by observing growth patterns in serially diluted samples (Garthright& Blodgett, 2003). This technique, standardized by the American Public Health Association (APHA, 2017), is widely adopted for its robustness and ability to quantify microbial loads in complex environmental samples.

Empirical studies have leveraged these methods to pinpoint pollution sources. Solo-Gabriele *et al.* (2000) employed Colilert® and MPN to trace *E. coli* to sewage outfalls in subtropical coastal waters, highlighting human waste as a dominant contributor. Similarly, Harwood *et al.* (2005) applied these techniques to assess agricultural runoff impacts, revealing elevated coliform levels linked to livestock manure. Environmental factors, such as temperature, pH, and nutrient availability, significantly influence microbial persistence and detection, necessitating site-specific analyses (Byappanahalliet *al.*, 2012). For instance, warmer temperatures can enhance *E. coli* survival in tropical waters, while nutrient-rich conditions may support non-fecal coliform growth, complicating interpretations (Ishii & Sadowsky, 2008).

III. METHODOLOGY

The methodology employed in this study was designed to provide a robust and reproducible assessment of microbial contamination across five sampling stations—Kurdi, KakorKalan, Katha, ShergarhGhat, and Veejalpur (Near Ayana)—using a combination of qualitative and quantitative techniques. These methods, rooted in standardized protocols, leverage the specificity of Colilert® technology and the statistical power of the Most Probable Number (MPN) method to detect and enumerate coliforms and

Escherichia coli (*E. coli*), key indicators of water quality (APHA, 2017).

i. Sampling Design

Water samples were collected from five strategically selected stations—Kurdi, KakorKalan, Katha, ShergarhGhat, and Veejalpur (Near Ayana)—chosen based on their proximity to potential pollution sources, including urban sewage outfalls, agricultural fields, and rural runoff zones (Solo-Gabriele *et al.*, 2000). Each sample consisted of 100 mL of water, collected in sterile, polyethylene bottles to prevent contamination, following guidelines outlined by the American Public Health Association (APHA, 2017). Sampling occurred during early morning hours to minimize diurnal fluctuations in microbial activity (Kinzelman *et al.*, 2003). To preserve sample integrity, bottles were immediately placed in insulated coolers at 4°C and transported to the laboratory for analysis within 6 hours, a timeframe critical to preventing bacterial die-off or regrowth (LeChevallier *et al.*, 1996). Duplicate samples were collected at each station to ensure reliability and account for spatial variability within the water body (Wright *et al.*, 2006).

ii. Coliforms and *E. coli* as Indicators

Coliforms and *E. coli* were targeted as primary indicators of fecal contamination due to their well-documented association with sewage, livestock runoff, and human waste (Tallon *et al.*, 2005). Their presence in water is a critical metric for assessing sanitary risks, as they often signal the potential co-occurrence of pathogenic microorganisms such as *Salmonella* and enteric viruses (Payment *et al.*, 2000). This indicator approach, widely endorsed by environmental health authorities, provides a practical and cost-effective means of evaluating water safety (Ashbolt *et al.*, 2001).

iii. Defined Substrate Technology (Colilert®)

The Colilert® system was utilized for rapid detection of total coliforms and *E. coli*. Each 100 mL sample was mixed with a Colilert® reagent packet containing ONPG and MUG substrates, sealed in a Quanti-Tray/2000, and incubated at $35 \pm 0.5^\circ\text{C}$ for 24 hours, adhering to IDEXX Laboratories' protocols (IDEXX Laboratories, 2018). Post-incubation, a yellow color change indicated coliform presence, while fluorescence under a 365 nm UV lamp confirmed *E. coli*, leveraging the method's high specificity (Fricker *et al.*, 1997). This technology's efficiency and accuracy have been validated across diverse water types, making it ideal for this study (Chao *et al.*, 2004).

iv. Multiple-Tube Dilution Technique

To quantify bacterial populations, the multiple-tube dilution technique was employed. Samples were serially diluted (10^{-1} , 10^{-2} , 10^{-3}) in sterile saline, and 1 mL aliquots were inoculated into triplicate tubes containing lauryl tryptose broth with Durham tubes for gas detection (Blodgett, 2010). Tubes were incubated at 35°C for 24–48 hours, with bacterial growth confirmed by turbidity and gas production, indicative of lactose

fermentation (Eckner, 1998). This method's triplicate design enhances precision in capturing microbial variability (Garthright & Blodgett, 2003).

v. Most Probable Number (MPN)

MPN values per 100 mL were calculated using statistical tables based on the number of positive tubes from the dilution series (APHA, 2017). For *E. coli* confirmation, positive tubes were subcultured into EC-MUG broth and incubated at 44.5°C for 24 hours, with fluorescence under UV light verifying presence (Eckner, 1998). Sanitary risks were assessed against WHO (2017) thresholds, where *E. coli* concentrations exceeding 1000 MPN/100 mL indicate severe health risks, providing a standardized benchmark for interpretation (WHO, 2017).

