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RESEARCH ARTICLE

The study of pathogenic bacterial species in the drinking water of different sources from Sehore District of Madhya Pradesh

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Abstract

Access to clean, sufficient drinking water is a fundamental human necessity. However, many regions, particularly developing nations, face challenges in providing clean drinking water. This study aimed to investigate the presence of pathogenic bacteria in drinking water samples from Sehore district of Madhya Pradesh, and highlight the importance of rapid detection methods. Water samples were collected from various sources including taps, borewells, water tanks, and the Narmada river across different villages of Sehore district. These samples were cultivated on MacConkey , Cetrimide and Xylose lysine desoxyscholate (XLD) Agar medium and incubated at 37°C till 24 to 48 hours for bacterial growth. Each randomly selected bacterial colony was then subjected to Colony PCR using bacteria-specific primers for the detection of *Pseudomonas sp., Escherichia coli*, and *Salmonella sp.* The Colony PCR results confirmed the presence of these pathogenic bacterial species, with high presence of *Escherichia coli*, moderate prevalence of Pseudomonas sp. while low prevalence recorded for *Salmonella sp.* The presence of these bacteria could pose significant health risks to the local population. The study also utilized molecular and biochemical techniques to identify *Escherichia coli, Pseudomonas sp.* and *Salmonella spp.,* The findings indicated varying levels of contamination across different water sources, with certain samples showing higher concentrations of pathogenic bacteria. Furthermore, the study evaluated the effectiveness of rapid detection methods such as polymerase chain reaction (PCR) in quickly identifying pathogenic bacteria in drinking water. These methods proved to be valuable tools for timely detection of bacterial contaminants, underscoring the importance of regular monitoring and swift action to ensure public health and safety.

Keywords: PCR, Psedomonas sp., *Escherichia coli*, *Salmonella sp*., Madhya Pradesh.

Introduction

Access to safe drinking water is a fundamental human right and a prerequisite for good health. Contaminated drinking water poses a significant threat to human health, particularly in developing countries where water treatment infrastructure may be limited. According to the World Health Organization (WHO 2017), contaminated drinking water is estimated to cause diarrhoeal disease in around 1.3 billion people annually, with children under five particularly vulnerable. Even in developed nations, occasional outbreaks occur, as reported by LeChevallier et al. (1990), who found coliform bacteria in 19% of disinfected drinking water samples in the United States. The situation is particularly dire in India, where studies like that by Chauhan et al. (2015) in Delhi have revealed alarming levels of contamination. In their research, every water sample tested positive for coliform bacteria, and concerning levels of pathogens such as E. coli, Salmonella, Staphylococcus aureus, and Pseudomonas aeruginosa were found.

Pathogenic bacteria are microorganisms that can cause illness in humans (Smith *et al*. 1999). Their presence in drinking water sources can contaminate the water and lead to a range of diseases, including diarrhea, typhoid fever, cholera, and dysentery (Kapembo *et al*. 2019). These illnesses can have severe consequences, especially for children and immunocompromised individuals. Contamination of drinking water with pathogenic bacteria can occur through various pathways. Inadequate sanitation practices, improper waste disposal, and insufficient water treatment

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infrastructure all contribute to the problem. Additionally, agricultural runoff containing fecal matter can pollute water sources, introducing harmful bacteria (Akhtar *et al.* 2021). Beyond India, studies like that by Bain et al. (2014) in Ethiopia indicate pervasive contamination, with E. coli present in 82% of rural household drinking water samples. Such bacteria, indicators of fecal contamination, can cause a range of illnesses, from diarrhea to typhoid fever.

This paper focuses on the Sehore district of Madhya Pradesh, India, where water quality issues persist despite efforts to improve infrastructure. This study aims to investigate the presence and distribution of pathogenic bacteria in drinking water from various sources in the region, employing microbiological techniques. Understanding the extent of bacterial contamination in Sehore's drinking water could inform strategies to enhance water quality and public health in the area.

Materials And Methods

All the drinking water samples were collected in gamma-sterilized bottles and were kept in an ice pack to prevent any significant change in the microbial flora of the samples during the transportation. The water samples were transported to the laboratory in vertical position maintaining the temperature 1–4 °C with ice pack enveloped conditions (Greeson, 977). The 60 water samples were collected various drinking sources (taps, borewells, water tanks, and the Narmada river across different villages) from Sehore, Madhya Pradesh. The serial dilution method was opted for the water samples inoculation on different selective media. The media that can be used to cultivate microorganisms and support the growth of a wide variety of non-fastidious organisms. The water samples were inoculated on MacConkey (March *et al.* 1986) , cetrimide (Brown VI, Lowbury 1965)and Xylose lysine desoxyscholate (XLD) agar medium (Taylor 965) and incubated at 37°C till 24 to 48 hours for bacterial growth. The each randomly selected appeared bacterial colony was taken in the individual autoclaved PCR tube and incubate at 96^{0}C for 7 minute. These bacterial sample were used for the Colony PCR for the detection of the *Escherichia coli Psedomonas sp.,and Salmonella sp.,* using bacterial gene specific PCR.

