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Microbial Profiling of the Jakham Reservoir in Tribal Region of Southern Rajasthan, India

Sampat Ram Meena[1*], S. M. Roy[2] and Ram Naresh Meena[3]

[1]Department of Zoology, Govind Guru Tribal University, Banswara (Rajasthan), India
[2]Fisheries & Limnology Research Laboratory, Department of Zoology, Government Post Graduate College, Pratapgarh (Rajasthan), India
[3]Department of Zoology, Government Post Graduate College, Rajgarh, Alwar (Rajasthan), India

**Corresponding author*

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A B S T R A C T

Jakham reservoir is an important fresh water reservoir for domestic and ecological needs in the tribal tract of Pratapgarh in southern Rajasthan. The lack of safe drinking water is still a major problem for indigenous areas. In this study, the microbial load of the Jakham water reservoir was evaluated to assess the waterborne health risks associated with it for the local tribal population. MPN was used to determine the microbial load and to isolate indicator organisms including total coliforms, faecal coliforms and faecal streptococci. A grand annual Log10 (X+1) transformed mean value were enumerated of FC 1.87±0.15MPN/100mL, TC 2.08±0.15MPN/100mL and FS 1.63±0.25MPN/100mL in 2023-24 but in second term 1.85±0.18MPN/100mL (FC), 2.09±0.23MPN/100mL (TC) and 1.57±0.18MPN/100mL (FS) were recorded. A total of eight opportunistic pathogenic bacterial strains, including *Escherichia coli*, *Pseudomonas, Klebsiella, Enterobacter, Salmonella, Clostridium, Streptococcus feacalis* and *Staphylococcus aureus* were recovered from the water samples. The peak isolation frequency was noted for *E. coli* and *Streptococcus faecalis* with their vigorous proliferation persisting throughout the entire observation period. Results demonstrated minimal microbial contamination levels that pose health risks to the affected population. Waterborne outbreaks can be reduced through community level water purification, protection of the infrastructure and periodic microbiological surveillance.

Introduction

Water is a good habitat of various microbes because it provides a wide range of nutrient, organic matter, hydro-geological chemical, metals, ions and diverse environment to grow easily. Microbial diversity in aquatic system encompasses the variety of microorganisms including bacteria, virus, fungi, cyanobacteria, algae, and protists. The field of aquatic microbiology uncovers a microscopic world that is essential for the health and functioning of aquatic ecosystems (Miao, 2023). Any biological system

biodiversity can influence both hetrotrophs and autotrophs. Bacterial population is omnipresent but it is not necessarily harmful in all cases, it may also be beneficial (Sharma and Vyas, 2022). Microbes regulate the health of water environment interact with its physical and chemical components. In aquifers microbial community directly enhances vital ecosystem services like catabolised pollutants, cycling of nutrient, and symbiotic relationships within aquatic environments (Miao, 2023; Bell *et al*., 2005). On the contrary, opportunistic microbes originating from various sources but major portion come from the gastrointestinal tract of warm-blooded animals. Excreta of humans, domestic animals, livestock, poultry and aquatic birds are contributed to aquifers enrich a numerous microbes that have a great concern in quality of drinking water. *Enterobacteriaceae* members are key sanitary indicator microorganisms of anthropogenic pollution of the water environment, indicating the presence of decaying organic matter and extremely unhygienic conditions (Erkinovich, 2022). These microorganisms are preferred to be nonpathogenic, have no or minimal growth in water, and reliably detectable at low concentrations. These microorganisms are most useful tool to unearth pathogens in aquatic environment (Ashbolt *et al*., 2001). In drinking water enumerate of coliforms bacteria, thermo tolerant coliforms, *E. coli,* fecal Streptococci, Enterococci, *Clostridia* and HPC commonly serving as the most reliable indicators of fecal contamination (Abdelrahman, 2011; Boehm and Sassoubre, 2014; Laad and Srivastava, 2016). The physicochemical characteristics and microbial loads of water provide precious information on the quality of water and their impacts on the morphology, physiology, and biodiversity of the biotic component in the body of water. This investigation assesses the microbial profile of Jakham Reservoir in Pratapgarh, Rajasthan to evaluate indigenous bacterial populations across key sectors of the reservoir. Multi-point water sampling was conducted to quantify nutrient dynamics, total microbial loads across the reservoir. The resulting profile provides essential baseline metrics for monitoring freshwater quality and understanding microbial abundance in a key inland reservoir.