IV. RESULTS AND DISCUSSIONS

This study provides a comprehensive analysis of microbial contamination across five sampling stations—Kurdi, KakorKalan, Katha, ShergarhGhat, and Veejalpur (Near Ayana)—using Colilert® technology and the Most Probable Number (MPN) method. The results reveal significant variability in coliform and *Escherichia coli* (*E. coli*) levels, reflecting diverse pollution dynamics and sanitary risks. These findings are contextualized within existing literature to elucidate contamination sources, environmental influences, and implications for water quality management.

i. Detection of Coliforms and *E. coli*

Colilert® analysis (Table 1) confirmed the ubiquitous presence of coliforms across all five stations, as indicated by a consistent yellow color change post-incubation, signifying enzymatic activity on ONPG substrates (Fricker *et al.*, 1997). *E. coli* was detected via fluorescence at Kurdi, KakorKalan, ShergarhGhat, and Veejalpur (Near Ayana), but notably absent at Katha. This pattern suggests variable fecal contamination, consistent with site-specific pollution sources such as sewage outfalls or agricultural runoff (Wright *et al.*, 2006). The universal detection of coliforms aligns with their environmental resilience, as they can originate from both fecal and non-fecal sources like soil or decaying vegetation (Byappanahalliet *et al.*, 2012). However, *E. coli*'s selective presence underscores its specificity as a fecal indicator, reflecting recent anthropogenic inputs at four of the five stations (Fujioka, 2001).

Table 1: Detection of Coliforms and *E. coli* Using Colilert® Technology

Sampling Station	Coliform Presence (Color Change)	<i>E. coli</i> Presence (Fluorescence)
Kurdi	Positive	Positive
KakorKalan	Positive	Positive
Katha	Positive	Negative
ShergarhGhat	Positive	Positive
Veejalpur (Near Ayana)	Positive	Positive

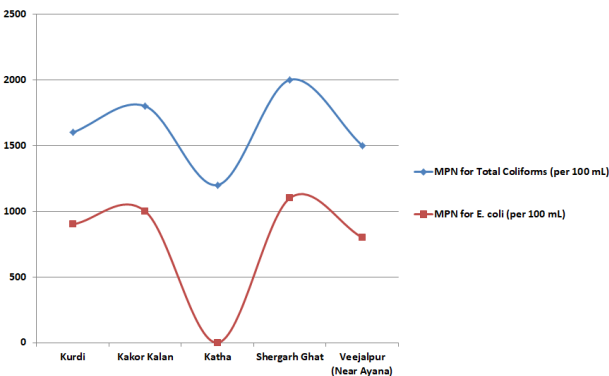
The absence of *E. coli* at Katha, despite coliform presence, is intriguing and may indicate natural attenuation processes, such as UV inactivation or predation by microbial communities, which disproportionately affect *E. coli* survival (Sinton *et al.*, 1998). Alternatively, Katha’s water source might lack direct fecal inputs, a hypothesis requiring further investigation into local hydrology and land use (Ishii & Sadowsky, 2008).

ii. MPN Quantification

MPN results (Table 2) provide quantitative insight into bacterial concentrations, with total coliforms ranging from 1200 MPN/100 mL at Katha to 2000 MPN/100 mL at ShergarhGhat, and *E. coli* from 0 MPN/100 mL at Katha to 1100 MPN/100 mL at ShergarhGhat. ShergarhGhat exhibited the highest microbial load, surpassing WHO (2017) recreational water guidelines (<500 MPN/100 mL for coliforms), while Katha’s *E. coli* absence stands out as a notable anomaly (McLellan & Salmore, 2003). Kurdi (1600 coliforms, 900 *E. coli*), KakorKalan (1800 coliforms, 1000 *E. coli*), and Veejalpur (1500 coliforms, 800 *E. coli*) also showed elevated levels, indicating widespread contamination.

