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Prevalence of *E. coli* isolates from different sources of water in Jabalpur city

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Abstract
The aim of the study was to observe the prevalence of *E. coli* by their morphological and biochemical test in various source of water in Jabalpur city. Out of 135 samples examined, 16 of *E. coli* were obtained showing an overall prevalence of 11.85%.The highest prevalence of *E. coli* was observed in Panipuri water (30.0%) followed by water from household purifier (13.33%), Narmada river water(10.0%), tap water/public place water (9.33%) and 0% prevalence in branded drinking water.

Keywords: *E. coli*, house hold purifier, Pani puri, water

Introduction
Water is a primary necessity of life. In its absence man and animals would survive for no more than a few days. As much as 70-75% and more than 90% of mammals and aquatic fauna are made up of water, respectively. Water contributes in a number of ways to the health, progress and enjoyment of living beings. The biological, agricultural and industrial applications of water have been known for centuries. The various uses of water are enumerated such as it is a universal solvent, efficient transport medium for carrying nutrients to body organs and tissues, facilitates the thermo-regulation of the body, helps in the maintenance of blood and plasma volumes, cellular osmotic pressure and assists in the secretary and excretory functions of the body. It is a major constituent of enzymes, hormones and valuable medium for administration of therapeutic and health promoting preparations. It is used for ensuring environmental sanitation, efficient disposal of waste on forms and in food processing plants, land irrigation and power generation.

The rapid increase in human and animal population had changes the global ecology and environmental quality. Therefore, safety of water has become a matter of great concern due to enormous increase of pressure on natural water resources. These water resources are contaminated with suspended impurities like organic and inorganic impurities resulting into diseases like fluorosis, cyanosis etc. Besides, viruses, bacteria, protozoa, helminths, fungi and algae are also leading to outbreaks of water borne diseases in humans and animals [1]. Most of the microorganisms do not actually produce overt disease on first exposure, but several of them may become potential threats to health if exposure periods are long and their densities in water high**.** Drinking water is a major source of microbial pathogens in developing regions due to poor sanitation. Poor water quality, sanitation and hygiene account for 2-3 billion episodes of diarrhea resulting some 1.7 million deaths a year world-wide (3.1% of all deaths and 3.7% of all DALY's –Disability-adjusted life years), mainly in developing countries through infectious diarrhoea. Nine out of 10 such deaths are in children from malnutrition, dehydration and other complications of waterborne bacterial infections [2]. At high risk are the two billion people living in poverty in the developing world. Waterborne bacterial diseases cause a wide range of syndromes including acute dehydrating diarrhea (cholera), prolonged febrile illness with abdominal symptoms (typhoid fever), acute bloody diarrhea (dysentery), and chronic diarrhea (Brainerd diarrhea). These diseases have been found to be fatal many times, particularly if insufficient medical treatment is provided [3].

Material and Methods
A total 135 samples was collected from different resources of water in and around Jabalpur city. It includes 15 samples each of branded drinking water (in bottles and pouches) and waters from household purifier, 75 samples of tap water/ public place water, 10 sample of Narmada

river water and 20 samples of panipuri water were collected from different sources of Jabalpur city. Samples were transported to laboratory on ice and stored at 4 °C till further processing. All samples were processed for isolation within 2 h of arrival in the laboratory.

Approximately 250 ml of water samples were collected from vendors, homes, institute, restaurant / hotel, public place, local food stall and different ghat of Narmada river of Jabalpur city in sterile bottles and brought to laboratory under sterile conditions on ice for bacteriological examination. Packed bottles and water pouches and panipuri water samples were collected as such and in sterilized polybags respectively from retail outlets and brought to laboratory in chilled and sterile condition.

Enrichment by MPN method, three tube set in triplicate was used. The first, second and third set of tube had taken 10 ml double strength MacConkey lactose broth, 5ml single strength MacConkey lactose broth and 5ml single strength MacConkey lactose broth respectively. The water sample of amount 10ml, 1ml and 0.1ml were added in respective first, second and third set of tube followed by incubation at 37 °C for 24 - 48 h and then observing production of gas and change in colour of the medium.

