Coliform bacteria presence in the household drinkable water

Sharat Chandra

ABSTRACT

Coliform bacteria are Gram negative non spore forming, oxidase negative, and rod shaped bacteria which are capable of aerobic and facultative anaerobic growth in the presence of bile salts (or other surface-active agents with similar growing inhibiting properties) and which are normally able to ferment lactose with production of acid and aldehyde within 48h when incubated at temperature of (36±2) ˚C **(Research Gate).**
They also possess the enzyme β Galactosidase and other decarboxylase enzymes it's tested by gas production in Durham's tube calculated by MPN table.

(IS: 16672)

Thermotolerant *E.coli* is coliform bacteria that are able to produce indole tryptophan within (21 ± 3) h at (44.0 ± 0.5) ˚C. **(Archieve.org)**

They also induces the enzyme β Galactosidase, give a positive confirmatory in methyl red test and can decarboxylate L –glutamic acid but can't produce acetyl methyl carbinol, utilise the citrate as the sole source of carbon or grow in KCN broth. Originally considered to represent only strains from the genera *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*. Classification of coliforms has been a difficult issue for decades **(Frontiersin.org).** Coliform differentiation was originally primarily based on the fermentation of sucrose and dulcitol, production of indole and acetylmethylcarbinol, and gelatine liquefaction. **(IS: 16672)**

Keywords: - Thermotolerant *E.coli*, Durham's tube, Kovac's Reagent, MPN Table,β Galactosidase, Coliform *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*, decarboxylase and L –glutamic acid.

Introduction

We collected the water sample from different sources of places in Gurgaon metropolitan cities metro areas, golf course, Honda chowk, Rajeev chowk etc. Then we perform the test its been conducted in different steps first the sample is been loaded in bottles tagged with different serial number from different sources and then preparing the media to confirmed the coliform test we concluded all steps very precisely. Liquid media is used MacConkey broth, media for confirming E.coli and Coliform, because this media will produced the gas production in Durham's tube (these are one side close and other open put inverted position). Then these are incubated for for 48h at this temperature of (36±2) ˚C. After this tubes is been taken out see the bubbling's of gas inside the Durham's tube , hence this test is primary to know the Coliform and E.coli is present inside the tube containing MacConkey broth media with sample water. For the E.coli test the secondary test confirmatory result we take the BGB broth media (Brilliant Green Bile Broth) is been placed in two different temperature one is set to (36±2) ˚C for 48h and other is set to the (21 ± 3)h at(44.0 ± 0.5)˚C.

E.coli tubes is been taken out from the incubator and adding the 2-3 drops of **Kovac's Reagent** is a biochemical reagent consisting of **isoamyl alcohol**, **para-dimethylaminobenzaldehyde** (**DMAB**), and concentrated **hydrochloric acid**. It is used for the important method for **indole test**, to determine the ability of the organism to split **indole rings** from the amino acid tryptophan (**Wikimili.com)**. There will be sudden change to the tube containing BGB medium broth pink colour of ring will be formed, since this confirmed test for the E.coli, Coliform will be taken out from the incubator and lactose broth is been prepared the lactose broth is been added to the tubes containing BGB which show false result in the red ring formation in it. If the gas is produced in the BGB broth it shows positive for Coliform bacteria. For isolation of the E.coli MacConkey media is prepared and autoclaving it at 121˚C for 15 min. And it's cooled down and media is poured in empty petri plate. Streaking with E.coli containing tubes will result in the pink coloured colonies as you can see in below fig.

Thermotolerant *E.coli* they can transmit the infection by the means of contaminated water supplies. These are highly adaptive to the temperature signifies the growth on the differential media. Infectious diseases caused by pathogenic bacteria, viruses and parasites are the most common and widespread health risk associated with drinking water. Nearly one-tenth of the **global disease burden** could be prevented by improving the water supply, sanitation, hygiene and the management of water resources **(WHO-1088)**

Water quality is affected by faecal matter, domestic and industrial sewage and agricultural and pasture runoff, in addition to a lack of awareness and education among the users .The detection of bacterial indicators in drinking water suggests the presence of **pathogenic organisms** that are sources of **waterborne diseases (Science Direct Pii)** .
Indicator microorganisms survive better and longer than pathogens, with uniform and stable properties, and may be easily detected using standard laboratory techniques.