Table 2: Most Probable Number (MPN) for Coliforms and *E. coli* (per 100 mL)

Sampling Station	MPN for Total Coliforms (per 100 mL)	MPN for <i>E. coli</i> (per 100 mL)
Kurdi	1600	900
KakorKalan	1800	1000
Katha	1200	0
ShergarhGhat	2000	1100
Veejalpur (Near Ayana)	1500	800



ShergarhGhat’s peak values suggest intense fecal pollution, likely from untreated sewage discharges, a common issue in urban-adjacent of River Yamuna. The high *E. coli* MPN at KakorKalan and Kurdi similarly points to fecal sources, potentially exacerbated by agricultural runoff carrying livestock manure (Harwood *et al.*, 2005). Veejalpur’s moderate levels may reflect a

mix of diffuse and point-source pollution, while Katha’s lower coliform count and *E. coli* absence could indicate dilution or environmental filtering (Jamieson *et al.*, 2004). These quantitative data, validated by the MPN method’s statistical rigor (Garthright & Blodgett, 2003), underscore the severity of contamination across most stations.

iii. Pollution and Sanitary Risks

Pollution levels and sanitary risks (Table 3) were categorized based on MPN values and WHO (2017) thresholds. ShergarhGhat was classified as very high (2000 coliforms, 1100 *E. coli*), exceeding the severe risk threshold (>1000 MPN/100 mL *E. coli*), indicating acute health hazards such as waterborne disease outbreaks (Rose *et al.*, 2001). Kurdi and KakorKalan, rated as high (significant risk), also pose substantial concerns with *E. coli* levels approaching or meeting the severe threshold (900–1000 MPN/100 mL). Veejalpur’s moderate-to-high classification (1500 coliforms, 800 *E. coli*) reflects notable but less extreme contamination, still warranting intervention. Katha, with moderate pollution (1200 coliforms, 0 *E. coli*), presents a medium risk, suggesting lower fecal impact despite coliform presence.

Table 3: Summary of Pollution Levels and Sanitary Risks

Sampling Station	Pollution Level (Based on MPN)	Sanitary Risk Level
Kurdi	High	Significant health risk
KakorKalan	High	Significant health risk
Katha	Moderate (<i>E. coli</i> absent)	Medium risk
ShergarhGhat	Very High	Severe health risk
Veejalpur (Near Ayana)	Moderate to High	Significant health risk

These findings align with studies linking elevated MPN values to sewage inputs, as observed by Hagedorn *et al.* (2011) in rural watersheds. ShergarhGhat’s severe risk profile mirrors conditions reported near urban sewage outfalls (Solo-Gabriele *et al.*, 2000), while Katha’s anomaly may reflect natural attenuation processes, such as sunlight inactivation or sediment trapping, reducing *E. coli* viability (Jamieson *et al.*, 2004; Sinton *et al.*, 1998). Environmental factors like water temperature, pH, or flow rates could further modulate these patterns, warranting site-specific follow-up studies (Boehm *et al.*, 2002). The sanitary risks identified—particularly at ShergarhGhat—highlight the urgent need for mitigation, as prolonged exposure to such water could elevate disease incidence (Converse *et al.*, 2009).

V. CONCLUSION AND RECOMMENDATIONS

This study revealed widespread microbial contamination across five sampling stations in Baghpat and Auraiya districts, Uttar Pradesh, with ShergarhGhat exhibiting the highest contamination levels (2000 MPN/100 mL coliforms, 1100 MPN/100 mL *E. coli*), posing severe sanitary risks. Kurdi (1600 coliforms, 900 *E. coli*) and KakorKalan (1800 coliforms, 1000 *E. coli*) also demonstrated significant fecal pollution, indicating acute health hazards from potential waterborne pathogens. In contrast, Katha's moderate contamination (1200 coliforms, 0 *E. coli*) suggests unique environmental or anthropogenic conditions mitigating *E. coli* presence, presenting a lower risk profile. These findings underscore the pervasive impact of untreated sewage and agricultural runoff on water quality, necessitating urgent and targeted interventions to protect public health and aquatic ecosystems.

To address these challenges, we recommend: (1) Routine monitoring using Colilert® and MPN methods to track contamination trends and enable rapid response, as demonstrated by Kinzelman *et al.* (2003) in assessing indicator bacteria in recreational waters. (2) Controlling pollution sources, such as sewage discharges and runoff, through engineered solutions like interceptor drains and vegetated buffers, a strategy supported by Boehm *et al.* (2002) for reducing fecal indicator loads. (3) Enhancing sanitation infrastructure, including the construction of decentralized wastewater treatment systems, as evidenced by Converse *et al.* (2009) in mitigating fecal pollution. (4) Investigating Katha's *E. coli* absence to identify natural attenuation factors (e.g., UV inactivation, dilution), building on Ishii and Sadowsky (2008), which could inform cost-effective mitigation elsewhere.

Immediate actions should prioritize ShergarhGhat, Kurdi, and KakorKalan with improved wastewater treatment and reduced agricultural runoff via sustainable farming practices. Community awareness campaigns can further curb pollution by promoting proper waste disposal. Strengthening regulatory frameworks to enforce water quality standards and integrating eco-friendly practices are critical to safeguarding these vital resources, ensuring long-term public health and environmental sustainability.

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