Materials and Methods

Study Site and Sampling:

Jakham Reservoir serves as a vital freshwater repository supporting domestic and ecological needs across the tribal tract of Pratapgarh in southern Rajasthan. 4 sites were select to conducted research on the basis of some anthropogenic activities that may affect be water quality. It poses a substantial threat to the health of tribal communities. A brief description of selected designated sites (JD-1 to JD-4) summarized in Table 1. Water samples were collected in sterilized bottles on the monthly interval across selected four sites over the period Nov 2023 – Oct 2025. The preparation was handled in an aseptic environment with appropriate precautions. Microbial exercises were conducted in microbiology lab, department of Zoology and Ozone Test House, Udaipur.

Table:1 Brief description of sampling stations select for investigation in Jakham Reservoir

Site	Sample Place	Geographical Location	Major Driver of Pollution
JD-1	Nakor	Latitude: 24.1475^0N Longitude: 74.62227^0E	Open defecation, litters, fishing wastes, recreational activities.
JD-2	Rohan Khera	Latitude: 24.10357^0N Longitude: 74.63912^0E	Cattle grazing open defecation, litters, fishing wastes, and recreational activities.
JD-3	Karma Khera	Latitude: 24.181944^0N Longitude: 74.63111°E	Picnic spot, boating and fishing, cattle grazing open defecation, wildlife movements.
JD-4	Main Jakham dam site	Latitude: 24.167083^0N Longitude: 74.58763°E	Tourism site, Picnic traffic, excreta of wildlife animal and human, fluvial debris.

Bacteriological Examination

Water samples were processed as per SOP given in IS 1622:1982 reaffirmed in 2019 utilizing MTF technique for enumeration of microbiological density. Presumptive total coliform density was evaluated by inoculating D-S-strength MacConkey broth containing inverted Durham vials. Tubes incubated at 37 ± 0.5 °C for 24–48 hrs. Tubes demonstrating both acid production and gas accumulation were scored as presumptive positive. Confirmatory testing for thermotolerant fecal coliforms was conducted by sub-culturing presumptive positive aliquots into 2% BGL broth maintained in a precision water bath at 44.5 ± 0.2 °C for 24 h. *E. coli* was confirmed via positive indole production in 1% peptone water at 44.5 °C upon addition of Kovac's reagent

(yielding a distinct cherry-red ring). Fecal streptococci were determined presumptively in ADB at 37±0.5°C (24–48 h) and confirmed by inoculation into BEA agar. Bacterial indices for all indicators were computed from the positive tube combinations using McCrady's statistical probability tables as specified in IS 1622: 1982 and expressed as MPN/100 mL. Different enteric and associated bacterial species were isolated and characterized perform Gram staining, biochemical test and morphological features on standard selective culture media.

Results and Discussion

The microbiological evaluation of the Jakham water exhibited wide variations in the bacterial densities across the four designated monitoring stations in defined time frame. Because microbial counts generally follow a highly skewed distribution with inherent zero-value possibilities. Log10 (X+1) transformation was executed to normalize the dataset before computing numerical averages and dispersion metrics.

Fecal coliform:

