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Occurrence of Escherichia coli in Raw and Disinfected Water Supply: Correlation with Enteropathogens

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Abstract: *The concept of using Escherichia coli as a signal of fecal pollution is a well established practice in the assessment of drinking-water quality. The relationship between the presence and/or concentration of* ***Fecal indicator (E.coli)*** *and the* ***Intestinal pathogens (Salmonella spp., Shigella spp. and Vibrio cholerae)*** *in drinking water supply of Neemuch, Madhya Pradesh over 12 consecutive months was explored. The correlation of E.coli with Salmonella spp. and V.cholerae was found to be weak in samples from all the three kinds of supplies and poor relationship existed between E.coli and Shigella spp. in source water whereas no relationship existed between the two groups in samples analyzed after the treatment. The correlations between densities of indicator bacteria and pathogens were overall weak but primarily positive. The regulatory assumption that E.coli* is *indicative of the presence of pathogens was not strongly supported by our findings. Concurrent presences of E.coli and pathogens in most of the samples (98.6%) were quite remarkable but due to the lack of strong correlation between their densities, it may be inferred that E.coli may not always be effective surrogate for the presence of intestinal pathogens.*

Key Words: *Correlation, Density, Enteropathogen, Indicator, Supply*

I. Introduction

The pathogens most frequently transmitted through water are those which cause infections of the intestinal tract; namely, typhoid and para-typhoid bacteria, dysentery (bacillary and amoebic) and cholera bacteria, and enteric viruses. The causative organisms of these diseases are present in the feces or urine of an infected person, and when discharged may gain entrance into a body of water that ultimately serves as a source of drinking water. Enteropathogens usually appear intermittently in low concentration in an aquatic environment that is not ideal for either their growth or extended existence. Hence, to guarantee the good health of a community, there is need for readily available methods to detect and enumerate pathogens from aquatic sources. Several limitations render detection of waterborne pathogens difficult. The low microbial densities usually found in water necessitate the analysis of large volumes of water to detect pathogens effectively. This may limit the number of samples that could be processed at one time and make the procedure costly. For example, the detection of *Giardia* cysts requires filtering a minimum of 380 liters and in the case of *Cryptosporidium* up to 1000 liters of water. Moreover, due to the large variety of pathogens and the complexity of testing methods, it is unrealistic and often difficult to test for individual species. Unfortunately, no single procedure is available for the detection of all waterborne pathogens. As such, the use of indicator organisms becomes attractive. The idea behind indicators is that certain nonpathogenic microorganisms are present in the feces of all warm-blooded animals. These microbes are easily isolated and quantified using simple microbial methods and their presence reveals that fecal contamination has occurred and enteric pathogens are likely to be present in the water. ***Escherichia coli*** that normally reside in the gastrointestinal tracts of humans and animals are used throughout the world to assess the microbiological quality of drinking waters. The primary goal of this research study was to isolate and enumerate *E.coli* from drinking water of Neemuch city. Secondly, the study attempted to establish a correlation between occurrence and/or concentration of *E.coli* and *Salmonella* species, *Shigella* species and *Vibrio cholerae* to determine whether any relationship between fecal indicator *E.coli* and pathogens of intestinal origin, in drinking water could be discerned.

II. Materials and Methods

2.1 Sampling

Samples were collected on a bi-monthly basis from three sampling sites from the drinking water supply of Neemuch, a city of 1,27,000 inhabitants. A total of 72 unprocessed (*dam water*) and processed (*outlet & household water*) samples were analyzed during the period from January 2013 to December 2013.

2.2 Quantitative Enumeration of *E.coli*

Direct Plating Method was employed to enumerate *E.coli* from the samples. One ml aliquot from the undiluted 100 ml water sample was poured into sterile Petri dishes in triplicates, onto which was added 18-20 ml **Eosine Methylene Blue** agar media (**Pour Plate Method**). After incubation, characteristic colonies

of *E.coli* which produced a distinctive metallic green sheen were counted after being confirmed through IMViC tests (Indole, Methyl red, Voges prokauer & Simmon citrate), Oxidase test and Triple sugar iron agar test. Some additional tests were performed to further characterize the isolates (Fig 3).

2.3 Quantitative Enumeration of Enteropathogens

Salmonella spp., *Shigella* spp. and *V.cholerae* were also isolated and enumerated by applying the same procedure used for *E.coli*, using **Thiosulfate Citrate Bile Salts Sucrose agar (selective)** for *V.cholerae* and **Salmonella Shigella agar** was opted as a selective and differential medium for the isolation of *Salmonella* spp. and *Shigella* spp. All the media were purchased from Himedia Laboratories, Mumbai, India.

2.3.1 Biochemical Confirmation of *Salmonella* and *Shigella* spp: Colonies of presumptive *Salmonella* spp. (colourless & black centered) and *Shigella* spp. (colourless) were counted after confirming their identity through some biochemical tests; Triple sugar iron agar test, Urease production test, Indole production test and Motility test.