Plating on selective media

The enrichment inoculums (0.1 ml) was streaked on MacConkey lactose agar (MLA, Hi-Media) plates and incubated at 37 °C for 24 h. Lactose fermenting pink, smooth, round colonies were then streaked onto eosin methylene blue agar (EMB, Hi-Media) plates and incubated at 37 °C for 24 h. Colonies showing metallic sheen were picked up and considered as presumptive *E. coli*. Suspected colonies from solid media were picked up and tested for biochemical reactions.

Morphological and biochemical identification of *E. coli* isolates

The presumptive isolates of *E. coli* were microscopically and biochemically characterized on the basis of colony morphology, gram's staining, motility, catalase test, oxidase test, IMViC pattern, triple sugar iron test, gelatin liquefaction test and urease test according to method described by [4, 5].

Microscopic examinations

Gram's staining

The colonies showing typical metallic sheen and black surrounded by a narrow green or green blue margin were selected for gram's staining. Gram-negative reaction with short rods and coccobacilli were presumptively considered positive for *E. coli*.

Motility

The motility of bacteria was examined after incubation of culture in brain heart infusion broth. The hanging drop method as described by C [4] was followed.

Biochemical identification

Suspected colonies from solid media were picked up and tested for biochemical reactions as per the method described by [4, 5] with certain modifications as and when required.

Catalase test

A drop of 3% (v/v) hydrogen peroxide was taken on a clean, grease free glass slide. The suspected colony was picked up and added with the help of flexiloop. A positive test was indicated by appearance of gas bubbles within 30 seconds.

Oxidase test

The suggestive isolates, as revealed on the selective media were picked up and tested for the oxidase reaction using the oxidase disc (Hi-media). With the flexiloop small amount of culture was smeared on the oxidase disc. The positive reaction was indicated by the development of violet colour within 30 seconds.

Indole test

In this test, the microorganism is incubated in tryptone broth (Hi-media) at 37 °C for 24 h followed by addition of Kovac's reagent (Hi-media, Appendix). Appearance of red coloured ring was taken as positive.

Methyl-Red (MR) test

Presumptive isolates was inoculated in tubes containing 5ml of glucose phosphate broth (Hi-media). After 48 h incubation at 37 °C, 5 drops of MR solution was added. Appearance of red colour immediately was taken as positive for MR test. In case of negative result (yellowish-orange colour), incubation of the broth was continued for an additional three days and retesting of the broth culture was done.

Voges-Proskauer (VP) test

In this test also Glucose phosphate broth (Hi-media) was used to test presumptive isolates of followed by 72 h incubation at 37 °C. After that Barrit's reagent solution A and B (Hi-media) was added. Appearance of pink burgundy colour was taken as positive for VP test, and in case of negative reaction copper/yellow colour was observed.

Citrate utilization test

For citrate utilization, commercially available Simon's citrate medium (Hi-media) was used. Slants were prepared and cultures to be tested were inoculated and incubated at 37 °C for 24 h. Change in colour from green to blue was taken as positive while no colour change was taken as negative for citrate utilization.

Gelatin liquefaction test

Isolates were examined for gelatinase production by stab inoculation in gelatin agar (Hi-Media). Pure cultures of isolates were stabbed individually into media and were incubated for 48 h at 37 °C. Thereafter, tubes were placed under refrigeration for 30 min at 4 °C. Tube that did not solidify after refrigeration was considered positive for gelatinase production.

Urease test

Some organisms produce urease enzyme which hydrolyses urea with the formation of NH_3 and CO_2. The test organism was streaked on the surface of christensen's urea medium slants and incubated at 37 °C for 24 - 48 h. Development of a pink colour in the medium due to the pH becoming alkaline indicated a positive result.

Triple sugar iron (TSI) agar test

For detection of H_2S production, triple sugar iron agar media (Hi-Media) was used. Slants were prepared and organisms to be tested were inoculated in both butt (stab inoculation) and slant and incubated at 37 °C for 24 h. Blackening of media was taken as positive for H_2S production.