Log transformation values presented in Table-2 illustrated that the spatial progression of FC contamination during entire period. During 2023-24, the lowest central biological density was recorded at JD-1 (1.80±0.15 $\log_{10}$ MPN/100mL; GM: 61.74 MPN/100mL), with raw data ranging tightly between 35 -105 MPN/100mL. Station JD-2 and JD-3 displayed identical mean values but varied slightly in their original back-transformed density. The maximum FC density observed at JD-4, peaking at a log-transformed value of 1.94±0.19 $\log_{10}$ MPN/100mL with a raw 175 MPN/100mL coordinating with GM 85.74 MPN/100mL. Similarly in 2024-25, FC prevalence was decreased at JD-1, mean log value of 1.69±0.19 MPN/100mL with a geometric mean 47.93 MPN/100mL. Similar decreasing trend followed by JD-2 as compared to previous year. But JD-3 and JD-4 displayed an escalation in bio-contamination during 2024-25. The log-transformed mean rose to 1.91±0.20 log10 MPN/100mL at JD-3 with a raw maximum spike of 200 MPN/100mL. JD-4 had the highest single concentration in the whole study with a mean MPN value of 1.98±0.21 log10 with a geometric mean of 94.29 MPN/100mL. Many researchers have recorded higher counts of FC microorganisms in different water sources compared to the Jakham water i.e. water bodies in Udaipur (Shivani and Sharma (2016); Sengupta *et al.* (2016); Angoori Barrage (Parihar *et al*, 2014); Sudhkar and Hemlatha (2015); Dholwad dam and Morwani dam in Ratlam (Rathore *et al*, 2016).

The grand annual faecal profile consolidates the spatial data to provide a holistic view of aquatic health of Jakham. Table-2, highlighted wide log mean stood remarkably stable across years in the water body, documenting 1.87±0.15log10 MPN/100mL in 2023-24 and slightly moderating to 1.85±0.18 $\log_{10}$MPN/100mL in 2024-25. Geometric mean is used to lessen the distorted effects caused by extremely large MPNs, thus creating a more accurate and realistic base line for long term microbial contamination for each site. Kothari *et al*. (2021) reported fecal coliform in drinking water in range of 20-300CFU/100mL that highly agreed with microbial counts of Jakham. Siliser and Jaisamand Lake, Alwar were also found highly contaminated due to high density of FC (Singh and Sharma, 2016). JD-3 and JD-4 indicate continuous organic enrichment and supply of domestic wastes possibly affected by local runoff events, open defecation and wastages from tourism and fishing. The faecal coliform densities were found to peak in the monsoon season due to surface wash-off (Vyas *et al*., 2015), which was similar to the results obtained in other freshwater bodies. These were some of the major practices for the persistent amplification of faecal coliforms.

Total coliform:

In our investigation, site wise TC counts were sustained across the all sampling sites. Table-2 stated that the raw TC concentrations ranged varied from 60 to 265 MPN/100mL in 2023-24.The grand mean of TC was noted constant at a log-transformed value of 2.08±0.20 $\log_{10}$MPN/100mL (GM: 118.94 MPN/100mL). On the other hand, there were slight increases in the prevalence of total coliforms (TC) over time. The prevalence of TC decreased significantly at site JD-1. The log-mean value of TC decreased to 2.04±0.23 log10 MPN/100 mL (GM: 108.93 MPN/100 mL). The TC contents of sites JD-3 and JD-4 were high. The mean at JD-3 was 2.11±0.23 log10 MPN/100 mL (GM: 128.73 MPN/100 mL) ranging from 60 to 350 MPN/100 mL. Station JD-4 was the highest single concentration tier for the whole two-year study, with a geometric average of 141.33 MPN/100 mL and a log10 value of 2.15±0.26 MPN/100 mL. Like the first term, sites JD-3&JD-4 also had high log and after log values of the total coliform count. Interestingly, the log

mean across the system was remarkably stable between years with a log mean MPN of 2.08±0.20 MPN/100 mL in 2023-24 compared to a slightly higher log mean MPN of TC of 2.09±0.23 reported in 2024-25 (Table 2). These relative stabilities in the log metrics translate into global geometric averages of 118.94 MPN/100mL and 121.76 MPN/100mL respectively. The averaged raw MPN of TC expanded to 60-265 MPN/100 mL (log mean 1.63±0.25; 2023-24) and 45-350 MPN/100 mL (log mean 1.57±0.18, 2024-25) as illustrated in Table 2, highlighted the high seasonal dynamism characteristic of Jakham reservoir. Compared to Jakham, Kumar *et al*., (2015) investigated extreme contamination of TC (15-3600MPN) and FC (4-240MPN) in different water body in Raipur. TC and FC concentrations across the study timeline, revealed a synchronized upward progression. Some studies aligned our findings *i.e.* Sudhkar and Hemlatha (2015); different water bodies in Udaipur (Shivani and Sharma (2016); Sengupta *et al.* (2016); Siliser and Jaisamand lake, Alwar (Singh, and Sharma, 2016); Amarja reservoir in Karnataka (Rizvi *et al.,* 2019). Nutrient and some environmental factors commonly favor to these microbial communities. The significant standard deviations coupled with raw TC maximum values reaching 265–350 MPN/100mL, findings highlight temporary surges in contamination, potentially driven by localized runoff events, open defecation practices.