Molecular identification of bacteria using colony PCR: Different random colonies were isolated form the plates and dissolved in 50 µL sterile distilled water in PCR tube and were subjected to PCR at 97^{0}C for 5 min for bacterial cell wall breakdown.

Results And Discussion

This study investigated the presence of pathogenic bacteria in drinking water samples collected from various sources in the Sehore district of Madhya Pradesh, India. The findings highlight the prevalence of bacterial contamination and emphasize the importance of rapid detection methods for safeguarding public health. The analysis using PCR identified varying degrees of contamination across water sources. *Escherichia coli* showed the highest prevalence, followed by a moderate presence of *Pseudomonas* sp. while low prevalence reported in case of *Salmonella* sp. These findings align with previous studies indicating *E. coli* as a common contaminant in water sources [Lee & Lee 2010]. The presence of these pathogenic bacteria poses a significant health risk to the population, as they can cause illnesses like diarrhea, dysentery, and abdominal cramps [WHO,2018]. The study successfully employed Colony PCR for the detection of target bacteria. This technique proved to be efficient and accurate in confirming the presence of pathogenic species. The rapid nature of PCR allows for timely identification of contaminated water samples, enabling prompt intervention

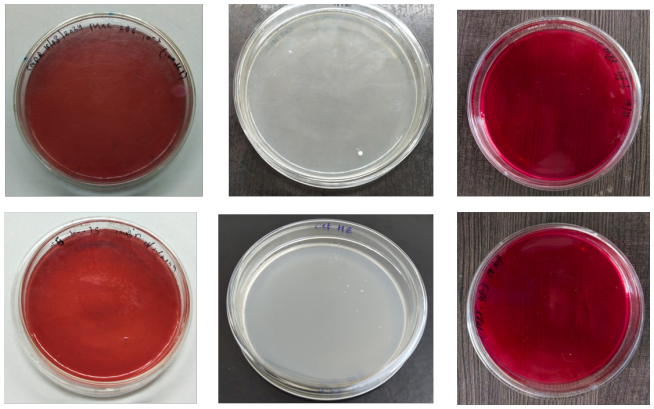

Figure 1: The figure showing selected images of isolated bacterial pure cultures during present study

Table 1: Colony PCR was performed using primers described in the table below

S No.	*Bacterial species*	*Primer*	*Sequence (5' to 3')*	*References*
		Pseu-OPRL FW	ATGGAAATGCTGAAATTCGGC	
1	*Psedomonas sp*	Pseu-OPRL Rv	CTTCTTCAGCTCGACGCGACG	Gholami *et al.*,2016
		Lac Z-F	CTTAATCGCCTTGCAGCACA	
3	*E. coli*	Lac Z-R	CAGTATCGGCCTCAGGAAGA	Foulds *et al.,* 2002
		ST 11	AGCCAACCATTGCTAAATTGGCGCA	
4	*Salmonella sp.*	ST15	GGTAGAAATTCCCAGCGGGTACTG	Lopes *et al.*,2018

to prevent outbreaks of waterborne diseases. This aligns with the growing emphasis on implementing rapid and reliable methods for water quality monitoring [Liu *et al.* 2012].

The varying levels of contamination observed across different water sources underscore the necessity for comprehensive water quality monitoring programs. Regular testing can help identify potential threats and ensure the delivery of safe drinking water to the public (Bartram and Ballance 1996) The findings of this study suggest that authorities in Sehore district should prioritize establishing a robust water quality monitoring system to safeguard public health (Chapman *et al.* 2021). The presence of pathogenic bacteria in drinking water samples from Sehore district highlights the public health threat posed by contaminated water. The study demonstrates the effectiveness of rapid detection methods like PCR (Eftekhari *et al.* 2021) and emphasizes the importance of regular water quality monitoring. By implementing comprehensive monitoring programs and prioritizing public health initiatives, steps can be taken to ensure access to safe drinking water and safeguard the well-being of the population in Sehore, Madhya Pradesh.