Table 1: Biochemical tests for identification of *E. coli*

S. No.	Biochemical test	Reaction
1.	Catalase	+ve
2.	Oxidase	-ve
3.	Indole production	+ve
4.	MR test	+ve
5.	VP test	-ve
6.	Citrate utilization	-ve
7.	Urease	-ve
8.	Gelatin liquification	-ve
9.	H_2S Production (blackening)	-ve

Result

Out of 135 samples examined, 16 of *E. coli* were obtained showing an overall prevalence of 11.85%.The highest prevalence of *E. coli* was observed in panipuri water (30.0%) followed by water from household purifier (13.33%), Narmada river water(10.0%), tap water/public place water (9.33%) and 0% prevalence in branded drinking water.

In panipuri water, highest prevalence was observed in water collected from vendors (30%) for *E. coli*. Similarly, the study in Narmada river water of different revealed that only Bargi dam had 50% presence of *E. coli*. In tap water/public place water home (20%), restaurant /hotel (10%), public place (0%) and local food stall (8.57%) and household purifier water home (0%), restaurant /hotel (0%) and institute (22.2%) prevalence observed and none of prevalence of *E. coli* had found in branded drinking water samples.

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

S. No.	Samples Type	No. of samples	No. of samples positive for *E.coli*	Prevalence of *E. coli* (%)
1.	Branded drinking water (bottles/ pouches)	15	-	-
2.	Water from household purifier	15	2	13.33
3.	Tap water /public place water	75	7	9.33
4.	Narmada river water	10	1	10
5.	Panipuri water	20	6	30
	Total	135	16	11.85

Table 3: Source wise prevalence of *E. coli* in water

S. No	Sample tested	Sample source	Number of samples	Number of samples positive for *E. coli*	Prevalence of *E. coli* (%)
1.	Branded drinking water (bottles/ pouches)	Brand	6	_	_
		Local	9	_	_
2.	Water from household purifier	Homes	3	_	_
		Institute	9	2	22.22
		Restaurant / Hotel	3	-	_
3.	Tap water /public place water	Home	15	3	20
		Restaurant/ Hotel	10	1	10
		Public place	15	_	_
		Local food stall	35	3	8.57
4.	Narmada river water	Bhedaghat	2	_	_
		Tilwaraghat	2	_	_
		Bargi dam	2	1	50
		Gwarighat	2	_	_
		Jilaharighat	2	_	_
5.	Panipuri water	Vendors	20	6	30