2.3.2 Biochemical Confirmation of *V.cholerae*: All the yellow (sucrose-fermenting) and blue-green (non-sucrose fermenting) colonies on TCBS agar plates were considered as total *Vibrio* colonies. To differentiate *V.cholerae* from other sucrose fermenting yellow vibrio's, all the yellow colonies were sub cultured on nutrient agar without added NaCl. The *Vibrio* able to grow on 0% NaCl and giving a positive oxidase test was confirmed as *V.cholerae* [1] and counted. Further biochemical characterization included Triple Sugar Iron agar test, Arginine Dihydrolase test, Ornithine & Lysine Decarboxylase test.

2.4 Statistical Analysis

Correlation analysis was conducted through MS Excel version 2007 using Log10 transformed values to determine the degree of correlation between counts of *E.coli* and pathogens. To test the stastical significance of correlation, one-way ANOVA was applied.

III. Results & Discussion

The results presented here represent occurrence, enumeration and seasonal prevalence of *E.coli* isolates in drinking water samples from three sampling sites; source (*dam water*), treatment plant (*outlet water*), and selected residences (*household water*). The presence and densities of fecal indicator organism; *E.coli* was correlated with the presence or absence of potentially pathogenic bacteria; *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae* to test the relationship between *E.coli* and pathogens.

3.1 Occurrence, Enumeration and Seasonal Prevalence of *E.coli*

Actual colony counts and log10 transformed values of *E.coli* before (*dam water*) and after (*outlet* & *household water*) treatment are summarized in tables 1, 2 and 3 respectively. It is evident from the tables that the isolation rate of *E.coli* was 100% in dam and household water samples (Table 1 & 3) and 95.8% in outlet water samples (Table 2). Figure 2 indicates rise and fall in the monthly mean log counts of *E.coli* obtained in dam, outlet and household samples. Statistical studies on log transformed counts proved significant differences ($P<0.05$) in *E.coli* counts among the three kinds of supplies, throughout the year. Several published studies address occurrence and survival of *E.coli* in drinking water habitats. Presence of *E.coli* in source water was also detected by Pratap Chandran et al. [2] in well water samples from ten different locations of Kanakkary Panchayath, Kottayam district, Kerala state and Dikobe et al. [3] who detected *E.coli* isolates in all the samples from Setumo Dam Mmabatho Area–North West Province, South Africa. According to Shar et al. [4] *E.coli* was thousand times higher than the WHO guideline values, in the samples studied. During the whole study period minimum *E.coli* count (1.82) in dam water samples was obtained in January (second sample-S2) and maximum (2.38) in July (first sample-S1). Study on seasonal variability in *E.coli* counts showed that seasonal average log count of *E.coli* was lowest in winter season (1.96) and highest in rainy (2.33) whereas in summer it was 2.20 (Fig 2). Seasons were categorized as summer: March-June, rainy: July-October and winter: November to February. The results of seasonal fluctuation study are similar to the findings of Hatha et al. [5] who obtained higher levels of *E.coli* counts during rainy months in Vembanadu Lake, along west coast of India and Shar et al. [4] who also concluded that the concentration of *E.coli* was significantly higher in summer months than in colder months ($p<0.01$).

The log counts in outlet water samples, as was expected, were significantly lower than were obtained in dam water samples ($P<0.05$), but significantly higher than WHO [6] and BIS [7] guideline values ($P<0.05$),

except in one sample of February (S1) which showed complete absence of *E.coli* in all the triplicates. Log counts in outlet water samples ranged from 0.51 (Apr S2 & July S1) to 1.98 (Oct, S1). It is quite noticeable that 95.8% samples from outlet water crossed the standard limit and only 4.16% had the zero count in accordance with WHO and BIS. Obasi et al. [8] in his study on Ero and Ureje dams in Southwest, Nigeria also found *E.coli* counts above the recommended standard of zero organism per 100ml of water as set by WHO 2002. According to the guidelines for drinking water quality, there is no tolerable lower limit for pathogens in water intended for consumption, preparing food, drink or for personal hygiene; it should contain no agents pathogenic to humans. But in contrast to this, throughout the whole study period, no sample from household water complied with the standard value of zero count. The log counts ranged from 1.36 (Feb, S1) to 2.11 (July, S2). Since *E.coli,* is the most preferred indicator organism whose presence in any water reflects the deterioration of bacteriological quality due to recent fecal contamination, its occurrence in each and every sample tested from the households is probably due to the inefficiency of *treatment plant*. This is hazardous for the health of those inhabitants of Neemuch who consume this unsafe municipal water. This result is in agreement to that of Juhna et al. [9] who also detected *E.coli* in biofilms from pipe samples and coupons in Drinking Water Distribution Networks and study of Daim et al. [10] in Al Gedarif city, in which raw waters, treated waters, main reservoirs, main pipelines, and sabeel zeer waters were highly contaminated with the coliforms and *E.coli*. One-way ANOVA analysis showed that during each month, all the household water samples showed significantly higher counts than the outlet water samples ($P<0.05$).

