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Microbial assessment and Molecular characterization of environmental bacteria isolated from domestic water of Selected Hotels, in Indore, Madhya Pradesh

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Abstract

The present study focused on microbial assessment and the physicochemical and bacteriological quality of water from selected hotels in Indore. The physicochemical parameters analyzed included pH, temperature, total alkalinity, turbidity, total hardness, dissolved oxygen, calcium, and magnesium. Bacteriological analyses, such as total bacterial count and coliform count, were performed to determine microbial contamination. In this study, five different bacterial isolates were obtained and characterized based on their morphological and biochemical properties. Among these, two isolates—*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*—were further identified using molecular characterization through the 16S ribosomal RNA method. The 16S rRNA gene sequencing revealed that these isolates are phylogenetically related to other known taxa and their closest phylogenetic neighbors. The findings of this study highlight concerns regarding water quality, showing that most hotel water samples were polluted based on the evaluated parameters. The results indicated that *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most prevalent pathogenic microorganisms detected in the water samples.

Keywords: Microbial assessment, 16s rRNA Identification, Microorganisms, drinking water and tap water

1. INTRODUCTION

Water used for household needs in many developing nations is often unsafe because it contains numerous chemical and biological contaminants. Disease-causing microorganisms—such as harmful bacteria and protozoa—further degrade water quality and create significant risks to public health (Cabral, 2010; Lin et al., 2022). Worldwide, individuals use an average of about 1.75 liters of water each day for household activities, including drinking, washing dishes, and general cleaning. Although some developing areas receive water through municipal systems, many families still turn to supplementary methods to ensure that their water is clean and consistently available (Abu Hasan et al., 2020). The World Health Organization (WHO) emphasizes that ensuring drinking water safety involves continuous oversight—from the original water source through treatment and final distribution. In line with this, the United Nations (UN) established Sustainable Development Goal (SDG) 6.1 in 2015, which focuses on guaranteeing safe and affordable drinking water for everyone by the year 2030, supporting global initiatives aimed at strengthening public health through better water management (Najjembe, C.A. *et al.* 2025). Indore is the cleanest city in India, access of safe drinking water to urban area and hotels major priority of city. Indore is rich industrial sector and known as special economic zone that exports and generate large scale employment which attract the business entrepreneurs. Indore city ha 369 registered/licensed restaurants in financial year 2022-23 report of Indore City Corporation. Irrespective lots of unregistered restaurants also exist as well that will not endorsing hygienic facilities that pose a risk to public health. The major source of water distribution in hotel and restaurant is groundwater.

The World Health Organization (WHO) emphasizes that ensuring drinking water safety requires continuous oversight at every stage, from the original water source through treatment and final distribution. In line with this, the United Nations (UN) incorporated Goal 6.1 into the Sustainable Development Goals (SDGs) in 2015, aiming for universal, affordable access to safe drinking water by 2030. Despite these commitments, the 2022 SDG

progress report shows that about 2.2 billion people still do not have safely managed drinking water services, underscoring the ongoing global challenge of improving water quality and availability (Irannezhad *et al.*, 2022).

The degree of contamination is often determined by examining the physical and chemical features of the water body. Any unfavorable change in the physicochemical properties of water causes water contamination. Microbiological culture method used to evaluate the microbiologic quality of drinking water and under this viable plate count significantly use to count the number of bacteria in drinking and tap water (Rompré, A. *et al.* 2002). Total coliform counts are the most widely utilized indicators of drinking and tap water safety. These tests are particularly good in detecting recent fecal contamination. The pathogenic bacteria spread through water distribution system and pollute fresh water by forming biofilms in spite of chlorination. Microbial aggregate form in water may dispersed in bulk water and their by increasing the risk communicable pathogen. Regardless of fecal contamination the risk of emerging water borne environmental pathogen resist with disinfectant would be the high risk in drinking and tap water. *Legionella* sp.., *Aeromonas* sp., *Mycobacterium* sp. and *Pseudomonas aeruginosa*are forms biofilm in pipes and grown in piped water distribution and may cause several outbreak (Sarker, Y. *et al.* 2016). Toxigenic *Vibrio cholerae* is a major waterborne pathogen capable of causing large outbreaks of severe watery diarrhea, with very high case-fatality rates when left untreated. Despite global progress, significant outbreaks are still reported in several districts. In comparison, waterborne infections caused by *E. coli*, particularly enterohemorrhagic strains, are less common than *Campylobacter* infections. However, these *E. coli* strains have an extremely low infectious dose—fewer than 100 organisms and can lead to serious complications such as hemolytic uremic syndrome and even death. Similarly, *Shigella* species remain important waterborne pathogens, responsible for over two million illnesses and nearly 60,000 deaths each year, mainly in developing countries. Waterborne outbreaks can occur with an infectious dose as low as 10 to100 organisms. Large and devastating outbreaks of typhoid fever are primarily associated with *Salmonella typhi*, whereas non-typhoidal *Salmonella* rarely causes waterborne outbreaks (Onifade, O.E. *et al.* 2019).