These indicator organisms include *Escherichia coli*, thermotolerant (faecal) coliforms,totalcoliforms,faecal streptococci and *Clostridium perfringens* .**(Jetir.org)**

The two methods commonly used to detect coliforms in water include the multiple fermentation tube technique and the membrane filter technique.
All well water is an important source of drinking and household water in both rural and urban areas. Studies the microbiological quality of drinking water has found varying rates of contamination (0–100%) with faecal coliforms and other heterotrophic bacteria. We conducted a cross-sectional study to analyse and compare the microbiological quality of well
Water in rural and urban households.

Methods

This pilot study was conducted in Gurgaon Haryana (population—0.877 million) in India, over a period of two months between July and August of 2009 **(Science direct).** Forty households each from rural and urban areas were selected through simple random sampling **(Science Direct).** "Rural area" refers to a place with a population of less than 5000 people, a population density of less than 400 people per sq. km and more than 25% of the male working population engaged in agricultural pursuits **(Science Direct).** It is widely believed that urban well water sources are of good quality due to the availability of **disinfectants** and awareness of the need for disinfecting water wells. **(Science Direct)**

Informed consent was obtained from the head of each household before the water sample was collected **(Ajfand.net).** Well water sources (dug wells) used as the main source of drinking and household water were included in the study, and wells that were not in use or wells that were declared unfit for use were excluded from the study **(Science direct)** .All of the wells screened were used by single families. Municipal water sources or water from stored containers was not included in the analysis. **(Science Direct)**

Sample Collection

Following World Health Organization (WHO) guidelines, clean, heat-sterilized bottles of 200 ml capacity were used for the water collection **(Science Direct).** A stone of a suitable size was attached to the sampling bottle using a piece of string **(Science Direct)** .The bottle was opened and lowered into the well; the bottle was completely immersed in the water, without touching the sides of the well and without hitting the bottom or disturbing any sediment **(Science Direct).** The bottle was filled and then removed by rewinding the string. Approximately 20–30 ml of water was discarded to provide sufficient airspace to allow shaking before the analysis to achieve a homogenous dispersion of the bacteria. After collection, the bottles were labelled with complete details, including the source of the water, the sample site, the address, and the date and time of collection, and delivered (within 2 h) to the laboratory in a light-proof insulated box containing ice packs **(Science Direct).** Before sampling the well water, 4–5 drops of aqueous **sodium thiosulphate** solution (100 g/l) was added to the sampling bottles to neutralize any residual chlorine **(Science Direct)**

Because a complete history of chlorination (quantity, time since last chlorination) could not be elicited, all the sources were neutralized with sodium thiosulphate soon after collection, regardless of the prior chlorination status.

Method of analysis

The water samples were processed using the multiple fermentation tube method to determine the presumptive coliform count/most probable number (MPN) of coliforms based on standard methods. Suspensions from positive tubes were subculture on MacConkey agar and incubated at 37 °C for 24–48 h. The resulting colonies were identified following standard operating procedures. The antimicrobial testing of the isolates against commonly used antibiotics was performed using **Kirby-Bauer's disc diffusion method** and was interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. The detection of extended-spectrum beta-lactamase (ESBL) production was performed with a phenotypic method using a double disc synergy test. The microbial quality of the water samples was assessed based on WHO guidelines. The results of rural and urban areas were statistically compared using the Chi-square test of association. The testing of the water samples was performed according to standard operating procedures, which were strictly followed in the pre-analytical, analytical and post-analytical phases. Analytical quality control measures, including duplicate sample testing, were performed. The culture media were subjected to sterility and performance evaluations before the samples were inoculated.

Results

The present study investigated the quality of household water from 80 samples collected at the source in rural and urban communities. The faecal contamination rate, as indicated by the growth of ***E. coli*** **or** ***Enterococcus*** spp., was 27.5% (22 samples: 12 urban and 10 rural). Total coliforms were found in 74 (92.5%) household water sources (urban—39, 97.5%; rural—35, 87.5%)

Table 1. Isolation rates of indicator bacteria from water samples.

Bacteria	Urban	Rural	Total no. of samples	Percentage
Escherichia coli	12	07	19	23.75
Faecal streptococci	00	03	03	3.75

Bacteria	Urban	Rural	Total no. of samples	Percentage
Total coliform bacteria	39	35	74	92.5

A total of 44 (55%) well water sources showed the absence of *E. coli* and *Enterococcus* spp. and the presence of ≥10 coliforms per 100 ml. Nine (11.3%) samples without *E. coli* or *Enterococcus* spp. had lower coliform counts (<10 coliforms/ml). The absence of *E. coli*, faecal **streptococci** and total coliforms was found in only 5 (6.3%) samples [**Table 2**]. A total of 48 (21 urban and 27 rural) samples showed a high MPN of >180. However, the statistical analysis (Chi-square test of association) did not reveal any significant difference in the quality of the water samples between the rural and urban households (P = 0.39).