3.2 Coherence between *E.coli* and pathogens detections

Relationships between indicator bacteria detection and pathogens detection were examined (Table: 4) and their co-occurrence was observed in most of the samples analyzed. The fraction of water samples that contained an indicator when a pathogen was detected, was 100% in source and household samples, and 79.1% in outlet samples. The frequency of false negatives (pathogen present and indicator absent) was only 4.16% in outlet samples (Table: 4).

3.3 Correlation between *E.coli* and Pathogens

Towards the end of the 19th century, it was increasingly realized that some bacteria were specifically associated with fecal matter, most notably ***Bacterium coli*** described by Theodore Escherich in 1885. As this organism appeared to occur in great numbers in all feces, particularly compared to the enteric pathogens, it was suggested that detection of this *B.coli* in water would be indicative of the presence of fecal matter and, hence, the possible presence of enteric pathogens. Over the years, however, it became apparent that *B.coli* was actually a group of closely related bacteria, the principal species of which is now named *Escherichia coli.* It occurs in high numbers in sewage and water that has been subjected to recent fecal pollution by human and animal feces. It is believed to be of purely fecal origin, and has been found to be present in fresh feces in concentrations as high as 10^9 per gram. Its presence is detectable by simple, inexpensive methods. For these reasons, and because environmental conditions are unlikely to support *E.coli* growth outside of the intestine, this organism has come to be the preferred indicator of choice for fecal contamination. However, their presence does not necessarily mean that pathogens are present, but rather indicates a potential health hazard and likewise their absence is not necessarily evidence of pathogen absence. The question posed by this study is: How effective are *E.coli* counts in predicting the counts of potential pathogens, in this study, specifically *Salmonella* species, *Shigella* species and *Vibrio cholerae* in local drinking water supplied to the whole Neemuch city. Relevant reports from the literature show contradictory findings. Harwood et al. [11] tested the validity of using indicator organisms (total and fecal coliforms, enterococci, *Clostridium perfringens*, and F-specific coliphages) to predict the presence or absence of pathogens (infectious enteric viruses, *Cryptosporidium*, and *Giardia*) in disinfected effluent from six wastewater reclamation facilities in the United States. In his study indicators were not predictive of pathogen presence, and the results yielded a high percentage of false-negative or false-positive results for all indicator-pathogen combinations. Hence, no strong correlation was found in his study for any indicator-pathogen combination. In the study of Wilkes et al. [12] fecal indicators were surrogates of pathogenic-bacteria in almost all samples of an agricultural setting. Sinton et al. [13] found *E.coli* as the best indicator of bovine fecal pollution in New Zealand, however, St. Pierre et al. [14] observed weak correlations between the *Campylobacter* spp. and thermotolerant coliforms in environmental water. Likewise, study by Polo et al. [15] has reported little to no correlation of indicator bacteria with *Salmonella spp.* while Efstratiou et al. [16] found a strong positive association between indicator bacteria and *Salmonella spp.* McEgan et al. [17] found weak linear relationships between biological indicators (*E.coli*/coliforms) and *Salmonella* levels. Schriewer et al. [18] evaluated the value of indicators (total coliforms, fecal coliforms and enterococci) to predict the occurrence of waterborne pathogens (*Campylobacter* spp., *Escherichia coli* O157:H7, *Salmonella* spp., and *Vibrio* spp.) in ambient waters. He observed correlations between some, but not all, indicator-pathogen combinations. Results of study by Ferguson et al. [19] showed some statistically significant correlations of pathogens with fecal

indicators but the correlations were very weak. Whereas contrasting result was obtained by Patra et al. [20] who showed positive significant correlation of fecal coliform bacteria with presumptive *E.coli, Shigella, Salmonella* and *Proteus/Klebsiella, total Vibrio, V.cholerae, V.parahaemolyticus* and *P.aeruginosa.* According to Hatha et al. [5] there was no significant correlation between high levels of fecal coliforms and incidence of specific pathogens.