There is great diversity of microorganism reported out of which some are emerging bacteria with high risk of communicable disease, more research is obviously needed to fully comprehend the relevance of the function that potential pathogens play in drinking water as well as the quantity and variety of all the bacteria introduced into the DWDS (Thom, C. *et al.* 2022). Therefore keeping in above view this research aimed to isolate and identify environmental bacteria from various water sources drinking and tap water from some selected hotels in Indore (M.P.) Water samples from multiple locations were tested for diversity by the application of a 16S rDNA-based clone library approach to examine the microbial community structure. The present research focus on the microbial status of domestic water collected from various hotels in Indore and identifies the environmental bacteria present through molecular analysis. By integrating conventional microbiological methods with advanced molecular tools, the study aims to provide valuable insights that can guide better water quality management, minimize potential health hazards for hotel occupants and staff, and support wider public health efforts within urban communities.

2. Material and Methods

2.1 Study area and experimental design

The work was conducted in the Department of Microbiology, SAGE University; Indore during the year April 2024. Water sample was collected from different hotels in Indore City (Figure 1). A totally random approach was used, with three replications. This study used two types of water: Drinking water and tap water and five water samples were taken from various water sources.

2.2 Sample collection and Media used

The water sampling was done from five various popular restaurants. One liter of water sample was collected in sterile sample bottle from each restaurant. Before collection of sample form from the tap, let it run for a while, then rinsed the containers three times. The container was then air tight to avoid any air contamination and sample are immediately transfer to the laboratory and stored 4^{o}C in laboratory for further investigation. Growth media used in this study was selective media and basal media such as Lactose broth, MacConkey agar, Eiosin Methylene Blue (EMB), Simmons citrate and Nutrient agar, for the microbial isolation. The experiment was conducted in three steps collection of water sample from different hotels in Indore City, in the second step isolation of microorganism and biochemical identification in the third step phylogenic analysis was done using mega x (Cabral, J. P. 2010).

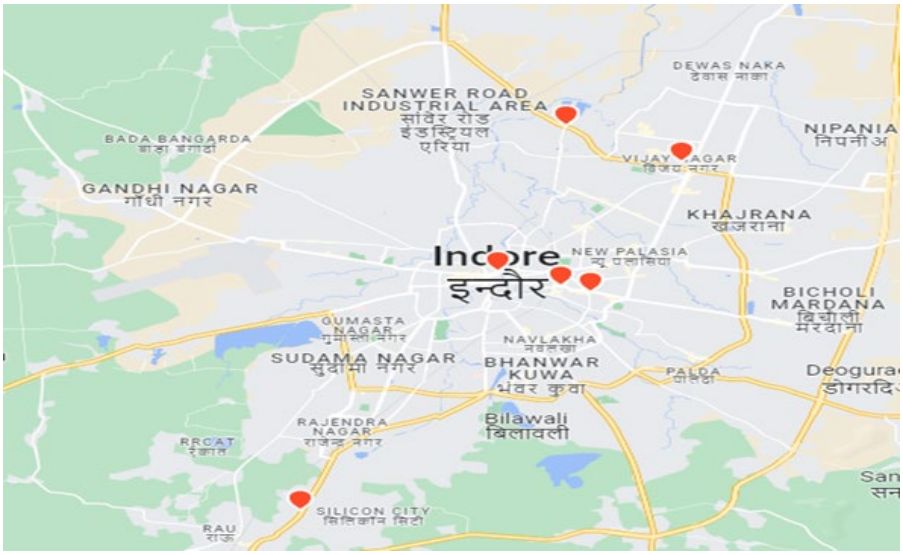


Figure 1. Location marked of different Hotels in Indore (M.P.) selected for sample collection.https://my.atlist.com/map/f1861b56-eb89-418f-a472-50fbd5553e70

2.3 Physicochemical analysis

The physiochemical qualities of water samples are tested for several physiochemical parameters such as temperature, pH, total dissolved solids, conductivity, dissolved oxygen, chemical oxygen demand, and biological oxygen demand using standard titrimetric method with slight modification (Haque, M.A. *et al.* 2019). Electriconductivity, Temperature, and pH of water samples are measured by thermometer, and pH meter, respectively.

