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Prevalence and incidence of *Escherichia coli* in different water sources in Jabalpur city

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Abstract
Jabalpur is the city with a large number of ponds and river tributaries which are the main source of water to drink. The study was needed for the quality of this water. The present study was done to observe the bacteriological quality of water in Jabalpur on the basis of isolation and characterization of *E.coli*. A total of 117 samples were collected from different water sources in Jabalpur city, comprising 20 each from different banks of river Narmada and public taps, 21 from tube wells, 35 from different ponds, 21 from hand pumps. The result showed an overall 15.38% prevalence of *E.coli* with sample wise prevalence of 00.00%, 4.76%, 10.00%, 15.00%, 34.00%, and 28.00% in tube wells, hand pumps, public taps, river Narmada and ponds, respectively. Characterization of *E.coli* with PCR revealed the presence of 83.33% *usp*A genes. Most of the samples collected ware having the presence of *E.coli* contamination.

Keywords: *Escherichia coli*, river Narmada, PCR, *usp*A

1. Introduction
Water is unique liquid, without which life is impossible. It contributes a number of ways to the health, progress and enjoyment of living beings. Human body contains almost 70% of it. It is having important functions like being universal solvent, thermo-regulation of body, maintenance of blood and plasma volumes, cellular osmotic pressure and assist in secretory and excretory functions of the body. Thus, water is an essential for life on earth. Visually clear and colourless drinking water is acceptable. However it should also be safe and free from chemical toxin and pathogenic microorganisms (Maheshwari, 2008) [1]. This "Elixir of Life" is facing a severe threat due to pollution. Poor sanitary practices lead to the growth of pathogens such as *Campylobacter jejuni*, enterotoxigenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholera* etc. causing mild to severe fatal form of diarrhoea. Abdominal pain or severe abdominal cramping often started suddenly. Watery diarrhea, beginning a few hours after the pain begins. It causes bright red bloody stools around a day later, resulting from the toxins damaging to the intestine (Medical News Today, 2017) [2]. Also, water on contamination by pathogens, may become dangerous to human health and cause various diseases.

Jabalpur is the third biggest urban agglomeration in Madhya Pradesh (Indian Population, 2017) [3]. It is an important trade, commerce, industrial, educational and administrative centre of regional and national importance. Good water resources are located around Jabalpur like river Narmada and many ponds, which is a source of animals and human consumption. River Narmada and ponds around Jabalpur receive a large amount of domestic wastes, sewage, agricultural and industrial effluents (Saxena *et al.*, 2016) [4]. There has therefore been an increased interest in the application of quantitative risk assessment for microbial load in drinking sources. This kind of pollution imparts a burden on public health which can be determined by the severity of the illnesses associated with pathogens, their infectivity and the population exposed. *Escherichia coli* is a typical member of the bacterial flora of the gastrointestinal tract of humans and other warm-blooded animals responsible widely used as indicators of faecal pollution in water quality assessment. *E.coli* may cause severe disease in the gastrointestinal tract and responsible for causing Haemolytic Uremic Syndrome (CDC, 2018) [5].

Thus the present study is proposed with the objective of the assessment of the bacteriological quality of water. Analyzing the viable coliforms along with other water born bacteria of three major community ponds which are used for human and animal bathing, washing clothes and also for drinking under water crises condition (winter). The data of this study may provide some important information about public health risks associated with the use of pond water in the region.

2. Materials and Methods
117 samples were collected from different sources of water located in Jabalpur city, comprising 20 each from different

banks of river Narmada and public taps, 21 from tube wells, 35 from different ponds, 21 from hand pumps.

Approximately 100 ml of water sample was collected from each source in a sterilized (autoclaved) sample collecting bottles. The bottles were tightly closed and labeled with the site and date of collection (Khadse, 2010) [6]. Then they were brought to the laboratory under sterile condition on ice for the bacteriological examination. All samples were processed for *E.coli* isolation within 24 hrs of arrival in the laboratory. For isolation of *E.coli* MacConkey lactose broth was used as enrichment medium and eosin methylene blue agar was used as selective media. Presumptive isolates were identified as *E.coli* on the basis of Gram's staining, motility and biochemical tests viz. catalase test, oxidase test, indole, methyl red, voges proskaeur and citrate utilization (IMViC) (Cruickshank *et al.*, 1975, Agarwal *et al.*, 2003) [7-8]. All the presumed isolates were subjected for polymerase chain reaction (PCR) based assays and screened for presence of *usp*A gene Primer used for PCR study is listed in Table No.1.