Our results show that there was no correlation between log numbers of the bacterial indicator *E.coli* and *Shigella spp.* within samples taken from the outlet ($r=0.09$, $P>0.05$) and households ($r=0.06$, $P>0.05$), whereas a weak correlation existed between the two groups in the samples analyzed from the source ($r=0.68$, $P>0.05$). The presence of fecal indicator bacteria was weakly correlated with *Salmonella* spp. ($r=0.37$ $P<0.05$, 0.29 $P<0.05$ & 0.28 $P>0.05$) and *V.cholerae* ($r=0.62$ $P<0.05$, 0.16 $P>0.05$ & 0.45 $P<0.05$) in all the three kinds of supplies (Tables 1, 2 & 3). In present study, concurrent presence of *E.coli* and pathogens lacking strong correlation between the densities does not strongly support the fact that *E.coli* is indicative of the presence of pathogens, because there is wide range of non-fecal sources responsible for the entry of pathogens into water bodies. Similar to this study, numerous publications have described the lack of correlation or presence of weak correlations between index microorganisms and pathogens in water [21,22,23]. Likewise, there are some other relevant studies which also reported little to no correlation between indicator bacteria and potentially pathogenic bacteria; Horman et al. [24] found no significant correlation between *E.coli* and isolated enteropathogens. Ahmed et al. [25] and Schriewer et al. [16] correlated fecal indicators, including *E.coli*, with potential pathogens, including *Salmonella spp.*, concluding that there was poor correlation between the two groups. DePaola et al. [26] examined the relationship between indicator bacteria and enteric viruses and found no significant relationship between the levels of indicator organisms and the presence of enteric viruses. In the study of Chao et al. [27] no *Salmonella* spp. were detected in any of the 107 samples positive for indicator presence.

Overall, on the basis of results obtained in present study and above mentioned studies it may be inferred that, there is clearly no one indicator that may be suitable for all pathogens for all environmental scenarios. In natural raw waters, the presence of pathogens may not result solely from a recent fecal contamination; indicators and pathogens concurrently in water, may come from different sources. Other possible factor might also be different densities of both the groups in original contamination sources which render the recovery of pathogens difficult from small sampling volumes. Hence, correlations between *E.coli* and pathogen's populations in *Jaju Sagar Dam* may not be explicit evidence of fecal contamination but rather of conditions favorable to the survival of all the groups of organisms. Although some correlation (weak) which existed between the numbers of *E.coli* and pathogens in *Jaju Sagar Dam* might be fairly site-specific and difficult to generalize. A lack of close correlation between the numbers of indicator and the three pathogens was also observed in treated and disinfected water supplies, which indicates that on subjecting to treatment strategies indicator and pathogenic bacteria responded differently. Following treatment and disinfection, the concentrations and frequency of detection of indicators and pathogens reduced at varying rates. Furthermore, survival and die-off of pathogen and indicator organisms in treated potable water depends on several factors including resistance to disinfection. Indicators can have persistence and survival characteristics different from those of pathogens. A true relation of indicator with pathogens within distribution system is not possible.

IV. Conclusion

For a variety of reasons, *E.coli* may not always be effective surrogate for the presence of intestinal pathogens. Pathogens and *E.coli* vary considerably with respect to a variety of factors that will influence their fate and transport in the environment; such as the size of the microorganism, abundance in feces, environmental fitness, and nature of hydrological processes that transport the organisms to and within the aquatic environment. *E.coli* may be considered as best indicator for recent fecal contamination instead of pathogen's presence.

Table 1: Correlation between counts of *E.coli* and Pathogens in unprocessed water samples taken from the dam

Months	*E.coli* Counts		Intestinal Pathogen's Counts					
			Salmonella spp.		*Shigella* spp.		*V.cholerae*	
	Count/ml	Log Count/ml	Count/ml	Log Count/ml	Count/ml	Log Count/ml	Count/ml	Log Count/ml
January	8.33	1.92	7.33	1.86	8.00	1.90	18.0	2.25
	6.66	1.82	6.00	1.77	9.30	1.96	19.6	2.29
February	9.00	1.95	8.00	1.90	7.00	1.84	10.6	2.02
	10.3	2.01	10.0	2.00	7.33	1.86	12.3	2.08
March	11.6	2.06	10.6	2.02	13.0	2.11	22.0	2.34
	14.0	2.14	11.6	2.06	8.66	1.93	21.6	2.33

April	16.6	2.22	9.00	1.95	17.3	2.23	16.3	2.21
	17.3	2.23	5.66	1.75	11.0	2.04	14.3	2.15
May	18.0	2.25	17.0	2.23	15.6	2.19	28.3	2.45
	15.6	2.19	14.3	2.15	9.00	1.95	27.0	2.43
June	16.3	2.21	13.6	2.13	16.3	2.21	22.6	2.35
	20.0	2.30	9.33	1.96	13.0	2.11	21.6	2.33
July	22.0	2.34	15.6	2.19	19.0	2.27	45.0	2.65
	24.3	2.38	14.0	2.14	17.0	2.23	41.0	2.61
August	18.0	2.25	13.0	2.11	18.0	2.25	38.6	2.58
	20.3	2.30	12.6	2.10	15.0	2.17	36.6	2.56
September	19.6	2.29	12.0	2.07	18.3	2.26	36.6	2.56
	23.0	2.36	10.6	2.02	14.3	2.15	33.6	2.52
October	24.0	2.38	8.00	1.90	12.3	2.08	25.0	2.39
	21.6	2.33	11.3	2.05	14.6	2.16	21.6	2.33
November	9.00	1.95	9.00	1.95	10.0	2.00	21.3	2.32
	12.0	2.07	13.0	2.11	15.0	2.17	18.3	2.26
December	8.00	1.90	14.0	2.14	11.5	2.06	20.6	2.31
	12.6	2.10	10.3	2.01	9.66	1.98	19.3	2.28
Correlation with *E.coli*			**0.37 (P<0.05)**		**0.68 (P>0.05)**		**0.62 (P<0.05)**	