2.4 Enumeration of water microbes

For enumeration of water microbes three techniques were used **(1) Estimation of bacterial population by standard plate count (SPC) method:** By inoculating Nutrient agar plates with 1ml of suitable dilution and results were expressed as colony forming units (cfu) per unit vol. enumerated after 48 h incubation at 25 ºC and 37 ºC. cfu count were measured by the serial dilution method on 10^{-4} dilution

(2) To access the quality of water, coliform count was determined by most probable number (MPN) method: Five test tubes containing 10 ml of double strength lactose broth and 10 test tubes containing single strength lactose broth with durhams tubes were taken. The collected water samples were inoculated in each lactose broth tubes i.e. 10 ml water sample was inoculated into each five tubes containing 10 ml double strength lactose broth, 1 ml water sample was inoculated into five tubes containing 5 ml single strength broth and 0.1 ml water sample inoculated into each 5 tubes containing 5 ml single strength lactose broth. All the test tubes were incubated at 35 ºC for 48 hr. After incubation, all the tubes were observed for acid and gas production. The production of acid and gas indicated the presence of coliforms and thus test was considered positive (Jiwintarum, Y. et al. 2018).

2.5 Microscopic characterization

After obtaining pure cultures based on distinct colony morphology, five different bacterial isolates were selected for microscopic characterization. The isolates were examined using Gram staining to determine their Gram reaction, cellular shape, and arrangement.

2.6 Biochemical characterization of bacterial isolates

The physiological features of bacteria from the Family Enterobacteriaceae, particularly *Escherichia* and *Enterobacter* produced from macConkey agar, colonies, are studied using biochemical IMViCtests. The green shiny colony is inoculated into tryptone broth, MR VP broth, and a simmons Citrate slant, respectively (Aditi, F.Y. *et al.* 2017).

2.7 Molecular characterization of selected isolates

The total genomic DNA of bacteria was extracted by using DNA Isolation Kit and protocol provided by (Clermont, O. *et al.* 2013). DNA for drying was kept overnight at room temperature, afterward dissolved in 150 µl of TE buffer and store at -20°C for further analysis The PCR amplification of the 16s rRNA gene was performed by primers 16s Forward Primer (395F) AAGGTCTGGAGCACGCTTAT and 16s CCGTGCCGTGGAATTAT. The PCR mixture contain 80-100 ng of template DNA, 20 µl volumes, with 2 µl of DNA material, 1× PCR buffer containing 0.16 mM dNTP, 20 pmol of forward and reverse primers, and 0.75 U Taq DNA polymerase.PCR reactions were performed in The PCR process consists of 6 minutes of denaturation at 95°C, 40 cycles of denaturation at 95°C for 30 seconds each, 1 minute of annealing at 50°C, and 1 minute of extension at 72°C. The final extension was 10 minutes at 72°C. The PCR findings were evaluated on a 2% agarose gel, then purified and sequenced. MEGA was used to reconstruct a phylogenetic tree. The phylogenetic tree was derived using evolutionary distances estimated using the Maximum Composite Likelihood approach (Hausiku, M.K. *et al.* 2020). BLAST analysis checks sequence data. The phylogenetic tree was built using evolutionary distances determined using the Maximum Composite Likelihood technique (Hausiku, M.K. *et al.* 2020). and sequence data validated by BLAST analysis (Z. Zhang et al., 2000).

3. RESULTS

3.1 Physiochemical characteristics of water sample

The microbiological, chemical, and physical properties were examined. Among the physical parameters are turbidity, electrical conductivity (EC), and pH. Table 2 displays the findings. During the rainy season; H002 (II) had the greatest pH of 8.17±0.30, while H005 (V) had the lowest pH of 7.85±0.12. During the winter season, H004 (IV) had the highest pH of 8.25±0.36 and H005 (V) had the lowest pH of 8.075±0.09, respectively. These values fit within the DFTQC limits. The concentration of hydrogen and hydroxyl ions represents water's pH (Rahman, A. *et al.* 2021). The acidity of water is determined by the presence of carbon dioxide, which dissolves in water to produce carbonic acid, increasing the acidity of water (Rahman, A. *et al.* 2021). Drinking water pH variation from normal (neutral pH) can have an impact on both public health and the water purification system. Chemical parameters were evaluated to determine the quantities of calcium, magnesium, total dissolved solids, total hardness, and dissolved oxygen.