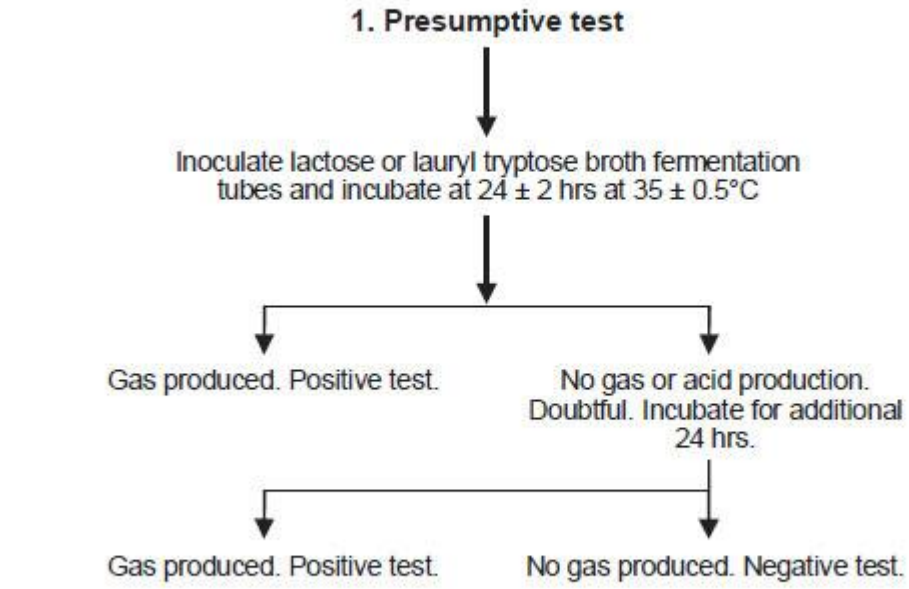
Table 2. Distribution of indicator bacteria and saprophytes in urban and rural water samples.

Isolates	Urban	Rural	Total
E. coli	2	1	3
E. coli and one coliform	3	1	4
E. coli and two coliforms	2	2	4
E. coli and a saprophyte	1	0	1
E. coli, a coliform and a saprophyte	4	3	7
Faecal streptococci	0	1	1
Faecal streptococci and a coliform	0	1	1
Faecal streptococci, a coliform and a saprophyte	0	1	1
One coliform other than *E. coli*	4	4	8
Two coliforms other than *E. coli*	6	6	12

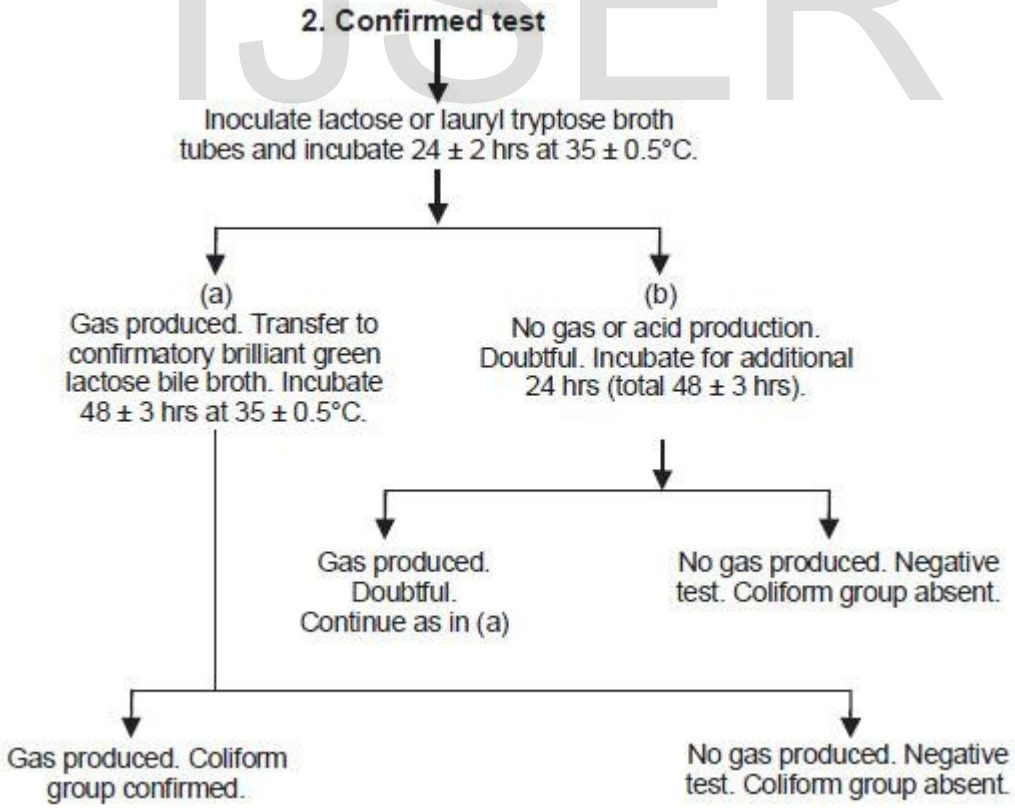
Isolates	Urban	Rural	Total
Three coliforms other than *E. coli*	0	1	1
A coliform and a saprophyte	11	12	23
Two coliforms and a saprophyte	6	3	9
Saprophytes	1	2	3
No growth	0	2	2
Total	40	40	80