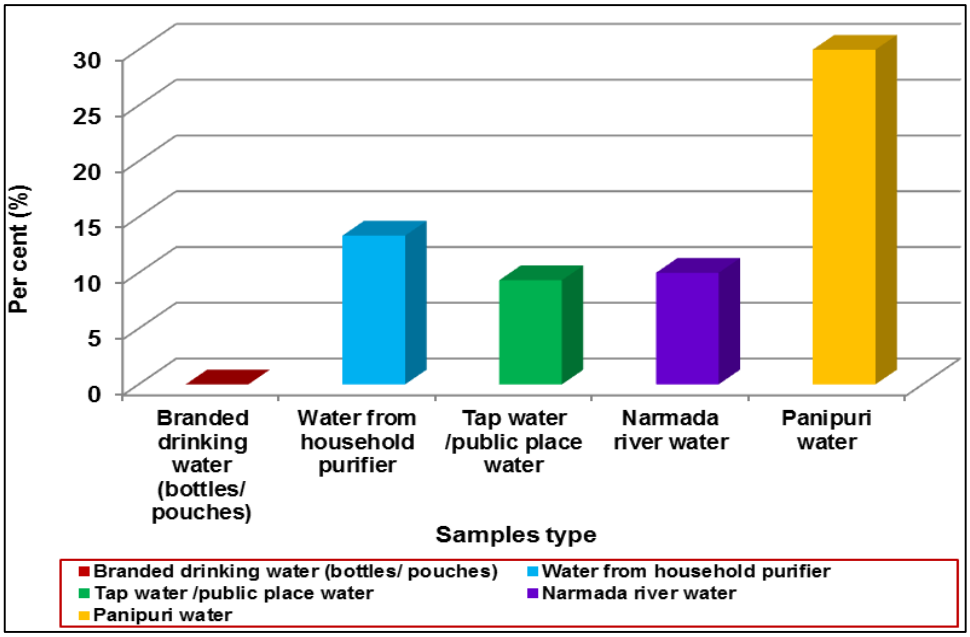

Fig 1: Sample wise prevalence of *E. coli* in water

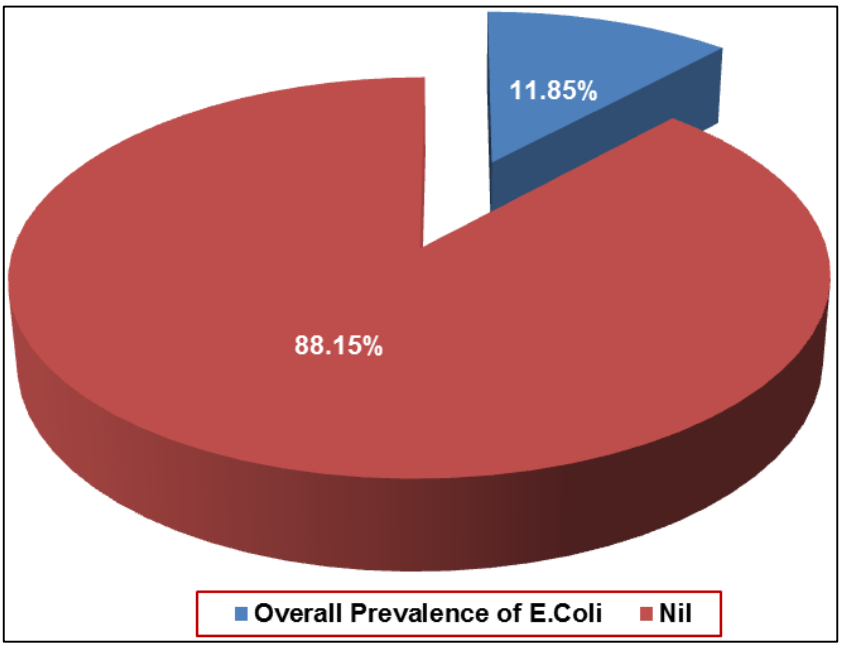

Fig 2: Prevalence of *E. coli* in water

Discussion

Out of 135 samples examined, *E. coli* were found in 16 samples. The higher prevalence was observed in panipuri water, water from household purifier, Narmada river water and tap water/public place water in descending order. *E. coli* was absent in branded drinking water.

Our study has also been supported by various co-workers [6]. In a study on microbiological quality of water from different sources, observed 82 (26.5%) *E. coli*. Similarly [7], examined 260 samples, and found 75 *E. coli* while [8], reported 79 *E.coli*. [9] Found 43% of *E.coli* in drinking water [10]. Revealed the presence of *E.coli* from Jaju Sagar Dam in M.P.

Co-worker from other countries like [11], examined tap water and bottled drinking water in Iran and found *E. coli* (7.58%), While [12] in USA, reported presence of *E. coli* in irrigated water [13]. Found the presence of *E. coli* (20%).

The presence of *E. coli* in drinking water or any kind of water is direct indication of fecal/sewage contamination and water is found to be unfit for direct consumption for humans. The presence of virulent strains of *E. coli* like O157:H7, verocytotoxin (stx), intimin (*eae*), enterohemolysin (*hly*) and enterotoxigenic (ETEC) is not considered in water because these strains are causing fatal diseases and are also associated with large numbers of outbreaks in and around world. These, pathogen are associated to precipitate disease in very less numbers (10-100). The presence of *E. coli* isn't recommended by any standard agency.

Conclusions

- The overall prevalence rate of *E. coli* was observed in water 11.85% with sample wise prevalence of 0.0, 13.33, 9.33, 10.0 and 30.0 percent in branded drinking water, water from household purifier, tap water/public place water, Narmada river water and panipuri water, respectively.
- *E. coli* considered as biological indicator of water and presence of *E. coli* in water it shows sewage contamination in water. *E. coli* is waterborne pathogen and causes waterborne diseases in humans and animals.
- *E. coli* is comparatively easy to detect, hence, waters are usually judged on their *E. coli* content.

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