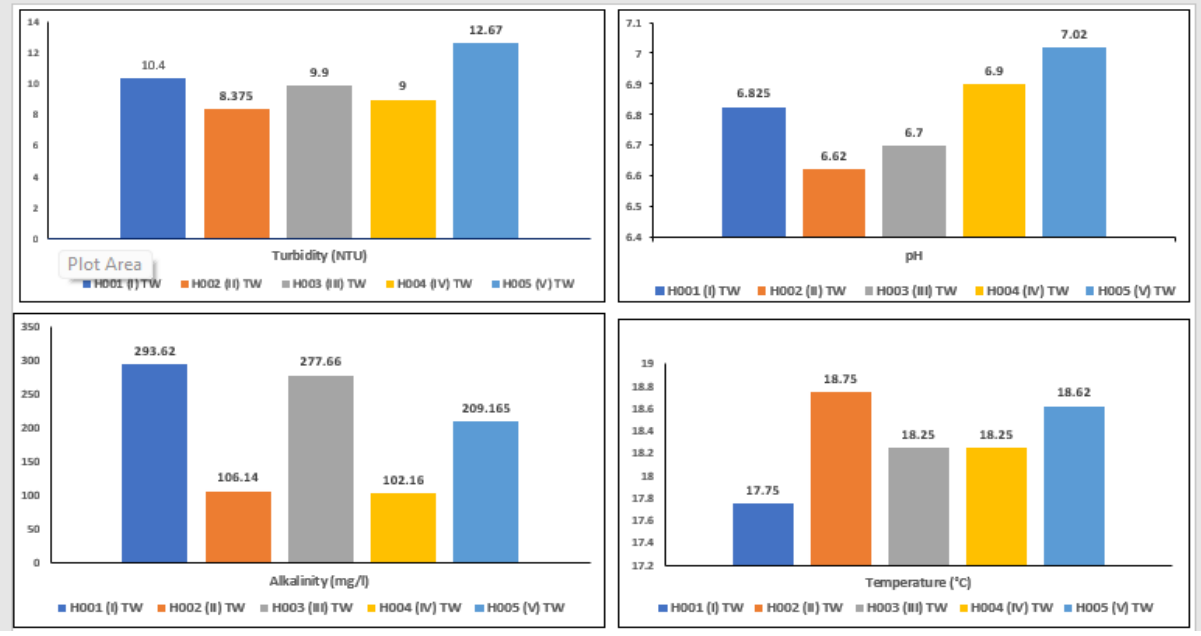


Figure -2. Temperatures, pH, Turbidity and Alkalinity levels in tap water from different selected hotel in Indore, M.P.

Chemical parameters were estimated to determine the concentrations of calcium, magnesium, and total dissolved solid, total hardness and dissolved oxygen of water sample collected. The results are shown in (Fig.3). The TDS is a

quantitative indicator of all dissolved organic and inorganic materials present in the water. The sampling station H004 (IV) and H001 (I) recorded a minimum 112 mg/l and maximum value was of 372 mg/l respectively. The probable reason of high TDS value is due to high salt concentrations because mixing of domestic sewage contamination. TDS value of water is depending on the type of pollution, the concentration of solids in polluted waters, according to (Adjovu, G.E. *et al.* 2023). TDS of water ranged from 400 to 2300 mg/l.

Dissolved oxygen is a basic requirement for plant life in any given body of water. Among the many hydro biological factors, the status of water DO content has been considered a critical parameter to indicate water patability and whether a system is suitable for aquatic organisms or not. The amount of dissolved oxygen depends on temperature and salinity of water content. However, the amount of dissolved oxygen has not any direct impact on health of individual but amount of dissolved oxygen can vary the taste of water and can taste unpalatable to some people. The DO content in the current tap water samples ranged from 6.00 to 12.73 mg/l, as good amount of DO therefore indicates better water quality.