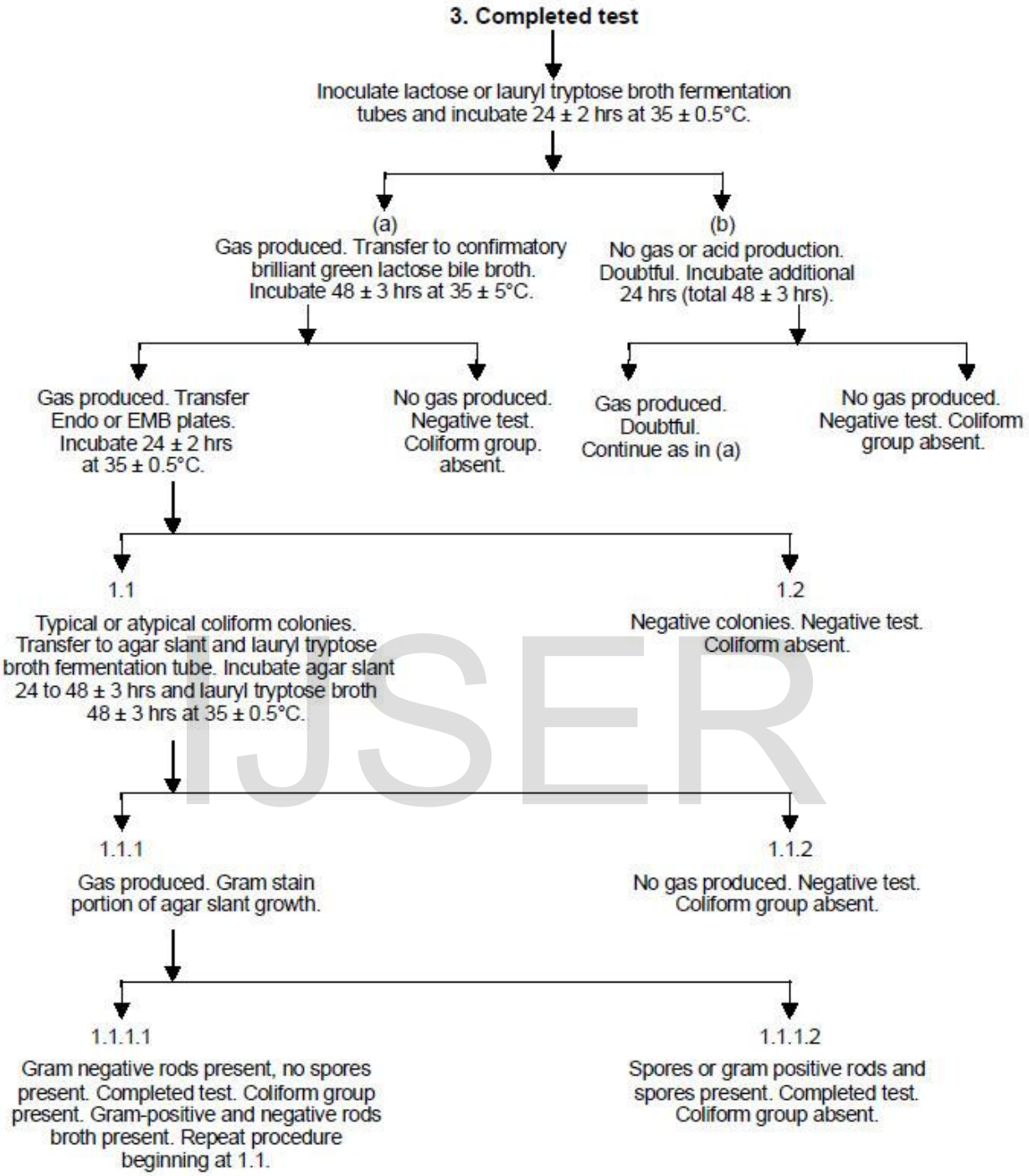
A total of 170 bacterial isolates were obtained and included coliforms (119, 70%), *Enterococcus* spp. (3, 1.8%) and saprophytic bacteria (48, 28.2%). The coliforms isolated included *Klebsiella* spp. (48, 28.2%), *Enterobacter* spp. (30, 17.6%), *Escherichia coli* (19, 11.2%), *Proteus* spp. (14, 8.2%) and *Citrobacter* spp. (8, 4.7%). The environmental saprophytes *Pseudomonas aeruginosa* (36, 21.2%) and *Acinetobacter* spp. (8, 4.7%) were also isolated from a significant number of samples [Table 3].

