Drinking quality assessment of well water with respect to *Escherichia coli* contamination from different sources[#]

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Abstract

The present study was undertaken to assess the drinking water quality of well water with respect to Escherichia coli contamination from different sources. A total of 138 drinking water samples contaminated with coliforms above 10 MPN Index per 100 mL formed the study material. A cross sectional survey was conducted for the sanitary evaluation of drinking water along with inspection of household wells and surroundings. A retrospective investigation was carried out to assess the contamination of E. coli-positive well water samples. The associated sources for E. coli contamination like, 93 human stool samples, 84 animal faecal samples, 25 bird cloacal swabs, 138 soil and 27 drainage water samples were also collected from the premises of the respective households in order to identify the source contaminating the well water. All the samples were subjected to isolation and identification of E. coli by conventional culture technique followed by molecular confirmation using polymerase chain reaction (PCR). Statistical analysis using Pearson's chi square test revealed highly significant association between the occurrence of E. coli in drinking water and sources viz., human stool, animal faeces and soil samples ($p \leq 0.01$). The unassociated sources of E. coli contamination in drinking water exhibited a highly significant association with the increasing distance of well from the septic tank as well as with the presence of linings in wells and animals in the households. Hence, the study conducted for the identification of sources of E. coli contamination of drinking water signifies the importance of revised construction rules to protect the wells from contaminating sources and the need of regular and effective water treatment protocols.

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Globally, 785 million people are deprived of access to safe and adequate drinking water (WHO, 2017). Gastrointestinal diseases account for over 50 per cent of the world's water-borne diseases that are responsible for over five million deaths annually (Malik *et al*., 2012). Diarrhoea, though recognized as a preventable and curable disease, is the eighth cause of death, worldwide (16,55,944 deaths) and the fifth cause, specifically, in children below the age of five (4, 46,000 deaths) (Troeger *et al*., 2016). *Escherichia coli* is one among the most important etiological agents of diarrhoea in the low-income countries (WHO, 2017). This organism belongs to the vast group of coliform organisms that reside exclusively in the gastrointestinal tract of the both humans and animals. Study conducted by the Centre for Water Resources Development and Management (CWRDM) in 2009 revealed that 70 per cent of wells in Kerala were exposed to contamination and more than 6,00,000 people seek medical help annually on account of gastrointestinal illness. The densely populated Thrissur district in Kerala was selected for the present study, in anticipation of finding evidence of exploitation of ground water for geogenic as well as domestic purposes, possibly by compromising the water quality and thereby rendering it unsafe for drinking. To the best of our knowledge, no studies have so far been conducted in Thrissur district to trace out the sources of *E. coli* contamination of well water. Keeping this in mind, the present study was carried out to assess the drinking water quality with respect to *E. coli* contamination.

Materials and methods

A total of 150 drinking water samples brought to the National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited laboratory at the College of Veterinary and Animal Sciences (CVAS), Mannuthy during the period September 2019 to July 2020 were analyzed and 138 samples that tested positive for coliform above the recommended limit of 10 MPN Index per 100 mL were subjected to detection and isolation of *E. coli*. A well-designed questionnaire was used for the sanitary evaluation of drinking water along with inspection of household wells and surroundings. Data on the presence of animals

and birds, presence of possible sources of contamination and its distance from the water source were also collected. A retrospective investigation was carried out to assess the contamination of *E. coli*-positive well water samples. In order to accomplish this, 93 human stool samples, 84 animal faecal samples, 25 bird cloacal swabs, 138 soil samples and 27 drainage water samples were collected from the premises of the respective households. All the samples were subjected to isolation and identification of *E. coli* as described by Barrow and Feltham (1993). The isolates obtained were further subjected to molecular confirmation for identification of the conserved genus specific 16*SrRNA* gene by PCR as per Manjushree *et al*. (2019).

The relationship between various factors (independent variables) and the presence of *E. coli* in well water (dependent variable) was determined using relevant statistical tools. Statistical analysis was done using Pearson's *chi*-square test with the help of IBM – SPSS version 24.0 to find significant association if any, between the occurrence of *E. coli* in drinking water and in associated samples *viz.*, human stools, animal faeces, soil and drainage water from the selected households of Thrissur district. The occurrence of *E. coli* in water was also compared with responses of the cross-sectional survey questionnaire in order to derive any possible associations between the same.

Results and discussion

The *E. coli* colonies on MacConkey Agar after incubation at 37 °C for 18-24 h, appeared as lactose fermenting, dry, flat and pink colonies due to precipitation of bile salts present in the media (Fig. 1). On Eosin Methylene Blue agar, dark colonies with a black center along with greenish metallic sheen (Fig. 2) were identified as *E. coli*. The isolates were tested for the presence of genus specific 16*SrRNA* gene with the amplicon size 231bp and the results showed that all the 258 samples subjected to PCR belonged to *E. coli* (Fig. 3).