Faecal Streptococci:

These are other indicator organisms that predominantly proliferate under aquatic ecosystems. The log mean of FS was lowest at JD-1 (1.55±0.25 log10 MPN/100 mL along with GM: 34.47 MPN/100 mL) and highest at JD-3 (1.69±0.26 log10 MPN/100 mL with GM: 47.64 MPN/100 mL) in 2023-24. While in the subsequent examination period, site 1 reached the bottom line with a long mean value of 1.45±0.23 log10 MPN/100 mL (range of raw 15-85; GM: 27.23 MPN/100 mL), but site 4 reflected the highest log mean (1.67±0.21 log10 MPN/100 mL), including raw ranging 19-110 MPN/100mL with a geometric mean of 44.7 MPN/100 mL. In Jakham water, the fecal streptococci appearance points out that there was an influx of excreta of human and domestic animal or wildlife origin in the water body, its potential to be health hazards. The grand mean of log value decreased from 1.63±0.25 to 1.57±0.18 MPN/100 mL previous to the last period with a decrease in FS raw values from 15-120 to 13-110. Table 2 illustrated ranges of averaged raw MPN of FS expanded to 60-265 MPN/100 mL (2023-24) and 45-350 MPN/100 mL (2024-25), highlighting the high seasonal dynamism characteristic of Jakham reservoir. Manjusha *et al*. (2014) reported a very high percentage of faecal streptococci in Kuppam river basin. Likewise, Chauhan *et al*. (2019) reported extreme counts of Streptococci and other faecal contaminants in the Chambal river basin of Kota due to the confluence of urban sewage and industrial effluent. The presence of faecal streptococci is an indicator of the presence of faecal pathogens in the water. They are capable for long periods in aquatic environment, and therefore, like fecal coliforms, fecal streptococci are accepted as indicators of water pollution (Manjusha *et al*., 2014; Motlagh and Yang, 2019).

Prevalence of enteric and other pathogenic bacteria:

In our study, we monitored eight different groups of bacteria with varying prevalence and with an overall increasing trend in isolation frequencies. Across all 4 monitoring stations of the Jakham water reservoir revealed persistent biological contamination, dominated by an absolute (100%) occurrence of primary fecal bioindicators *E. coli* and *Streptococcus faecalis* between both observation cycles (Figure-1), highlighted in deep crimson red color box in heatmap (Figure-1). Garode and Bhusari (2017) also were reported 100% presence of *E. coli* in Chikhli river basin. These consistent baseline indicators formed strong co-occurrence patterns with secondary enteric and opportunistic pathogens reported across the reservoir basin. Specifically, *Salmonella spp.* had an annual recovery of 20.83% to 22.92% and co-existed with *E. coli* through common transmission routes. Figure 2 illustrates the constant appearance of *Salmonella* (16.70-25.00%) within all sites. The coliform matrix was further characterised by a heavy recovery of *Klebsiella* spp. (47.92% to 66.67%) and *Enterobacter* spp. (39.58% to 43.75%). The highest number of *Klebsiella* was reported at site JD-3 with 75.00%, whereas *Enterobacter* was 58.33% at JD-3 & 4 (Figures 1 & 2). Soil and aquatic environments serve as reservoirs for *Klebsiella* spp., predominantly found due to anthropogenic contamination (Barati *et al*., 2016). Simultaneously, the Gramme-positive spore-forming anaerobe *Clostridium* spp. was found to recover annually by 45.83% to 54.17%, tracking closely with *Enterococcus* counts, particularly at transitional and impounded stations JD-3 and JD-4 (individual sites show 66.67% but vary year on year, Figure 1). *Staphylococcus aureus* maintained steady isolation frequencies of 22.92% to 25.00%. However, *S. aureus* marginally