SCHEMATIC OUTLINES FOR THE E.COLI TEST PROCEDURE



Schematic outline of presumptive test for coliform detection.




3. Completed test
Inoculate lactose or lauryl tryptose broth fermentation tubes and incubate 24 ± 2 hrs at 35 ± 0.5°C.
(a)
Gas produced. Transfer to confirmatory brilliant green lactose bile broth. Incubate 48 ± 3 hrs at 35 ± 5°C.
(b)
No gas or acid production. Doubtful. Incubate additional 24 hrs (total 48 ± 3 hrs).
Gas produced. Transfer Endo or EMB plates. Incubate 24 ± 2 hrs at 35 ± 0.5°C.
No gas produced. Negative test. Coliform group. absent.
Gas produced. Doubtful. Continue as in (a)
No gas produced. Negative test. Coliform group absent.
1.1
Typical or atypical coliform colonies. Transfer to agar slant and lauryl tryptose broth fermentation tube. Incubate agar slant 24 to 48 ± 3 hrs and lauryl tryptose broth 48 ± 3 hrs at 35 ± 0.5°C.
1.2
Negative colonies. Negative test. Coliform absent.
1.1.1
Gas produced. Gram stain portion of agar slant growth.
1.1.2
No gas produced. Negative test. Coliform group absent.
1.1.1.1
Gram negative rods present, no spores present. Completed test. Coliform group present. Gram-positive and negative rods broth present. Repeat procedure beginning at 1.1.
1.1.1.2
Spores or gram positive rods and spores present. Completed test. Coliform group absent.

Schematic outline of presumptive confirmed and completed test for total coliform detection.

Gram Staining
Reagents

1. Ammonium oxalate-crystal violet (Hucker's)
2. Lugol's solution
3. Counter stain
4. Acetone alcohol.

Procedure

1. Prepare a light emulsion of the bacterial growth on an agar slant in a drop of distilled water on a glass slide.
2. Air-dry or fix by passing the slide through a flame and stain for 1minute with ammonium oxalate-crystal violet solution.
3. Rinse the slide in tap water and then apply Lugol's solution for 1minute.
4. Rinse the stained slide in tap water.
5. Decolorize with acetone alcohol till the stain is just removed.
6. Counter-stain with safranin for 15 seconds and then rinse with tap water.
7. Blot dry with blotting paper and view through the microscope.
8. Cells that decolorize and accept the safranin stain are pink and are defined as gram negative. Cells that do not decolorize but retain the crystal violet stain (deep blue) are defined as gram positive.

Steps in the gram staining are shown in the following table.

Step	*Procedure*	*Results*	
		Gram + ve	*Gram – ve*
Initial stain	Crystal violet for 30 sec.	Stains purple	Stains purple
Mordent	Iodine for 30 sec.	Remains purple	Remains purple
Decolonisation	95% ethanol for 10-20 sec.	Remains purple	Becomes colourless
Counter stain	Safranin for 20-30 sec.	Remains purple	Stains pink

Computation MPN Table (Most Probable Number)

The number of positive finding of coliform group organisms resulting from the multiple portion decimal dilution planting should be computed as the combination of positives and recorded in terms of the Most Probable Number (MPN). The MPN for the variety of planting series are presented in table in Appendix III. The values are at the 95% confidence limit for each of the MPN determined. These values are prepared for 10, 1 and 0.1mL combination. If the combination is 100, 10, 1mL, the MPN is 0.1 times the value in the table. If on the other hand a combination corresponding to 1, 0.1, and 0.01 mL is planted, record MPN as 10 times the value shown in the table.

