Original Article

The Rapid Pcr Detection Of Potentially Pathogenic Bacteria In The Drinking Water Of Different Sources From Bhopal District Of Madhya Pradesh

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Abstract

One of the essentials for human survival is access to clean, sufficient drinking water (WHO, 2015). But the major problems in the world, especially in developing nations, is the lack of availability of clean drinking water and challenges with its quality, Hence the present study were planned to study the presence of bacteria in the drinking water samples of Bhopal, Madhya Pradesh.The 200water samples were collected from household point-of-use (POU) in a pre-autoclaved vial. Nutrient Agar is a nutrient medium has usedfor the to cultivate of microorganisms. The NAM plates were incubated in incubator at 37 ^{0}C overnight for the desired bacterial growth. The each randomly selected appeared bacterial colony was taken in the individual autoclaved PCR tube, preformed the Colony PCR for the detection of thePsedomonas sp., Aeromonas sp., Escherichia coli and Salmonela sp. using bacteria specific primers for PCR.The Colony PCR accurately confirmed the presence of pathogenic bacterial species with moderate prevalence of Psedomonassp.,Aeromonas sp. and Salmonella sp. but Escherichia coli sp. showed high prevalence in drinking water samples from Bhopal districts of Madhya Pradesh, the presence of all pathogenic bacterial species could be one of the major threats to the health of the human population of the study area.

Keywords: Psedomonas sp., Aeromonas sp., Escherichia coli,Salmonela, Colony PCR.

INTRODUCTION:

Out of the top twenty significant health risk factors, consuming contaminated water is the potent cause. For instance, in 42 poor nations, contaminated drinking water was to blame for 90% of deaths under the age of five year old children. Diarrheal diseases, the main contributor to these preventable child fatalities, accounting for 88 percent of diarrheal illnesses, along with inadequate sanitation and poor hygiene [Pruss-Ustun*et al.*,2019].Human activity and environmental changes have put drinking water quality at danger, even in developed areas [Delpla I et al 2009].

In developed nations, "water-borne" diseases are characterised by disease epidemics linked to microbiological water pollution. A fecal-oral pathway is used to spread water-borne pathogenic pathogens [Levantesi C et al 2012]. Water-washed, water-based, water-related insect vector, and waterborne are the four different types of water-related diseases. The majority of them are caused by water of poor quality [Gleick PH et al 1993]. Septic tank waste, domestic animal waste, and waste from wildlife may be dumped into surrounding fresh water sources.Humans consume microbial organisms that exist in water through drinking water.

By examining the presence and concentration of faecal pathogen indicator bacteria, microbiological water quality can be evaluated. Both a qualitative and a quantitative approach can be used to examine the microbiological water quality. The qualitative approach seeks to determine whether bacteria are present in the sample [Clarke R et al 2017]. Simple and straightforward qualitative exams are available. To demonstrate the presence of bacteria in a sample, typically only a few millilitres of sample are combined with reagent. This technique works well for basic water quality monitoring but is less effective for determining the degree of contamination in the water. If one wants a more in-depth investigation of the water quality, one might employ a quantitative method after using a qualitative method as a first step.

The main sources of microbial pollutants in water, which cause microbial risks and outbreaks, include Salmonella, E. coli, Vibrio, and Campylobacter. The gastrointestinal tract of people and animals, which has a high demand for drinking water, is a common habitat of such bacterial infections. Excreta from people and animals that have these faecal indicators (enteric pathogens) can damage the environment, including water supplies, and give people a variety of diseases. To determine the effects of exposure, it is necessary to explore the role that aquatic environments play in the spread of highly pathogenic bacteria to different hosts. Nonetheless, culture methods remain popular because of their ease and simplicity.