Table 1: Details of primer used for PCR reaction

Pathogen	Gene	Primer	Reference
E.coli	*usp*A (F)	CCGATACGCTGCCAATCAGT	Osek (2001)
	*usp*A (R)	ACGCAGACCGTAAGGGCCAGAT	

Template DNA incorporated in PCR reactions were prepared by boiling and snap chilling method. Overnight grown brain heart infusion (BHI, Hi-Media) broth cultures measuring 1.5 ml were taken into sterilized microcentrifuge tubes. The tubes were centrifuged at 10,000 rpm for 5 min. The supernatant was removed and the pellet remained as sediment was washed twice with sterile distilled water and after final washing the crude lysate was resuspended in 1 ml sterile distilled water. Further, tubes were incubated in boiling water bath for 20 min followed by immediate chilling on crushed ice for at least 20 min. Finally, tubes were centrifuged at 10,000 rpm for 2 min and 5 µl of clear supernatant was used as template DNA in PCR assay (Tripathi, 2015) [9].

PCR protocol

PCR reaction was setup in 25 µl of the reaction volume for amplification of the gene. The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture used in the PCR assay, by adjusting the annealing temperatures and cycling conditions as per need. To investigate the virulence potential of *E.coli*, isolates were subjected to PCR methodology with necessary modifications (Osek, 2001) [10]. In the present study, PCR was optimized with individual primer pair targeting the gene. Following initial optimization trials, reaction mixture was standardized in 25 µl volume containing 5 µl of purified DNA template, 2.5 µl of 10X Taq DNA polymerase buffer (20 mM Tris-HCl, pH 8.0, 1mM DTT, 0.1 Mm EDTA, 100 mM KCl, 0.5% Nonidet P40, 0.5% Tween 20 and 50% glycerol and 20 mM $MgCl_2$), 0.2 mM dNTP mix, 10 pmol of each forward and reverse primer, 1 unit Taq DNA polymerase and volume make up was done by autoclaved milli Q water. The reaction mixture was properly mixed and the amplification cycles were carried out in thermocycler (Bio-Red) with preheated lid (105°C). The standardized amplification reaction started with an initial denaturation at 94°C for 5 min, followed by 35 cycles each having denaturation at 94°C for 1 min, annealing at 52°C for *uspA* gene and extension at 72°C for 1 min, with a final extension for 10 min at 72°C.

On completion of PCR, amplified products were analyzed by agarose gel electrophoresis. Following agarose gel electrophoresis, PCR amplicons were visualized as a single compact band of the expected size under UV transillumination and documented by gel documentation system (Bio-Rad) and data were recorded photographically (Singh, 2016) [11].

3. Results

The identification of *E.coli* was done by various morphological and biochemical tests as described in materials and methods *viz.* Gram's staining, motility, catalase test, oxidase test and IMViC pattern.

Out of 117 samples examined, 18 isolates were obtained showing an overall prevalence of 15.38%. The highest prevalence of *E.coli* was observed in pond water (34.28%) followed by water from different banks of river Narmada (15%), public taps (10%) and hand pumps (04.76%). None of the water samples from tube well had *E.coli* as depicted in table No. 02. Isolates were further subjected for molecular identification using universal stress protein (*usp*A) gene which is a marker to differentiate pathogenic *E.coli* from other Gram negative. *usp*A gene was detected in 15 out of 18 isolates *i.e.*, 83.33%, as shown in table 03.

Table 2: Prevalence of *E.coli* from different water sources

S. No.	Samples Type	Number of samples	Number of samples positive for *E.coli*	Percentage of prevalence of *E.coli*
1.	Different banks of river Narmada	20	03	15.00
2.	Ponds	35	12	34.28
3.	Hand pumps	21	01	04.76
4.	Tube wells	21	00	00.00
5.	Public taps	20	02	10.00
Total		117	18	15.38

Table 3: Molecular characterization of *E.coli* isolates

S.No.	Isolates from various sources	Number of *E.coli* isolates	*E.coli* positive for *usp*A	
			Number of positive samples	Percentage of positive samples
1	Different banks of river Narmada	03	03	100.00
2	Ponds	12	10	83.33
3	Hand pumps	01	01	100.00
4	Tube wells	00	00	00.00
5	Public taps	02	01	50.00
Total		18	15	83.33

4. Discussion

Presence of coliform indicates the contamination of water with fecal and sewage material. It also indicates presence of intestinal origin pathogens. The present study was designed to detect the coliforms bacteria in water samples and to determine the water supply system being operated correctly for the availability safe water for drinking or food preparation. During the present study, the coliform bacteria have been found in all kinds of samples tested. In this study isolates showing characteristic colonies with a green metallic sheen on eosin methylene blue (EMB) agar were characterized on the basis of Gram's staining and IMViC pattern. Of the total samples examined, 18 *E.coli* isolates were procured with an overall prevalence of 15.38%. Category wise prevalence in case of different banks of river Narmada - 15.00% (3 / 20), ponds - 34.28% (12 / 35), hand pumps - 4.76% (1 / 21) and in public taps - 10.00% (2 / 20). None of the water samples from tube well had *E.coli* which may be due to water coming from a deeper stratum of earth that provides some degree of filtration.