fluctuated between all sites, ranging from 16.67 to 33.33%. Furthermore, non-fermenting, pigment-producing *Pseudomonas* spp. recorded elevated isolation frequencies across the basin (58.33% to 62.50%), consistently peaking in water fractions characterized by high chemical and biochemical oxygen demands. Site JD-1 recorded *Pseudomonas* spp. 41.67% (lowest, 2024-25 Figure-1 yellow shade), but the highest counts were noted at JD-3&4 (75.00%). The persistent presence of *E. coli* and *Streptococcus faecalis* is indicative of the continued and uninterrupted disposal of faecal matter into the Jakham Reservoir. The proliferation of these is evidence that the water source is directly unfit for drinking purposes.

E. coli and *Salmonella* shared the co-transport mechanism that originated from sewage discharge and catchment runoff with coliforms. *Salmonella* sustained detection (reaching 25.00% at downstream reaches) signals risks of typhoid and paratyphoid and bacillary gastroenteritis among riparian communities utilizing raw reservoir water. Vyas *et al*. (2015) also reported *Enterobacter and E. coli* in drinking water in Junagarh. Suthar *et al*. (2009) isolated 10 bacterial pathogens from drinking water of northern Rajasthan, which supports the present study findings. Co-occurrence of faecal streptococci (enterococci) and *Clostridium* species is a reliable biomarker for long-term and chronic contamination.

Table.2 Microbial pollution indicators (FC, TC and FS) density across sampling stations and annual cycles (2023–24 and 2024–25)

Sampling Station	Log Mean + SD		Range (Min-Max) of raw values of the coliform		Geometric Mean	
	Fecal Coliform					
	2023-24	2024-25	2023-24	2024-25	2023-24	2024-25
JD-1	1.80±0.15	1.69±0.19	35-105	24-100	61.74	47.93
JD-2	1.86±0.14	1.81±0.18	42-127	40-145	71.89	62.83
JD-3	1.86±0.11	1.91±0.20	48-110	45-200	72.17	80.85
JD-4	1.94±0.19	1.98±0.21	50-175	38-170	85.74	94.29
Grand Annual FC	1.87±0.15	1.85±0.18	35-175	24-200	72.40	69.25
Total Coliform						
JD-1	2.08±0.19	2.04±0.23	68-230	45-240	118.8041	108.9235
JD-2	2.08±0.21	2.05±0.23	60-257	50-300	117.8798	110.8936
JD-3	2.09±0.20	2.11±0.23	65-240	60-350	120.8755	128.729
JD-4	2.08±0.22	2.15±0.26	62-265	70-325	118.2267	141.33
Grand Annual TC	2.08±0.20	2.09±0.23	60-265	45-350	118.9409	121.7601
Fecal Streptococci						
JD-1	1.55±0.25	1.45±0.23	15-85	13-80	34.47	27.23
JD-2	1.61±0.26	1.54±0.14	18-90	21-63	40.46	33.64
JD-3	1.69±0.24	1.62±0.17	17-95	21-80	47.64	41.16
JD-4	1.67±0.26	1.67±0.21	20-120	19-110	46.03	44.73
Grand Annual FS	1.63±0.25	1.57±0.18	15-120	13-110	41.83	36.05
E. coli						
JD1-JD4/Grand Annual *E. coli*	100% frequency of occurrence					

Note: *All bacterial counts are represented in MPN/100mL. Log transformations were performed using the ($log_{10}(X+1)$ formula to normalize the skewed microbial data distributions; Geometric Mean (GM) values are back-transformed and adjusted accordingly ($10^{\wedge (Mean)} - 1$).* ***E. coli data is represented as the annual percentage frequency of detection, where 100% indicates continuous presence across all 24 sampling months.*** *TC = Total Coliform, FC = Faecal Coliform, FS = Faecal Streptococci.*

Figure.1 Heat-map representing Site-wise and annual basis occurrence of bacterial contaminations in Jakham water reservoir (2023-2025).