Polymerase Chain Reaction (PCR) is one of the most widely used molecular methods for detection of a wide variety of microorganism in various clinical samples. The PCR assays have been developed for detection of Salmonella spp., V. cholerae, and E. coli in a wide variety of sample types such as water [Momba MN et al 2006].
In Present study we were focused to evaluate the selected bacterial species (*Psedomonas sp., Aeromonas sp., Escherichia coli* and*Salmonela sp.)*in drinking water of different sources from Bhopal district of Madhya Pradesh.

MATERIALS AND METHODS

Thewater 200 sampleswere collected from household point-of-use (POU)in apre-autoclaved vial from the various drinking sources in Bhopal, Madhya Pradesh. The serial dilution method was opted for the water samples inoculation on Nutrient Agar Medium. Nutrient Agar is a nutrient medium that can be used to cultivate microorganisms and support the growth of a wide variety of non-fastidious organisms.Nutrient agar is widely used because it can support the development of a wide range of bacteria and contains numerous nutrients required for bacterial growth. The NAM plates were incubated in incubator at 37 ^{0}C overnight for the desired bacterial growth. The each randomly selected appeared bacterial colony was taken in the individual autoclaved PCR tube and incubate at 97 ^{0}C for 7 minute. These Bacterial sample were used for the Colony PCR for the detection of the*Psedomonas sp., Aeromonas sp., Escherichia coli and Salmonela sp.*, using bacteria specific PCR.

Molecular identification of bacteria using colony PCR:

Different colonies were isolated form the plate and dissolved in 50 µl sterile distilled water in PCR tube and was subjected to PCR at 97^{0}C for 5 min for bacterial cell wall breakdown.

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

S/No.	Bac. Species	Primer	Sequence (5' to 3')	Reference
1	Psedomonas	Pseu-OPRL FW	ATGGAAATGCTGAAATTCGGC	Gholami et al.,2016
		Pseu-OPRL Rvas	CTTCTTCAGCTCGACGCGACG	
2	*Aeromonas*	Aer 2F	AGCGGCAGAGCCCGTCTATCCA	Mulamattathil et al., 2014
		Aer 2R	AGTTGGTGGCGGTGTCGTAGCG	
3	E. Coli	Lac Z-F	CTTAATCGCCTTGCAGCACA	Foulds et al., 2002
		Lac Z-R	CAGTATCGGCCTCAGGAAGA	
4	*Salmonela sp.*	ST 11	AGCCAACCATTGCTAAATTGGCGCA	Lopes et al.,2018
		ST15	GGTAGAAATTCCCAGCGGGTACTG	

RESULTS AND DISCUSSION

In present investigation, bacterial colonies of from water samples were grownon Nutrient Agar Medium (Figure 1.) The isolated bacterial colonies were subjected to use for colony PCR as per standard protocol (Gholami et al.,2016: Mulamattathil et al., 2014;Foulds et al., 2002; Lopes et al.,2018)