A total of 76 positive *E. coli* isolates were detected in drinking water as against 51 in human stool, 53 in animal faeces and 46 in
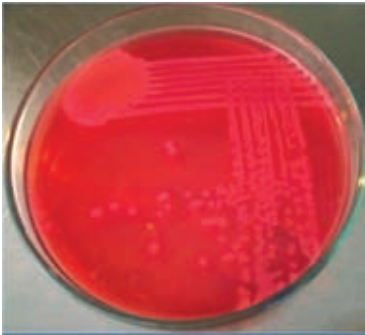
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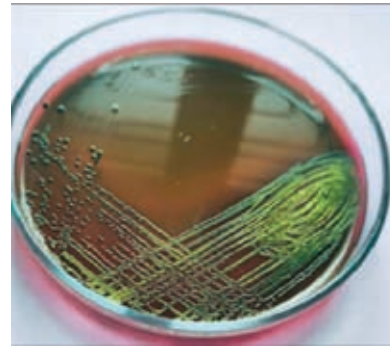**Fig. 1.** Pink colour Lactose fermenting colonies of *E.coli* on MaConkey Agar

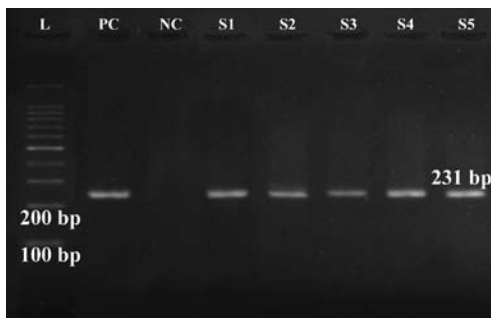**Fig. 2.** Greenish metallic sheen colonies of *E. coli* on Eosin Methylene Blue agar

Fig. 3. Detection of 16*SrRNA* gene (231 bp) by PCR
(L-100 bp ladder, PC-positive control NC- negative article and S-samples)

Fig. 4. Occurrence of *E. coli* from multiple sources

soil samples. The overall occurrence of *E. coli* in drinking water and other samples *viz.,* human stool, animal faeces, bird cloacal swabs, soil and drainage water samples was observed as 50.67, 54.83, 63.09, 84, 33.33 and 18.52 per cent, respectively (Fig. 4). As shown in Table 1, a highly significant association was observed between the occurrence of *E. coli* in drinking water and sources *viz.,* human stool, animal faecal and soil samples (p ≤ 0.01). Schriewer *et al*. (2015) documented that 46 and 97 per cent of the household drinking water sampled had human and animal faecal indicators in them, respectively. The presence of *E. coli* in human faecal samples could be related to improper handling and usage of contaminated drinking water. The occurrence of *E. coli* in the soil indicates faecal pollution. The presence of faecal pollution could be from the animal sources near the premises or the seepage from the nearby septic tanks. Widespread contamination of the household environment with faecal bacteria could play a significant role in exposure of children to diarrhoeal pathogens, considering
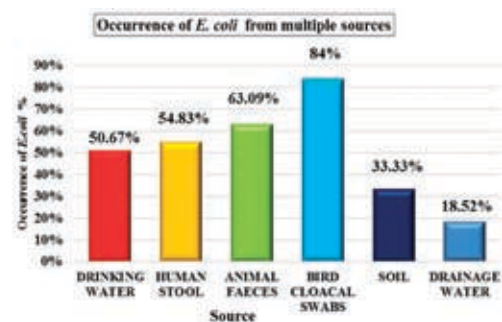
that they are likely to have frequent hand-to-soil and hand-to-mouth contact. Hence, strict and appropriate hand hygiene practices should be adopted to prevent the spread of the pathogen. Similarly, occurrence of *E. coli* in drinking water and the presence of animals in the households showed highly significant association. Of the total households surveyed in the study, the practise of rearing animals and washing them near the well premises was noticed in 60.14 and 44.20 per cent, respectively. Barnes *et al*. (2018) observed significant association between the presence of domestic animals and contamination of drinking water in households in Kenya. Pickering *et al*. (2012) observed that, presence of animal faeces in the courtyard was significantly associated with increased contamination of the soil. Carelessly managed animal faeces in the yards need to be given ample importance. Infiltration of rainwater contaminated with animal faeces especially during periods of heavy rainfall and flooding is a likely cause of the presence of animal faecal contaminants in well water (Table 2).