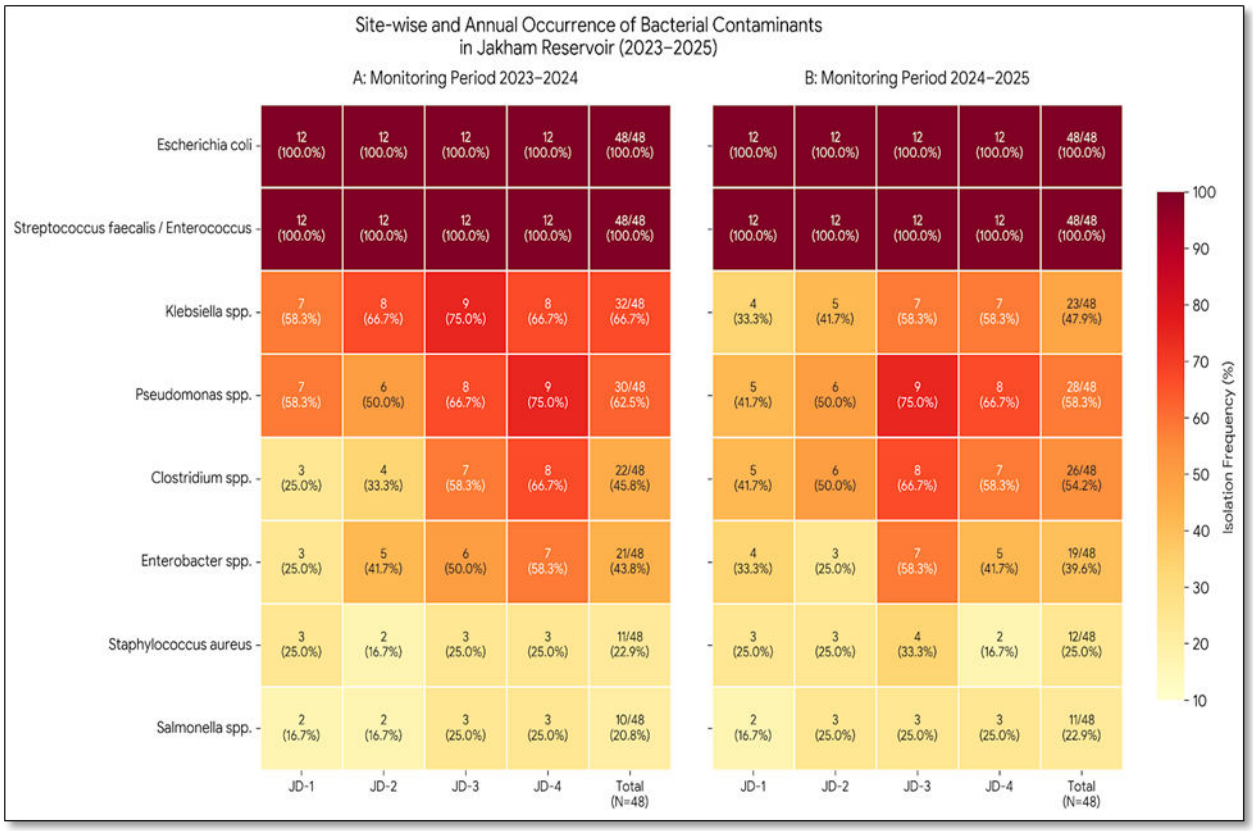
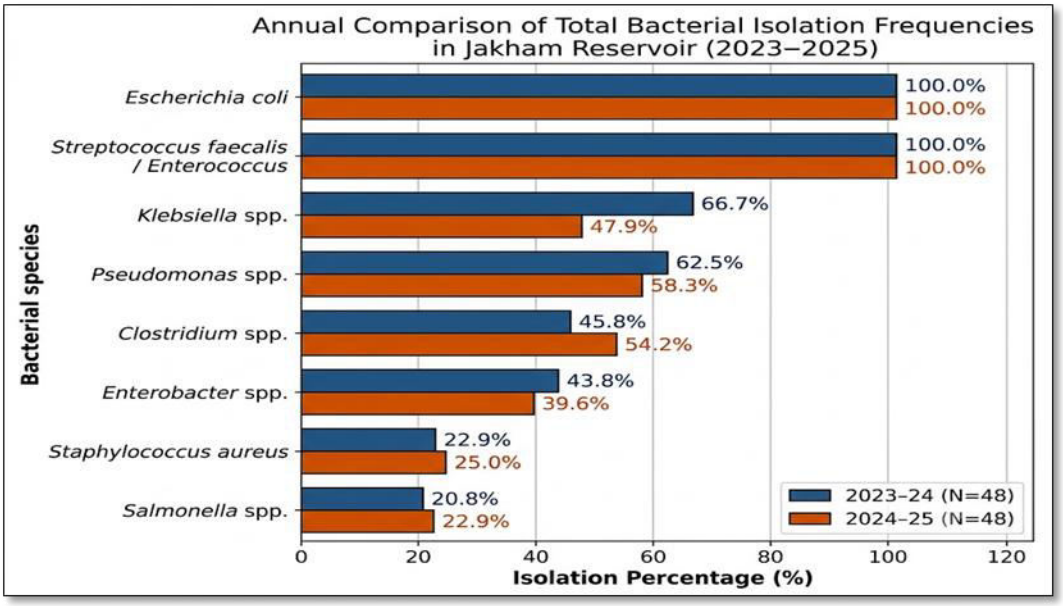