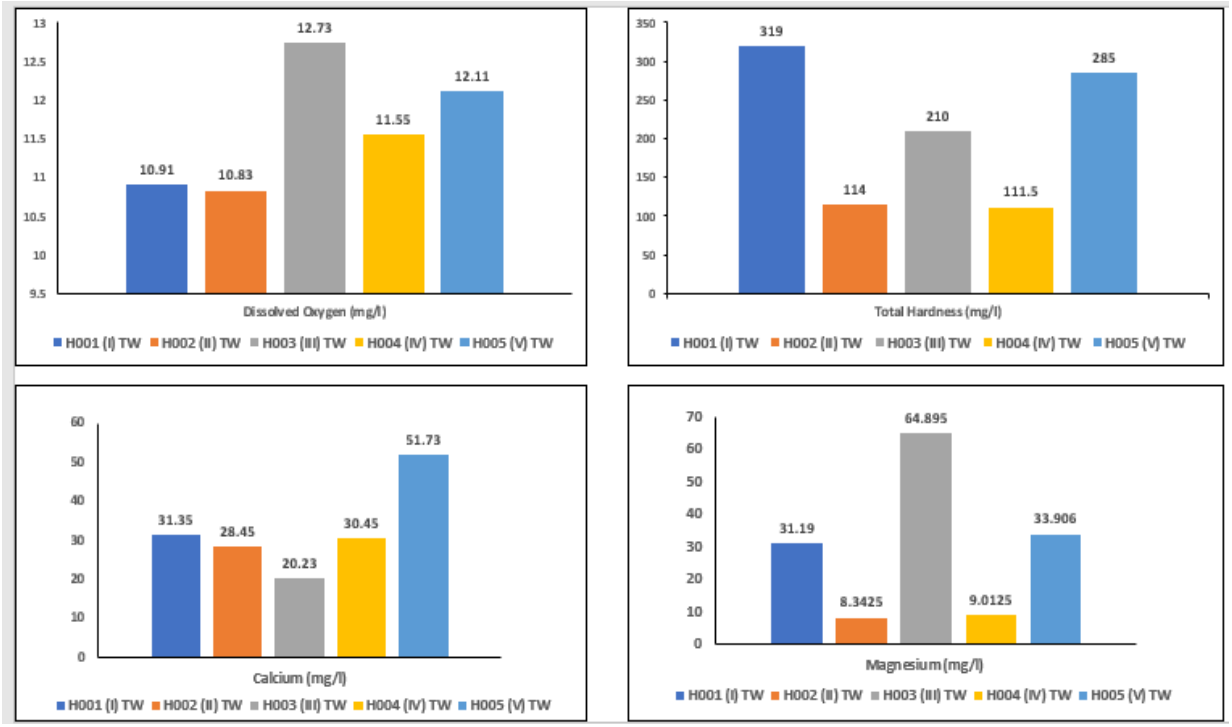


Figure -3. Total Hardness, Calcium, Magnesium and DO levels in tap water from different selected hotel in Indore, M.P.

3.2 Enumeration of water microbes

The total viable counts by the SPC method of all the five sites are presented in Table 2. The Total viable count (TVC) count was found in tap water in compare to drinking water in all the samples. Sample no. 1 and 3 was showing the maximum no. of TVC count.

TVC	H001	H002	H003	H004	H005
Tap (cfu 10^{-4})	53.33±6.8	33.33±6.8	61.23±5.81	32.66±7.8	26.33±6.8
Drinking (cfu 10^{-4})	25±6.81	10.68±3.61	34.33±2.16	16.33±7.60	12±4.33

±Standard deviation

3.3 MPN Analysis

Total Coliform was higher in tap water of sample H001 and H003 in MPN test (Table 2).

Water Sample	Type of Water	Media	Incubation				Positive Control	Negative Control
				10ml	1 ml	0.1 ml		
H001 (I)	Tap Water	MacConkey Broth	37°C for 24 hours	4	3	2	Positive	Negative
	Drinking Water			-	-	1		
H002 (II)	Tap Water			-	2	-		
	Drinking Water			-	-	-		
H003 (III)	Tap Water			5	4	3		
	Drinking Water			2	1	1		
H004 (IV)	Tap Water			-	-	-		
	Drinking Water			2	1	-		
H005 (V)	Tap Water			2	1	1		
	Drinking Water			2	-	-		

To isolate and purify, several colony bacteria were investigated (Fig 4). Total five bacteria were successfully isolated and purified from domestic water sample of selected hotels in Indore (M.P.). Five isolates (AT, LD, SD, SH and GK) investigated. Gram staining indicated that AT, LD, SD, SH and GK was a Gram negative-bacterium.