Table 1. Association of presence of *E. coli* in drinking water with presence of *E. coli* in associated sources

Sl. No	Associated sources	*E. coli* in drinking water				Total	χ^2 Value & p value
		Absent		Present			
		No.	%	No.	%		
1	Human	28	68.3	13	31.7	42	34.478**
		5	9.6	47	90.4	51	(<0.01)
2	Animal	23	82.1	5	17.9	38	56.875**
		3	4.8	60	95.2	53	(<0.01)
3	Soil	38	76.0	12	24.0	92	48.117**
		3	6.4	44	93.6	46	(<0.01)
4	Drainage water	4	57.1	3	42.9	22	3.56 ns
		0	0.0	5	100.0	5	(0.316)

** Significant at 0.01 level; ns non-significant

Table 2. Association of different factors with respect to the presence of *E. coli* in drinking water

Sl. No.	Factors	Score	*E. coli* in drinking water				Total	χ^2 Value & p value
			Absent		Present			
			No	%	No	%		
1	Distance between well and septic tank	0	19	30.2	44	69.8	62	14.351**
		1	28	57.1	21	42.9	49	(0.001)
		2	18	69.2	8	30.8	27	
2	Well and neighbours' latrine	0	10	66.7	5	33.3	15	2.615ns
		1	38	45.2	46	54.8	83	(0.271)
		2	17	43.6	22	56.4	40	
3	Presence of waste water drainage near the well	0	50	49.5	51	50.5	111	0.873ns
		1	15	40.5	22	59.5	27	(0.350)
4	Lining	0	1	7.7	12	92.3	25	8.946**
		1	64	51.2	61	48.8	113	(0.001)
5	Covering	0	27	48.2	29	51.8	52	0.047ns
		1	38	46.3	44	53.7	86	(0.829)
6	Animals present	0	39	81.3	9	18.8	55	34.446**
		1	26	28.9	64	71.1	83	(<0.001)
7	Well and animal pit	0	18	32.1	38	67.9	56	0.764ns
		1	8	23.5	26	76.5	34	(0.382)
8	Washing animals	0	17	34.0	33	66.0	77	1.431ns
		1	9	22.5	31	77.5	61	(0.232)
9	Plantation	0	53	51.0	51	49.0	104	2.524ns
		1	12	35.3	22	64.7	34	(0.112)

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant

In the present study, 44.93 per cent wells in households, were present at a distance of <7.5 m from the septic tank. Highly significant statistical association was observed between the occurrence of *E. coli* in drinking water and the increasing distance of well from the septic tank and the lining on the well ($p \le 0.01$). Occurrence of *E. coli* was observed most often in cases where septic tanks were at a distance of less than 7.5 m from the well water sources. Even though a distance of 15 m between wells and possible sources of contamination is recommended (Park, 2017), the provisions of Kerala Building Rules (2017) have been revised such that is distance is 7.5 m. In the present study, lining of wells was absent in 18.12 per cent of the wells. The occurrence of *E. coli* was relatively more in wells without lining in its wall when compared to wells with lining. Ananth *et al*. (2018) observed significant association between the occurrence of *E. coli* in drinking water and close proximity of septic

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tanks to the wells in Trivandrum and this was reported as the chief factor making well water unsafe. Similar observations were made by Megha *et al*. (2015) who also reported that poor planning, design of the wells and improper siting of wells from latrines to be the possible reasons altering the well water quality. Harikumar and Madhav Chandran (2013) documented that the lining of wells and location of latrines at a distance of more than 7.5 m from the wells could help reduce the occurrence of *E. coli*. Charles *et al*. (2012) stated that for every one metre increase in distance between dug-well and pit latrine, there was a corresponding decrease of 3.38 faecal coliforms /100mL in terms of contamination, which was in consonance with the findings of the present study (Table 2).

In the present study, covering of wells was absent in 37.68 per cent of the wells. Compared to the present study, where covering of wells did not exert significant difference in the coliform load of the well, conflicting observations were made by Megha *et al*. (2015) wherein, the authors observed significant statistical difference in coliform contamination between covered and uncovered wells. The presence of neighbour's latrine and animal disposal pits at < 7.5 m from well water in 10.87 and 2.89 per cent of the households, respectively did not show statistical association with occurrence of *E. coli* in drinking water ($p \geq 0.05$). Similarly no association was observed in the case of washing of animals, waste water drainage and plantation area near the wells in 44.20, 19.57 and 24.64 per cent of the households, respectively. Contamination of the wells by the above sources could occur through infiltration into groundwater sources, surface water seepage and water run-off following a heavy rain (Table 2).

Conclusion

The present study emphasized the importance of revisiting the house building rules by ensuring more stringent provisions to reduce potential contamination of wells. The wells should be sited at a minimum distance of 7.5 m from a potential contamination source. High risk wells need improved construction to block contamination pathways, improved hygiene practices around the well and comprehensive management measures to protect wells from anthropogenic activities. There is need for enforcement of public laws on construction of latrines, and guidelines for the construction of wells. Quality and frequency of chlorination of wells also need to be ensured. In urban areas, where land is limited, public water supply may be encouraged along with assurance of quality and monitoring of water quality at correct intervals.

Acknowledgement

Conflict of interest

The authors declare that they have no conflicts of interest.

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