Figure.2 Bar plot representing annual comparative isolation frequency



Substantial non-enteric anthropogenic pressures, particularly open-water bathing and laundry, are supported by significant correlation of *Staphylococcus aureus* with particulate-associated faecal streptococci. The occurrence of *Pseudomonas* spp. reflects saprophytic adaptation to high carbonaceous loads. High BOD and COD regimes are taken advantage of by opportunistic bacilli for persistence in the environment. For this, Bhumbla *et al*. (2020) isolated *E. coli* which were the predominant bacteria along with *Klebsiella pneumoniae* and Coagulase Negative *Staphylococcus* (CONS) in the water of Lake Pichhola and Swaroop sagar. Physico-chemical parameters in water stimulate microbes and affect public health (Shukla *et al*., 2017). The results from Jakham are in agreement with the investigation of Pant *et al*. (2022). They reported qualitative and quantitative enumeration of coliform bacteria in song river water in the rural area of Dehradun, India. Some health threatening bacteria like Salmonella spp., *Klebsiella* spp., *E. coli, Pseudomonas* spp., and faecal coliform were reported in the water. Pathogenic Gram positive and negative microorganisms were isolated from the water source that poses health threat to consumers (Sandhya *et al*., 2014). Similar water borne enteric bacterial species in north Kerala reported by Ambili and Sebastian (2019); Amarja reservoir in Karnataka (Rizvi *et al.,* 2019); Belagavi City Mishra *et al.* (2019).

In Conclusion, the present study was conducted to evaluate the microbial diversity and concentration in water of Jakham reservoir. Various viable and recreational human activities such as fishing, boating, livestock grazing, open defecation and bathing that can significantly affect water quality take place in the area. The present study results indicate that the concentrations of Total Coliforms, Faecal Coliforms and Faecal Streptococci were significantly higher than the set safety levels at all four sites of the ecosystem. Furthermore, we observed a high prevalence of various members of the *Enterobacteriaceae* family that originate from gastro intestinal environment. Apart from these enteric bacteria and its associated Gram-positive bacteria from fecal sources were highly proliferating in the water system. The presence of these microorganisms in the Jakham basin points to significant pollution and indicates high levels of organic contamination. Direct consumption of such polluted water could adversely affect the health of the local community. Consequently, from a microbiological perspective, the water quality is poor. It must be treated to be safe for consumption

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Author Contributions

Sampat Ram Meena: Contributed to data collection, data analysis and prepared draft of the manuscript; S. M. Roy & Ram Naresh Meena: Contributed to writing and editing the manuscript study design.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Conflict of Interest

The authors declare no competing interests.

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