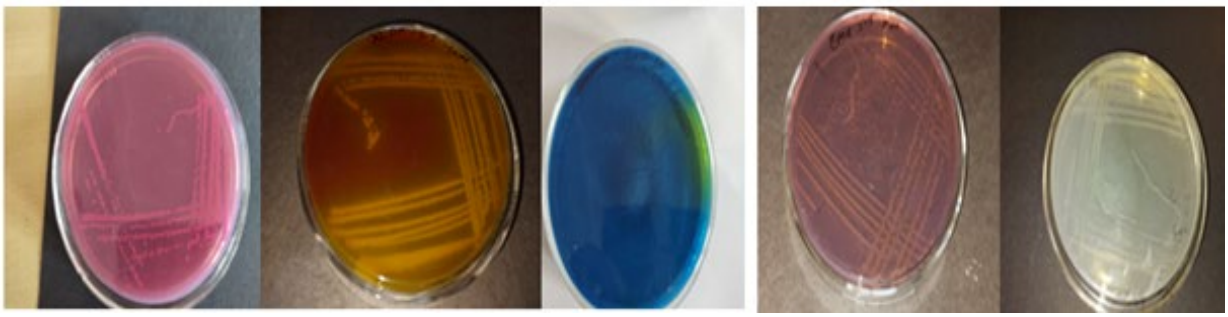


Figure 4 Purified cultures of bacteria on MacConkey agar medium, EMB, Simmons citrate and Nutrient agar medium

3.3 Colony Morphology in different medium and gram staining: colony morphology of all five isolates and gram reaction shows in Table 3

Table 3 Morphological characteristics and gram reaction of the isolated bacteria

Isolates	Color	Size	Form	Margin	Texture	Elevation	Opacity	Gram Staining
AT	Bluish Coloured	Medium	Circular	Entire	Smooth, Moist	Raised	Opaque	Gram -ve
LD	Yellow, green	Medium	Circular	Entire	Smooth	Slightly	Transluce nt	Gram -ve
SD	Cream Coloured	Large	Circular	Entire	Mucoid, Sticky	Convex	Opaque	Gram -ve
SH	Off White	Medium	Circular	Entire	Smooth	Slightly	Opaque	Gram -ve
GK	Pale	Medium	Circular	Entire	Smooth	Slightly	Transluce nt	Gram -ve

3.4 Biochemical characterizations of microorganism isolate

The isolated bacteria displayed a unique colony with various colours, texture and shapes. Taxonomic identification of each isolate was confirmed by biochemical and sugar fermentation tests which include methyl red, Indole, citrate utilization, Voges-Proskauer, oxidase, urease, catalase activity, Triple sugar iron agar, H_2S production and carbohydrate fermentation (Table 4). The results of isolates were compared with Bergey's Manual of Determinative Bacteriology. The isolate AT and LD selected for molecular characterization by 16S rRNA sequencing and phylogenetic

Table 4 Biochemical test of isolates bacteria

S.No	Isolate num	Tests			
		Indole	Methyl Red	Voges Proskauer	Citrate
1	AT	-	-	+	+
2	LD	-	-	+	+
3	SD	+	+	-	-
4	SH	-	-	-	+
5	GK	-	-	+	+

3.5 Molecular Identification by16SrRNA sequencing

Total two isolate were subjected to 16SrRNA sequencing and their identities are as shown in Table.2. The 16S rRNA sequencing and BLAST results confirmed isolate identity as *Pseudomonas aeruginosa* LC726426 and *Klebsiella pneumonia* LC726427 are Enterobacter sp., The isolate AT strain DSM 50071was found to bear the closest phylogenetic relationship to *Pseudomonas aeruginosa*with a percentage identity of 98.53% and isolate LD strain DSM 30104 with a percentage identity of 99% and 100% *Klebsiella pneumonia, Klebsiella pneumoniae*e identified Enterobacteriaceae family organisms based on genetic distance and phylogenetic trees

Table 5. Molecular characterization and Accession no of isolated bacterial species

Isolate No.	**Strain name**	**Accession no.**
AT	*Pseudomonas aeruginosa*	LC726426
LD	*Klebsiella pneumoniae*	LC726427