Table 2: Correlation between counts of *E.coli* and Pathogens in processed water samples taken from the outlet

Months	*E.coli* Counts		Intestinal Pathogen's Counts					
			Salmonella spp.		*Shigella* spp.		*V.cholerae*	
	Count/ml	Log Count/ml	Count/ml	Log Count/ml	Count/ml	Log Count/ml	Count/ml	Log Count/ml
January	3.33	1.52	1.00	1.00	4.33	1.63	3.00	1.47
	5.00	1.69	2.66	1.42	5.33	1.72	2.00	1.30
February	0.00	0.00	0.00	0.00	2.00	1.30	1.00	1.00
	2.00	1.30	4.00	1.60	0.00	0.00	1.66	1.22
March	6.33	1.80	1.66	1.22	3.00	1.47	4.00	1.60
	4.00	1.60	2.00	1.30	6.66	1.82	4.66	1.66
April	3.66	1.56	1.00	1.00	2.00	1.30	0.33	0.51
	0.33	0.51	0.33	0.51	1.00	1.00	2.66	1.42
May	2.66	1.42	1.33	1.12	5.00	1.69	5.00	1.69
	5.00	1.69	3.66	1.56	4.00	1.60	5.66	1.75
June	6.00	1.77	3.00	1.47	2.33	1.36	4.33	1.63
	4.33	1.63	5.00	1.69	4.00	1.60	4.66	1.66
July	0.33	0.51	2.60	1.41	5.33	1.72	5.66	1.75
	2.33	1.36	3.33	1.52	6.33	1.80	6.66	1.82
August	7.00	1.84	4.00	1.60	4.00	1.60	3.66	1.56
	5.66	1.75	0.00	0.00	1.00	1.00	4.00	1.60
September	4.66	1.66	2.00	1.30	2.66	1.42	3.66	1.56
	8.00	1.90	1.00	1.00	5.66	1.75	1.00	1.00
October	9.66	1.98	4.33	1.63	0.33	0.51	4.00	1.60
	8.33	1.92	0.00	0.00	5.00	1.69	4.33	1.63
November	6.00	1.77	0.33	0.51	3.66	1.56	2.00	1.30
	5.33	1.72	2.66	1.42	2.00	1.30	5.00	1.69
December	3.00	1.47	4.66	1.66	0.00	0.00	4.33	1.63
	7.66	1.88	3.00	1.47	3.33	1.52	3.00	1.47
Correlation with *E.coli*			**0.29 (P<0.05)**		**0.09 (P>0.05)**		**0.16 (P>0.05)**	

Table 3: Correlation between counts of *E.coli* and Pathogens in processed water samples taken from the households

Months	*E.coli* Counts		Intestinal Pathogen's Counts					
			Salmonella spp.		*Shigella* spp.		*V.cholerae*	
	Count/ml	Log Count/ml	Count/ml	Log Count/ml	Count/ml	Log Count/ml	Count/ml	Log Count/ml
January	4.00	1.60	5.66	1.75	12.0	2.07	8.00	1.90
	6.33	1.80	8.00	1.90	7.33	1.86	10.3	2.01
February	2.33	1.36	7.00	1.84	8.00	1.90	7.33	1.86
	5.00	1.69	9.33	1.96	2.00	1.30	9.00	1.95
March	8.66	1.93	6.33	1.80	15.6	2.19	12.0	2.07
	9.00	1.95	10.0	2.00	8.33	1.92	13.3	2.12
April	4.00	1.60	5.33	1.72	12.3	2.08	11.3	2.05
	4.33	1.63	11.0	2.04	6.66	1.82	13.6	2.13
May	7.00	1.84	7.00	1.84	13.6	2.13	15.3	2.18
	6.33	1.80	10.6	2.02	10.3	2.01	18.3	2.26
	9.66	1.98	11.3	2.05	8.00	1.90	13.6	2.13