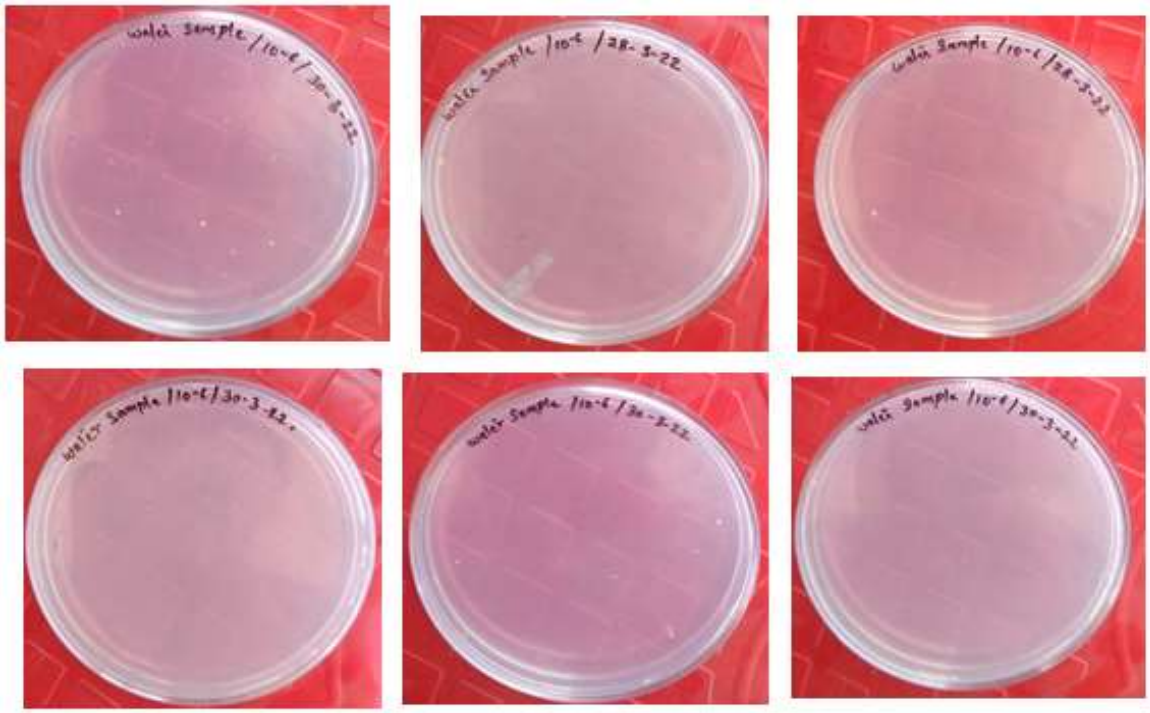

Figure 1: The figure showing selected images of isolated bacterial pure cultures during present study.

We have successfully performed the colony PCR ,and reported the presence of the Psedomonas(504 bp), Aeromonas (416 bp), E. Coli (180 bp) and Salmonela(429 bp) , pathogenic bacterial species in collected household point-of-use (POU) water samples from Bhopal district of MP., using earlier established protocols.