The MPN for combination not appearing on the table or for other combinations of tubes and dilutions may be estimated by Thomas' simple formula:

$$\text{MPN/100 mL} = \frac{\text{No. of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}}$$

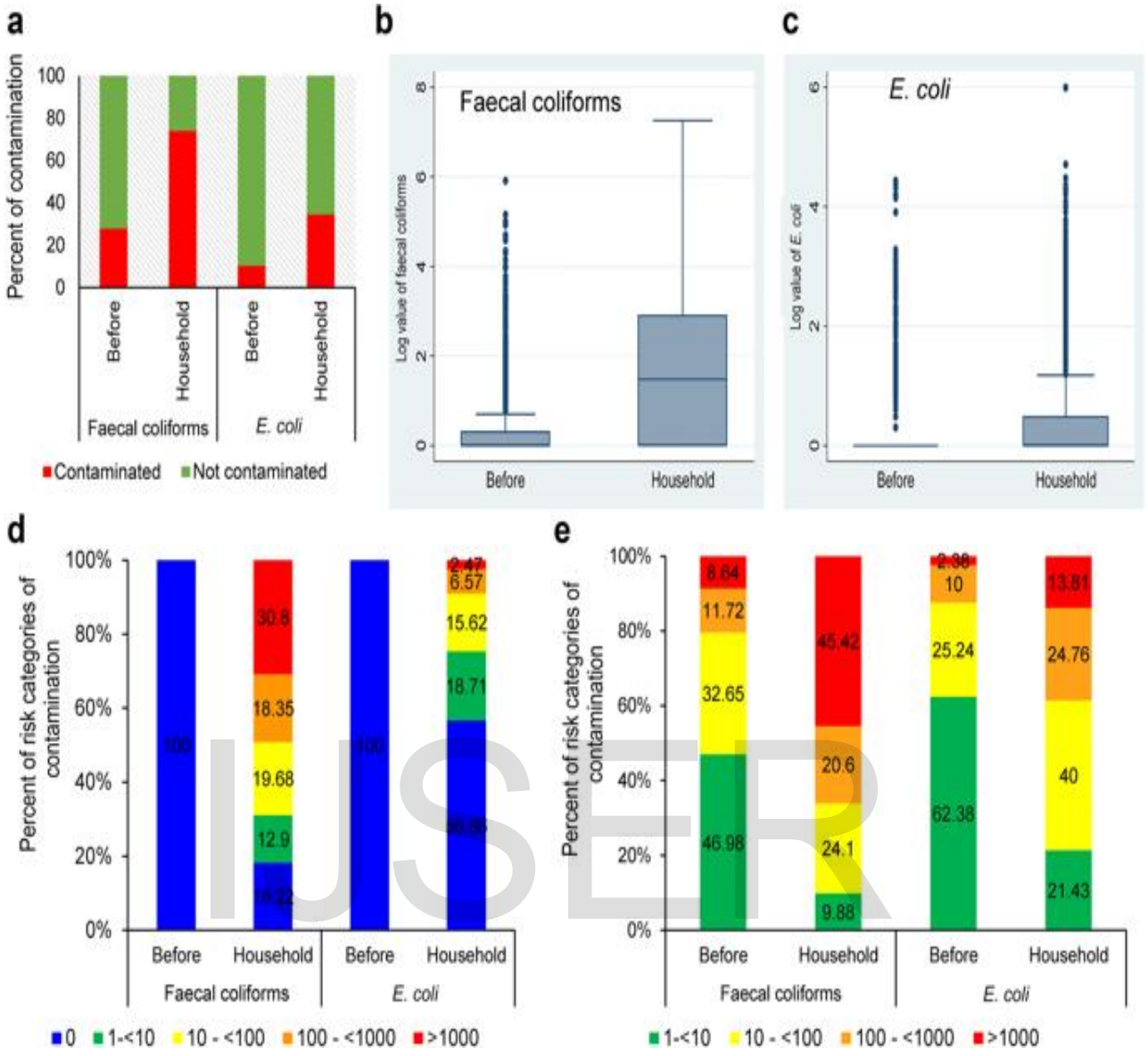
Introduction

Water is unsafe for human consumption when it is contaminated with pathogenic microorganisms, and an acceptable quality of water supply must be ensured for all. The prevalence of water-borne diseases including diarrhoea, cholera, typhoid fever, and dysentery, has been mainly attributed to unsafe water and unhygienic practices. Faecal contaminants going into the water supply could lead to a serious form of water contamination leading to the transmission of enteric pathogens such [3,4,5]as *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, and *E. coli*. These pathogens are usually found in human and animal feces and could possibly reach the sources of community water supply through leaching or other. Any potable water may be contaminated microbiologically due to insufficient sanitation and unhygienic practices. In order to estimate the number of microbes present and to find out microbial types, different microbiological water analysis methods are used in different labs. It is a very expensive and strenuous procedure to examine all the possible microbial pathogens in water, and therefore, a specific group of microorganisms that come from the same source as human pathogens is used to indicate the presence of pathogens. In order to indicate the presence of faecal contamination in water, indicator microorganisms were approved for the studies of coliform. If indicator