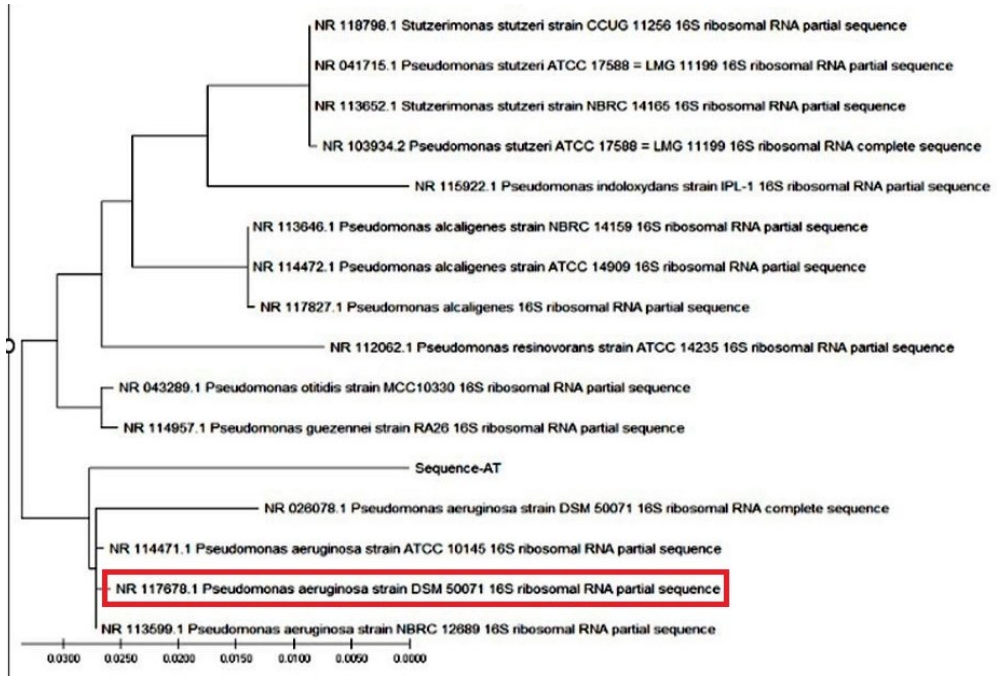
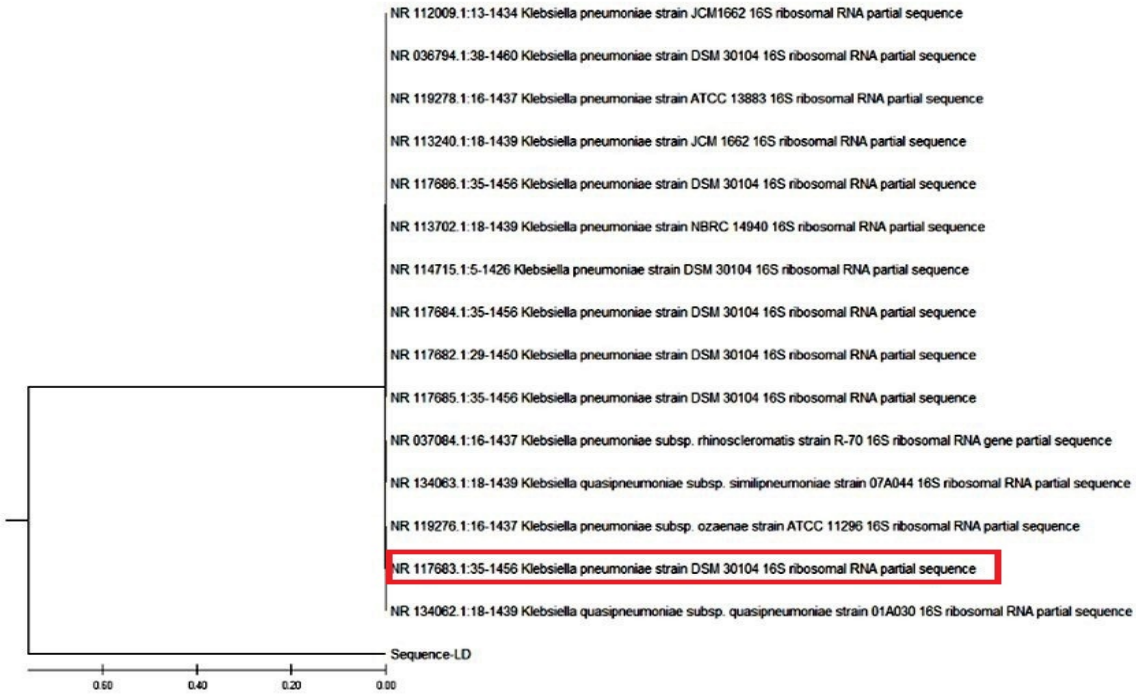


Figure. 5 Molecular phylogenetic showing the genetic relationship of the ATisolate using maximum on basis of likelihood method bacterial 16S ribosomal RNA sequencing



fication kit (Qiagen). They were then sequenced with a

Figure. 6 Molecular phylogenetic shows the genetic relationship of the AT isolate using maximum on basis of likelihood method bacterial 16S ribosomal RNA sequencing.