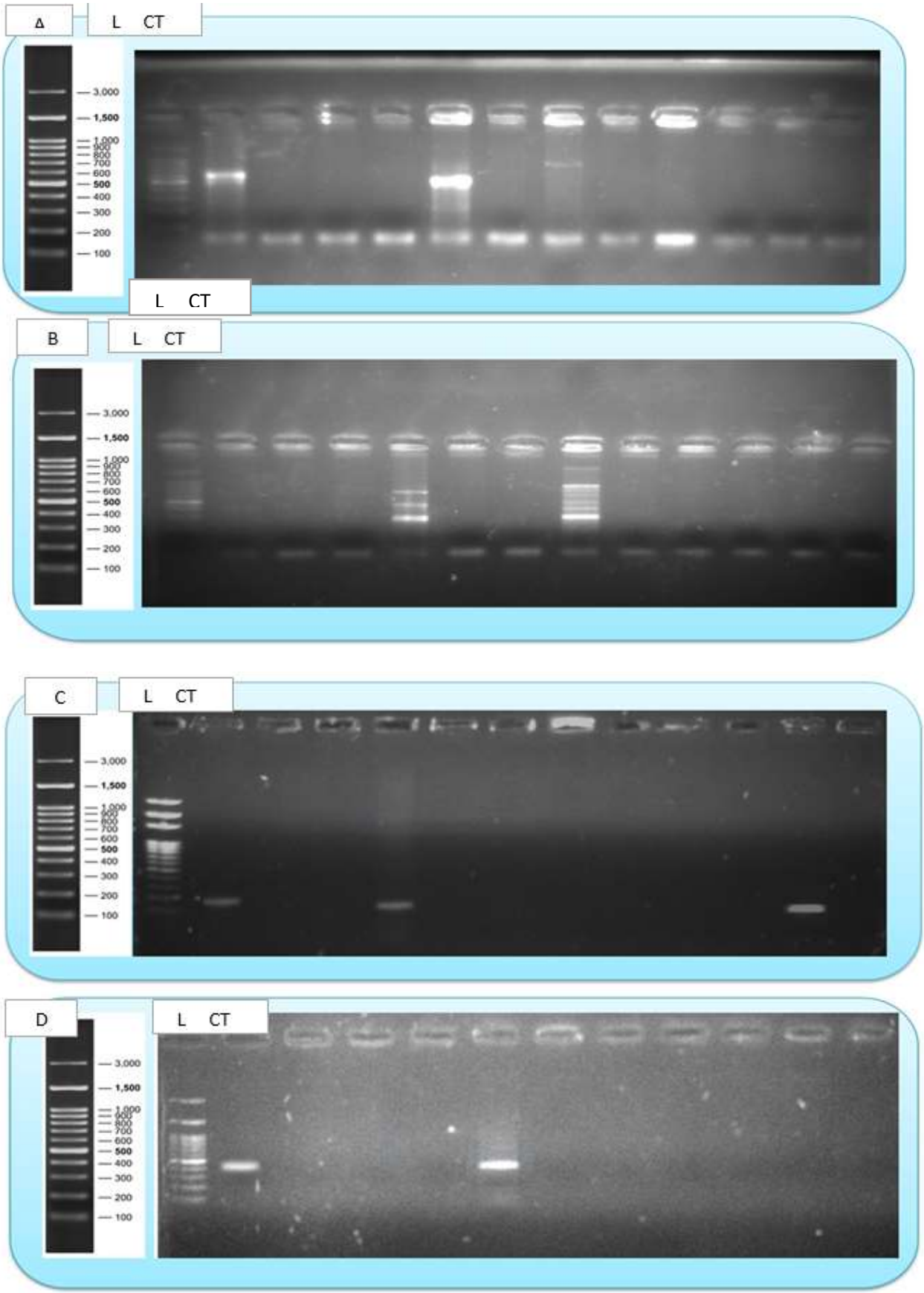

Figure 2 : Figure A showing the PCR product of Psedomonas sp. (504 bp), B Aeromonas (416 bp), E. Coli (180 bp) and Salmonela sp. (429 bp).

Table 2 : Table 1 showing the frequency of the bacteria species in collected 200 water samples.

S. No.	Bacteria	Targeted Bacteria gene	Bacteria present in 200 sample
1	*Psedomonassp*	OPRL	18
2	*Escherichia coli sp.*	Lac Z	22
3	*Aeromonas sp.*	AER	15
4	*Salmonelasp*	ST	06
Total			61

The earlier research articles studies also revealed that POU water is more contaminated than those of their sources (Wright et al., 2004; Mahmud et al., 2019). In this study we have reported the frequency of E coli was 11 %, in studied samples.*Escherichia coli*pathotypes have been identified as the pathogens mainly responsible for moderate to severe diarrhoea in low and middle-income countries (Navab-Daneshmand*et al.*,2018).
We have also identify the different prevalence rate of the bacterial speciessuch as *Psedomonassp (9%), Aeromonas sp. (7.5 %), Salmonelasp (3%)*in water sample of household point-of-use.Importance of detection of *Aeromonasspp*have increased in recent years due to its pathogenic properties for human, It may cause septicaemia , and diarrheal disease in Human (Janda,1991). In present study we have noticed its significant presence in drinking water (table 2). This study also shown the occurrence of *Salmonelasp*in water samples (table 2), the Salmonella ranks high among the pathogens causing foodborne disease outbreaks, According to the Centers for Disease Control and Prevention, salmonella contributed about 53.4% of all foodborne disease outbreaks from 2006 to 2017, and consumption yields about 32.7% of these foodborne salmonella outbreaks (Liu & Li,2018).

CONCLUSION OF THE STUDY:

The present investigation confirmed the presence of pathogenic bacterial species with moderate prevalence of*Escherichia coli sp, Pseudomonassp, Aeromonas sp.* and *Salmonellasp* in drinking water samples from Bhopal Districts of Madhya Pradesh, this could be one of the reasons for the frequent bacterial disease in the study area.

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