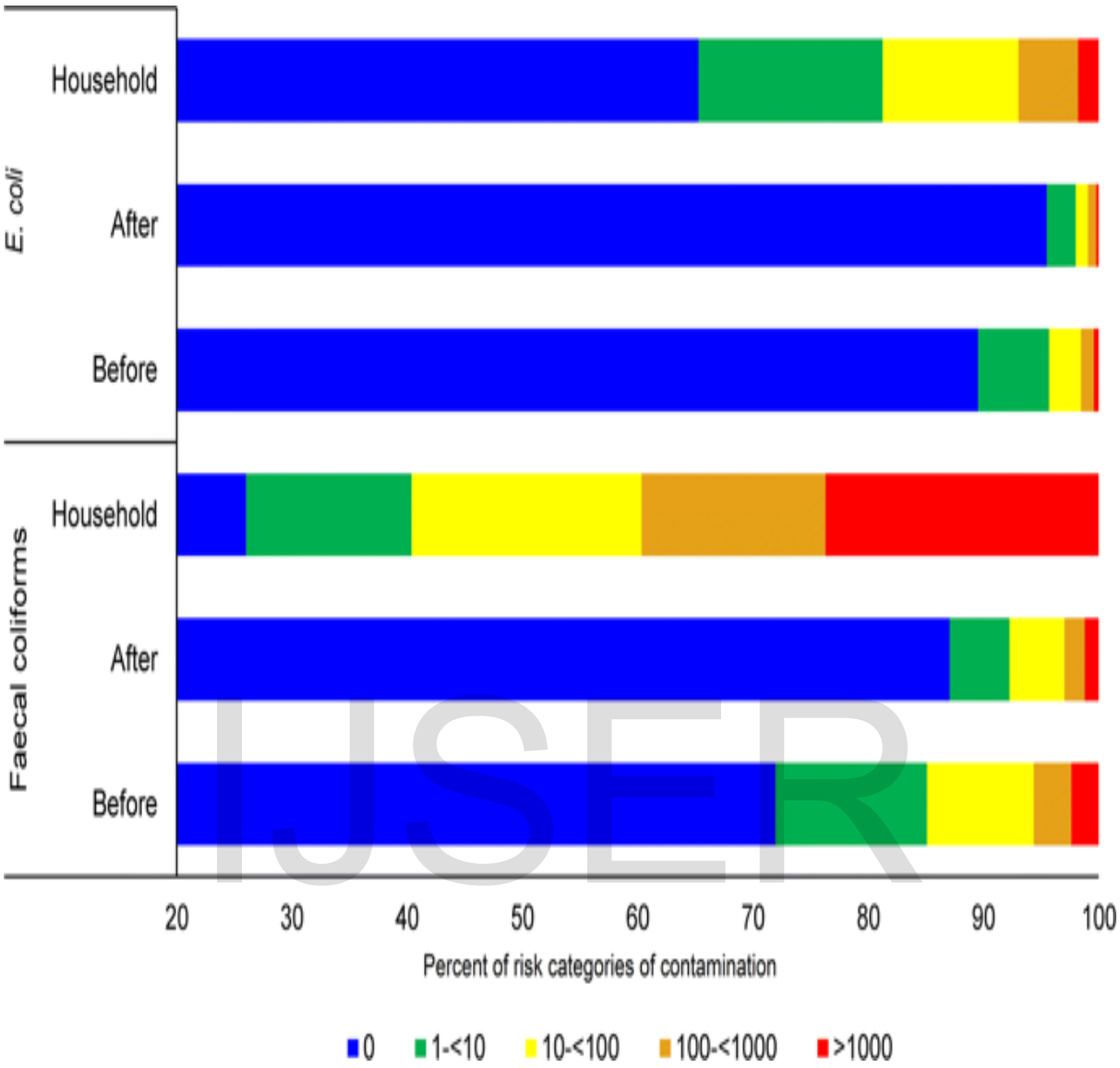
microorganisms are observed in a substance, it designates the presence of faecal contamination and therefore, pathogenic microorganisms might be present in that water.