June	5.33	1.72	5.33	1.72	12.0	2.07	12.6	2.10
July	6.00	1.77	10.3	2.01	10.6	2.02	19.0	2.27
	13.0	2.11	14.0	2.14	13.6	2.13	20.6	2.31
August	10.0	2.00	8.30	1.91	8.66	1.93	16.3	2.21
	7.66	1.88	13.0	2.11	11.3	2.05	18.3	2.26
September	9.33	1.96	7.00	1.84	7.33	1.86	10.6	2.02
	12.6	2.10	12.3	2.09	10.6	2.02	8.33	1.92
October	5.66	1.75	4.00	1.60	11.6	2.06	9.00	1.95
	11.0	2.04	5.66	1.75	6.00	1.77	12.6	2.10
November	8.33	1.92	6.66	1.82	4.33	1.63	14.6	2.16
	12.0	2.07	8.00	1.90	14.6	2.16	17.0	2.23
December	8.00	1.90	9.00	1.95	3.00	1.47	13.3	2.12
	10.6	2.02	5.00	1.69	5.66	1.75	11.3	2.05
Correlation with *E.coli*			**0.28 (P>0.05)**		**0.06 (P>0.05)**		**0.45 (P<0.05)**	

Table 4: Presence-Absence (P/A) of Indicator and Pathogens; Correlation between indicator and pathogens detection

Sample	% of samples	*E.coli*	Intestinal Pathogens			Correlation
			Salmonella spp.	*Shigella* spp.	*V.cholerae*	
Dam water (N=24)	100%	+	+	+	+	Positive
Outlet water (N=24)	4.16%	-	-	+	+	Negative
	8.33%	+	+	-	+	Positive
	8.33%	+	-	+	+	Positive
	79.1%	+	+	+	+	Positive
Household water (N=24)	100%	+	+	+	+	Positive

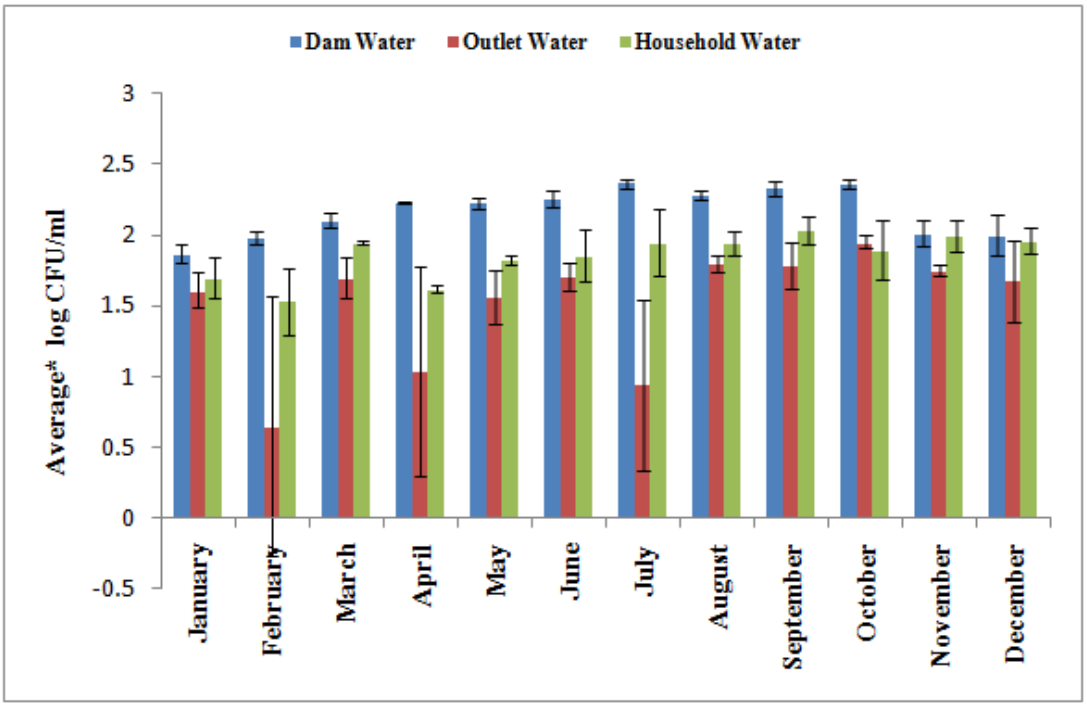


Fig 1: Densities of *E.coli* in the three kinds of samples
*Mean of fortnight readings