4. Discussion

Microbial pollution of domestic and tap water can be used to identify the presence of bacterial population (Eusebius, A. et al. 2024). The existence of pathogenic bacteria in tap or domestic water not only indicates the fecal contamination but also raises possible health concerns for humans (Haque, M.A. *et al.* 2019). Their presence in water source may indicate the occurrence of other infectious organism causing diseases such as cholera, gastroenteritis, dysentery, and typhoid fever. During this research the microbial contamination of tap and domestic water of selected hotel was assessed and monitored with both indicator and pathogenic bacteria. Current study found bacteriological counts of total coliforms (2400 MPN/l00ml) and fecal coliforms (920 MPN/l00ml) in various water bodies. The results show that water bodies are microbial contaminated, indicating potential public health hazards. The maximum allowable limit of total coliforms in drinking water is 1/100ml, according to ICMR (1975) and 10 /100ml, according to WHO (1993). E. coli can be utilized as a bio-indicator of underwater ecological systems, and their presence can help to assess water quality. Because coliform are considered as normal flora of intestinal tracts of humans and other warm-blooded animals, their presence indicated the evidence of fecal contamination. Bacterial genera such as E*. coli, Kelbsilla*sp, *Enterobacter* sp*, Shigella*sp, and *Salmonella* species were prevalent in water samples, which could be attributed to domestic and sewage waste contamination by numerous human activities.

The current studies show that bacterial densities in all samples collected increase the safe limits of drinking water standards, indicating that the water is unfit for drinking. According to (Chandra Mohan, N. *et al.* 2009). the main cause of water quality deterioration appears to be a lack of proper sanitation and domestic sewage mingling. Sewage is the most common leachate and one of the most important sources of pathogenic microbial contamination in domestic water. It includes a diverse range of pathogenic microbes. The study result also demonstrates the effect of season on drinking water and bacteriological quality. The rainy season had the highest feacal contamination, while the winter season had the lowest. It appears that during the winter, water levels drop and microbes do not reach lower levels (Venugopal, T. *et al.* 2009). Investigated the environmental impact and seasonal variation of drinking water.

Another study found that greater TC and FC levels were substantially associated with rainfall and sewage mixing (Crowther, J. *et al.* 2001). Whereas, the greater number of fecal coliform during the summer season may be responsible for high organic matter and excellent growth-supporting nutrients that promote bacterial development. The current studies indicate that all stations observed contamination, though the degree varied within the functional limit and the quality was rated unsatisfactory. The present condition of water supply in India is far from satisfactory.

Our study was isolate the gram-negative bacteria found in intestinal part in our body. Three colonies' isolates were obtained when using routine bacteriological procedures from domestic water sample (Tap and Drinking water) of some selected hotels during this investigation, and their physical, cultural, and biochemical characteristics were examined. A total of three microorganisms were isolated from domestic water sample and out of these two are screened further. The genera isolated in this study, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, have not previously been reported.

Pathogenic microorganisms, including bacteria, viruses, and protozoa, are primarily responsible for the health concerns associated with drinking water. Maintaining water sources' purity and protecting them from contamination present formidable challenges (Lin, L. *et al.* 2022). Two isolates of bacteria were identified as belonging to the genera *Pseudomonas and Klebsiella* (Lin, L. *et al.* 2022) and (Abada, E. *et al.* 2019) revealed that the most common bacteria in tap water samples were pseudomonas spp. The most significant microbiological illnesses spread through water include cholera, poliomyelitis, amoebic dysentery, bacillary dysentery, typhoid fever, and infectious hepatitis. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were found in mixed amounts in the water samples taken from some selected hotels. This might be the result of a pipeline leak causing sewage water and drinking water to mingle. Regularly checking the water quality for improvements not only guards against disease and dangers, but also stops further pollution of the water resources. Providing clean water as much as possible requires protecting water supplies from contamination by human and animal waste, which can harbour a variety of bacterial, viral, protozoan, and helminthic parasites.

5. Conclusion

Indore is the cleanest city in India, access of safe drinking water to urban area and hotels major priority of city. Indore is rich industrial sector and known for its special economic zone and city has registered/licensed restaurants. The major source of water distribution in hotel and restaurant is groundwater and it is the major challenge for the city is to provide safe and hygienic facilities that pose a risk to public health. Therefore, this

study aims to evaluate the bacteriological and physicochemical quality of tap and distilled water of some selected hotels in indore. It is concluded that most of the water sample are unacceptable and positive for total coliform test for E. coli which indicates fecal contamination of water source. Therefore, we recommend proper sanitation of water source and periodic bacteriological assessment of water supply. Besides this regular monitoring of damaged pipe and water distribution system will be effective in proper management system and reduce the of public health

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Conflict of Interest

The authors declare no conflict of interest.

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