Conclusion

We reviewed the available literature to identify studies that evaluated the relationship between microbial indicators of drinking water quality and diarrhoea. We found that studies using *Escherichia coli* as an indicator of household drinking water quality reported consistent effect estimates, that when pooled suggested a significant association with increased diarrheal illness. Results from studies using thermotolerant ("faecal") coliforms, on the other hand, were inconsistent, and suggested no association with diarrhoea when pooled. In this review, we also note several areas where the design and reporting of the included studies could have been improved, and make recommendations for future studies. The results from our review suggest that EC has value as a faecal indicator organism, but that use of FC should be considered carefully in contexts where an association with diarrheal disease outcomes is important. These results clearly indicate that there is a need for improved water storage, handling and transportation methods. Future studies must focus on working within communities to develop better water management strategies and foster community driven, sanitation focused, education initiatives. Combined, improved water testing, education and management could potentially solve the problem of contaminated drinking water within the SNP and SNP Buffer Zone and help support increased tourism.



Contamination scenario of faecal coliforms and *E. coli* in source and point of use water samples. **A** Bar diagram showing percent contamination of faecal coliforms and *E. coli*, before decontaminating the mouth of the tubewell and household (POU) water. **B** Box and whisker plot of log value of faecal contamination before decontaminating the mouth of the tubewell and POU water. **C** Box and whisker plot of log value of *E. coli* before decontaminating the mouth of the tubewell and POU water. **D**, **E** Bar diagram showing percent of risk categories of contamination of faecal coliforms and *E. coli*, before and after decontaminating the mouth of the tubewell and POU water



Risk categories of contamination. WHO (2011) has developed a classification and color-codes scheme for *E. coli* colonies per 100 mL water sample which (ii conformity with WHO guideline—blue (0/100 mL); (ii) low risk—green (1–< 10/100 mL); (iii) intermediate risk—yellow (10–< 100/100 mL); (iv) high risk—orange (100–< 1000/100 mL); (v) very high risk—red (> 1000/100 mL.

Results and discussion

Point-of-use water is far more contaminated than that of its source

A total of 3186 bottles were tested for faecal coliforms and *E. coli*, and we found that 28% (n = 893) were contaminated with faecal coliforms and 10.5% (n = 333) with *E. coli* (Fig. 1a). The contamination levels of household point-of-use (POU) water samples were far worse: a total of 6278 samples were tested and 73.96% (n = 4644) were found contaminated with faecal coliforms and 34.7% (n = 2179) with *E. coli* (Table 1 and Fig. 1a). Other studies also showed that POU water(s) are highly contaminated than those of their sources [8, 15]. It is well established that faecal coliforms can survive in water for longer periods than *E. coli*, so this leads to the thinking that the sources that are contaminated with faecal coliforms and not with *E. coli* might not be contaminated recently. *E. coli* is considered as the indicator of recent faecal contamination, and selection of *E. coli* is common because it is economical to detect and often present where faecal contamination is a problem [7]. *Bacteroides* spp. which is now being used as an experimental indicator of human faecal contamination and considered more reliable than *E. coli* as well as being species specific, is considerably more expensive [16, 17]. The odds of being contaminated by faecal coliforms in POU water are 7.42 times relative to source water (Table 2). On the other hand, the odds of POU water being contaminated by *E. coli* are 4.68 times than that of its source water (Table 2). We have determined and compared the risk-of-drinking of water from a contaminated source to non-contaminated source, and it was observed that the contamination rate increased in the highest risk category wherein the source was contaminated (Fig. 1d, e). It can be assumed that if the source water is not contaminated, then household water becomes contaminated due to unhygienic practices. Of note, our data suggest that when the source water is contaminated, then higher numbers of POU water samples could be graded in the highest risk category, which eventually increases the risk of infection many folds. In this study, only the source and POU water quality were monitored but how the POU water was contaminated was not determined. The contamination of POU could have multiple origins; from the vessels, or from the source water or could occur due to the unhygienic practices of the user. Nevertheless, the contamination of POU water is manifest, and the people are drinking it. Therefore, the determination of the source of contamination is highly important to target intervention, such as cleaning the vessels or treat the water with chlorination. In a challenging environment like Gurgaon camps with limited resources, it is recommended to build awareness of hygienic practices as well as provide interventions such as

chlorination of POU water to ensure safe drinking water. Although illiteracy or lack of formal education are barriers to understanding, awareness can be enhanced following the interventions, but an actual change of behaviour could often be low. Such awareness build up requires a long term effort, but short term interventions including on-site household decontamination are urgently required (chlorination, local low-cost UV purification units, etc.)

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