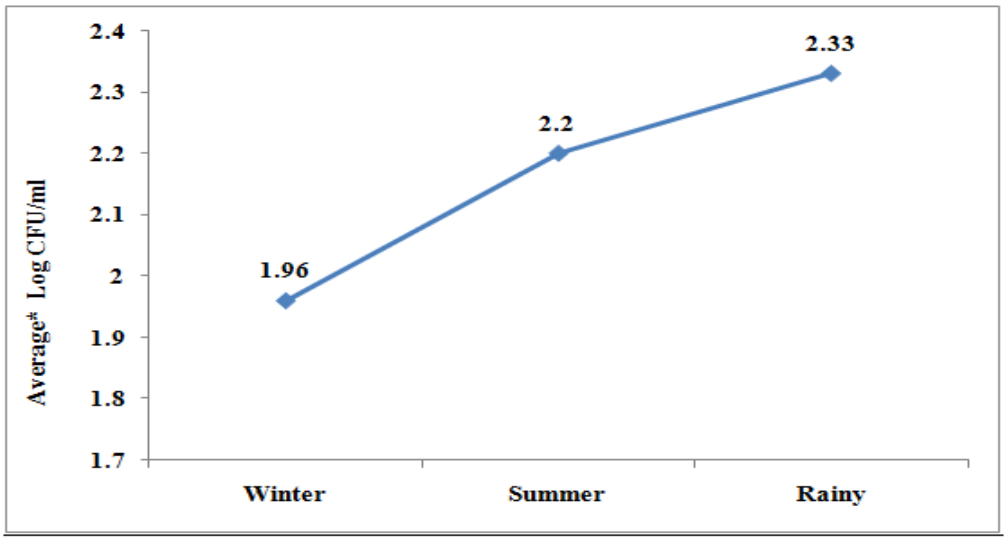
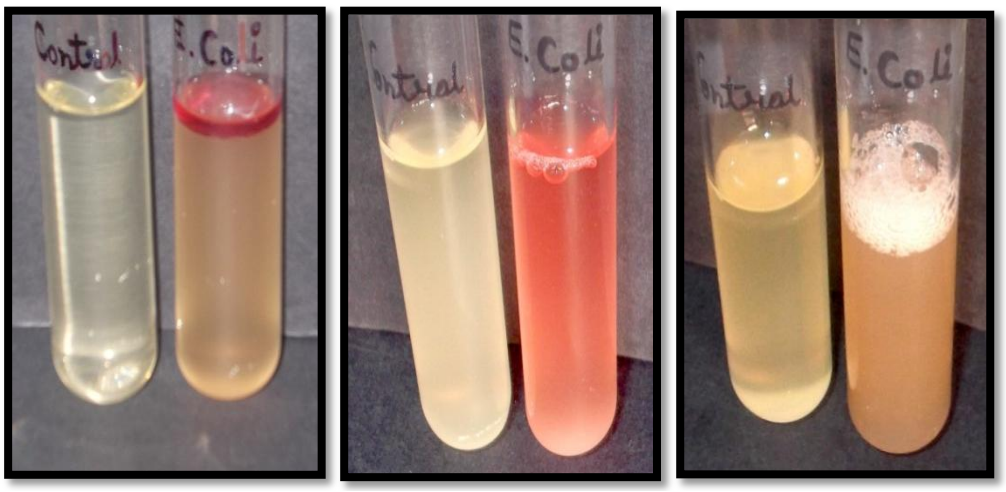
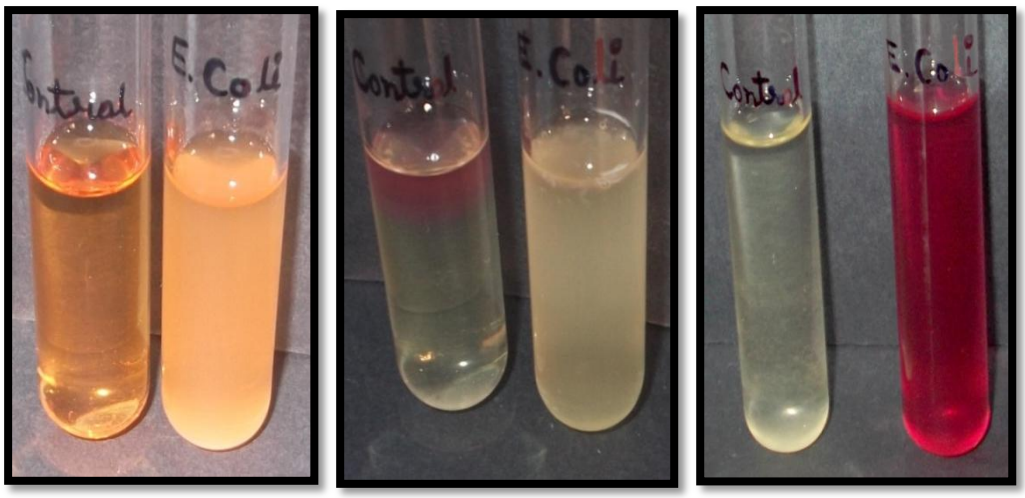


Fig 2: Seasonal shifts in *E.coli* counts in dam water samples
*Mean of four readings