In present investigation, bacterial colonies of from water samples were grown on selective medium according to bacterial species (Figure 1). The isolated bacterial colonies were subjected to use for colony PCR as per standard protocol (Lopes *et al.* 2018) We have successfully performed the colony PCR ,and reported the presence of the *Psedomonas* (504 bp), *E. coli* (180 bp) and *Salmonella* species (429 bp) , bacterial species in collected from different water sources of Sehore district of Madhya Pradesh using established protocols (Figure 2).

As the Table 2 showed the most frequently found bacteria in the samples was Escherichia coli sp, with a

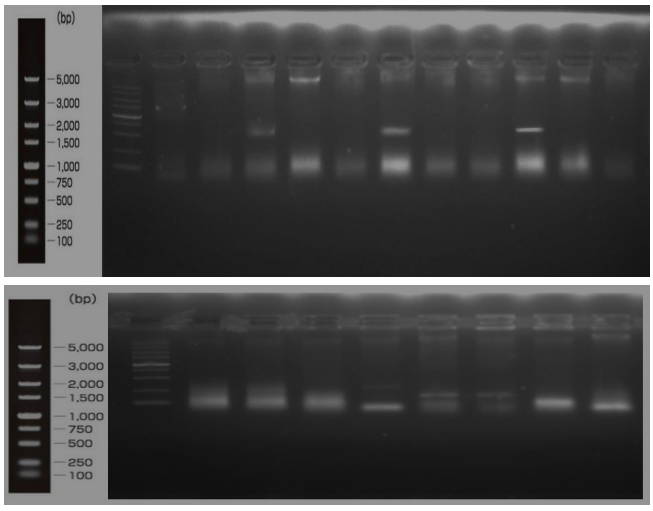

Figure 2 : Figure showing the PCR product of *Psedomonas sp.* (504 bp), and *E. coli* (180 bp)

Table 2 : Table showing the frequency of the bacteria species in collected 60 water samples

S. No.	*Bacteria*	*Targeted Bacteria gene*	*Bacteria present in 60 sample*
1	*Psedomonas sp*	OPRL	27
2	*Escherichia coli sp.*	Lac Z	41
4	*Salmonella sp*	ST	2

presence in 41 out of the 60 samples. This represents a frequency of approximately 68.3%. Escherichia coli sp is a common bacteria found in the environment, foods, and intestines of people and animals (Smith *et al.* 2019). It is most commonly associated with contaminated water or food and can cause serious food poisoning in their hosts. The second most common bacteria found was *Pseudomonas* sp, which was present in 27 out of the 60 samples, representing a frequency of approximately 45%. Pseudomonas sp is a common bacteria that can be found in most man-made and natural environments (Donnik *et al.* 2020). Special features of *Pseudomonas aeruginosa* strains in animal and poultry farms in the regions with various levels of man-made pollution. It is known for its metabolic versatility and can degrade a variety of pollutants. However, some species of *Pseudomonas* can cause infections, especially in people with weakened immune systems (Horcajada *et al.* 2019). Whereas 2 cases of *Salmonella* sp was reported in 60 samples. *Salmonella* sp is a bacteria that is most commonly associated with foodborne illnesses, often resulting from the consumption of contaminated food or water (Popa & Papa 2021).

The presence of these bacteria in the water samples could indicate possible contamination. The high frequency of *Escherichia coli* sp is particularly concerning, as it is often associated with fecal contamination, which could indicate the presence of other potential pathogens. The presence of *Pseudomonas* sp, while not as immediately concerning as *Escherichia coli* sp, could still pose potential health risks, especially for individuals with weakened immune systems. These results highlight the importance of regular water testing and treatment to ensure the safety of the water supply. Further studies should be conducted to identify the source of these bacteria and to determine the most effective methods for removing them from the water supply (Zulkifli *et al.*2018).

Conclusion of the study

The study on the presence of pathogenic bacteria in drinking water from various sources in the Sehore district of Madhya Pradesh has revealed significant findings. The study confirmed the presence of pathogenic bacterial species such as *Pseudomonas sp., Escherichia coli*, and *Salmonella* sp in the water samples. The prevalence of these bacteria varied, with a high prevalence of *E. coli*, moderate prevalence of *Pseudomonas sp*. but low prevalence of *Salmonella sp*

were reported. These findings indicate a potential health risk to the local population due to the consumption of contaminated water. The study also highlighted the effectiveness of rapid detection methods like colony PCR in identifying these bacterial contaminants swiftly. This underscores the importance of regular monitoring of water sources and immediate action to ensure public health and safety.

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