Isolates were further subjected for molecular identification using universal stress protein (*usp*A) gene which is a marker to differentiate pathogenic *E.coli* from other Gram negative enterobacteria. *usp*A gene was detected in 15 out of 18 isolates *i.e.,* 83.33%, so our study indicates better association between this gene and isolates than Pandey *et al*. (2015) wherein he reported only 31.48% association (Pandey *et al.,* 2010) [12], Rani (2016) reported 100.00% association (Rani, 2016) [13].

Similar results with prevalence ranging from 11.82% to 23.64% were also reported by Mupidwar and his co-workers (Mupidwar *et al.,* 2015) [14] in their study conducted in Salburdi river and Kamptee river Maharashtra. Borah and others have found over 78.00% of tested ponds samples contaminated with *E.coli* (Borah *et al.,* 2010) [15] *a*nd Gogoi and Sharma also reported three ponds of Dibrugarh district having faecal coliforms *i.e., E.coli* (Gogoi and Sharma, 2013) [16]. Our study disclosed that ponds water was contaminated with coliform organism although with lower percentage comparatively. Improperly treated septic - sewage discharges, animal manure, storm water runoff washed into rivers or ponds may be responsible for this. One study conducted by Rani in Narmada river revealed 10.0% prevalence of faecal coliform organism which is in accordance to our findings (Rani, 2016) [13]. Nontongana and co-workers have also reported various *E.coli* pathotypes from the Kat river in South Africa Nontongana *et al.,* 2014) [17].

Hand pumps and tube wells are generally used to access to shallow / deep groundwater and considered microbiologically safe but in many cases they may contain significant levels of faecal indicator bacteria such as faecal coliforms and *E.coli*. Islam and others reported in their study that 41.00%, tube well water samples were contaminated with total coliforms and 13.00% with *E.coli* (Islam *et al.,* 2001) [18]. Coliform bacteria are found in shallow wells compared to deeper wells because bacteria are naturally filtered out by soil and rock as is noticed in tube well because surface water infiltrates into the ground. Though sometimes, deeper wells / tube wells may also contain coliform bacteria, if surface water gets entry into the well or if they are improperly constructed or poorly maintained. The contamination may be due to improperly treated septic and sewage discharges or leaching of animal manure. Aboh and his coworkers in 2015 found 20% of *E.coli* in his study for microbiological assessment of well waters in Samaru, Zaria, Kaduna, State, Nigeria. In our study 4.76% prevalence of *E.coli* was noticed in case of water samples from hand pumps but none of the water samples of tube well was found contaminated with *E.coli* organism (Aboh *et al.,* 2015) [19].

Modernization and industrialization in cities influence the quality of water directly or indirectly (Van, 2003) [20], (Wellen *et al.,* 2015) [21]. Presence of *E.coli* in public tap water indicates contamination either through a faulty distribution system or it indicates improper / insufficient treatment of drinking water. Irrigation with water of poor microbiological quality can elevate levels of bacyeria on produce. Benjamin and Mandrella in 2013 studied climate and management variables associated with generic *E.coli* in irrigation water on leafy green produce farms, where they found 13.8% prevalence of this bacteria (Benjamin and Mandrella, 2013) [22]. As such presence of coliform bacteria in drinking water does not always cause illness because most of these bacteria are harmless to humans. *E.coli* is the only member of the total coliform group of bacteria that is found only in the intestines of mammals, including humans, so total coliforms and *E.coli* are used as indicators to measure the degree of pollution and sanitary quality of drinking water. Momtaz and coworkers detected *E.coli* in tap water as well as in bottled drinking water in Isfahan, where they found 7.58% prevalence (Mumtaz *et al.,* 2013) [23]. The presence of *E.coli* in water indicates recent fecal contamination and may indicate the possible presence of disease causing pathogens, such as bacteria, viruses, parasites, etc (Park, 2011) [24].

5. Conclusion

In this study, most of the samples collected ware having the presence of *E.coli* contamination. This is indicative of the population located around these water resources is at public health threat. The water is needed to be treated prior to use of it and direct consumption should strictly be avoided.

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