Positive Indole Test **Positive MR Test** **Negative VP Test**

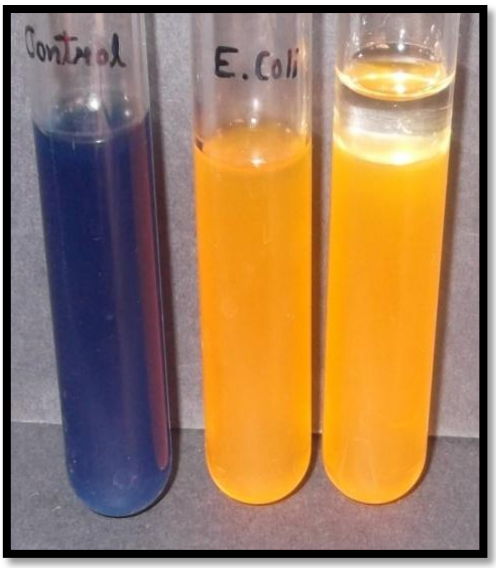


Negative Urease Test **FTG Test- Facultative** **Positive NR Test**

Negative SC Test

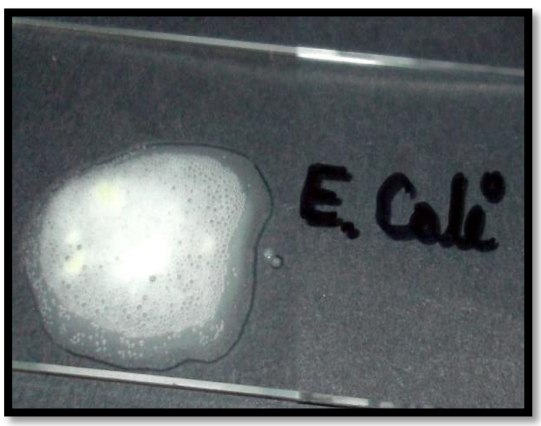
Negative Oxidase Test

Positive Mannitol Test

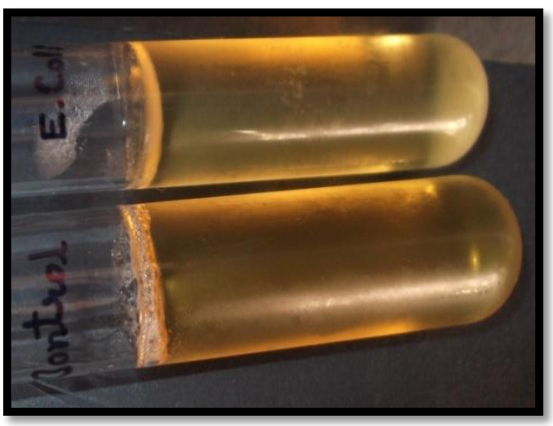


OF Test-Fermentative

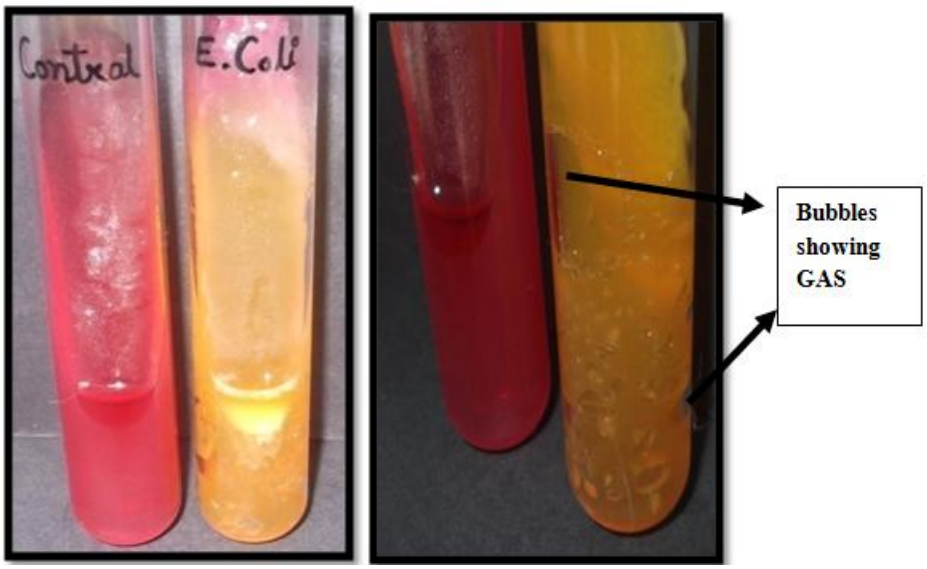
Negative Starch Test



Positive Catalase Test



Negative Gelatinase Test



Positive TSIA Test **Backside of the tube showing gas production**

MR- Methyl red, VP- Voges proskauer, SC- Simmon citrate, NR- Nitrate reduction, TSIA- Triple sugar iron agar, OF- Oxidative fermentative, FTG- Fluid thioglycollate

Fig 3: Biochemical results of *E.coli*

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