Water Practice & Technology

IWA PUBLISHING

Water Practice & Technology Vol 17 No 3, 708 doi: 10.2166/wpt.2022.017

Comparative analysis of molecular and conventional methods for bacteriological water quality assessment in drinking water resources around Chennai

Divakar Raj Dinakaran [a], Hemaiswarya Shanmugam [a], Indumathi M. Nambi[b] and Mukesh Doble [a,*]

[a] Bioengineering and Drug design lab, Department of Biotechnology, Indian Institute of Technology, Madras, Tamil Nadu, India
[b] Environment and Water Resources Engineering Division, Department of Civil Engineering, Indian Institute of Technology, Madras, Tamil Nadu, India
*Corresponding author. E-mail: mukeshd@iitm.ac.in

DRD, 0000-0002-9363-1746; HS, 0000-0003-1060-1064; MD, 0000-0002-4335-0698

ABSTRACT

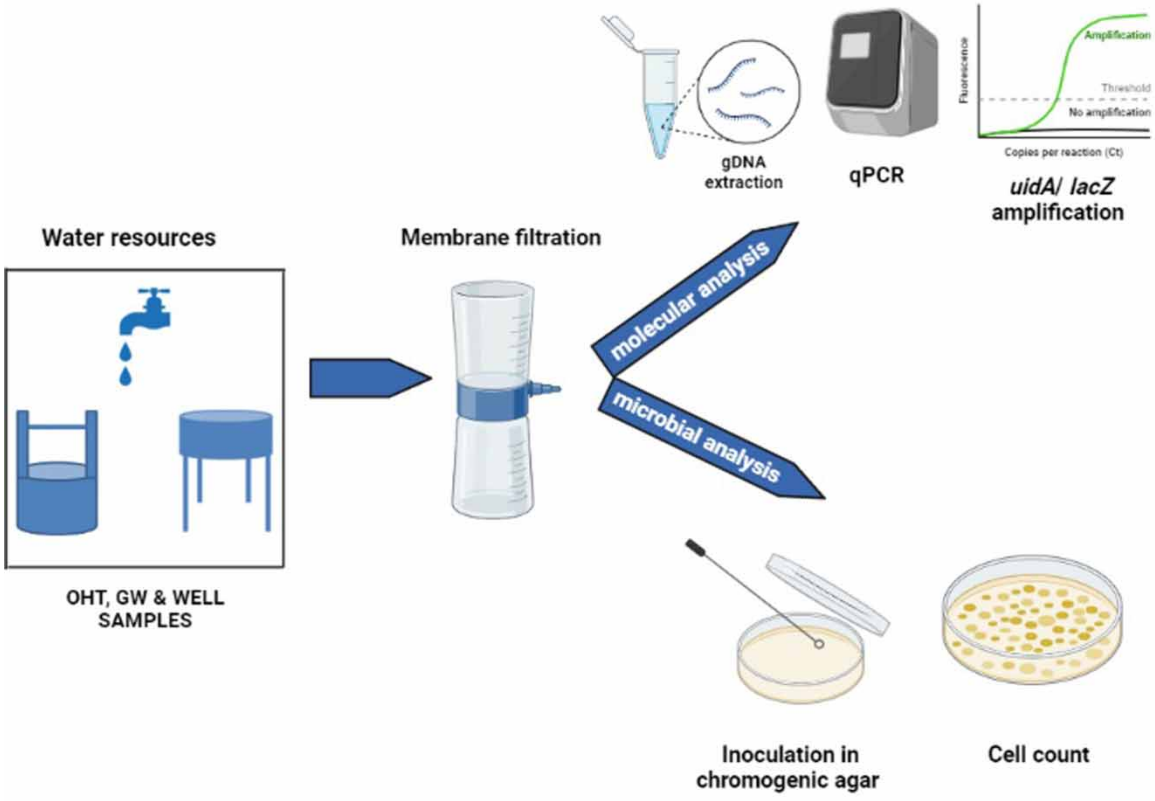
Coliforms and *Escherichia coli* represent the fecal contamination in drinking water and hence its potability. The present study estimates *uidA* and *lacZ* genes as distinctive biomarkers for rapid and efficient detection of contaminated water resources. In total, 39 environmental water samples were collected in and around the metropolitan city of Chennai, India. The results were compared with culture-based method using selective medium. The study shows that the performance and amplification efficiency of uidA and *lacZ* are 99.6% and 103.4% respectively. The sensitivity of the qPCR method in selectively identifying *E. coli* was 71.4% in a total of 92.3% coliform contamination. The conventional method showed that 10 samples were positive for *E. coli* and 12 samples positive for coliforms from a total of 39 samples. A lower positive predictive value of the biomarkers observed is due to the insignificant association between the two methods and is determined to be 59.0% and 66.6% for *E. coli* and coliforms respectively. The study reports 50 and 70% of the overhead tank (OHT) samples were contaminated with *E. coli* and coliform respectively, which indicates sanitation measures through these water supplies are not adequately taken care of. High throughput molecular detection technique is reported here, which can be used for monitoring environmental samples more quickly.

Key words: biomarkers, coliform, *Escherichia coli*, fecal indicators, water monitoring, water pollution, qPCR technique

HIGHLIGHTS

- Comparison of microbiological plate assay and molecular qPCR technique for pathogen detection.
- Several water resources in and around Chennai tested for microbial pollution.
- Proposed as an efficient tool for monitoring environmental drinking water samples.
- Evaluation of diagnostic parameters.
- High throughput molecular detection of water samples.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Microbial contamination in water resources is a major problem leading to several diseases. Multiple microbes lead to water contamination, whereas in most cases *Escherichia coli* and coliforms are applied as indicator organisms to monitor microbial contamination of water. The coliforms are Gram-negative, non-spore forming bacilli with the ability to ferment lactose to produce acid and gas. *E. coli* is a group of bacteria that are used as indicators of fecal contamination in water and is commonly found in many other environments such as aquatic, soil and vegetation. *E. coli* and coliform bacteria should not be detected in any water that is intended for drinking (Cotruvo 2017). The presence of these microorganisms implies a lack of system integrity and poor general sanitary quality of the water, leading to greater water-borne diseases (Maheux *et al.* 2014). Fecal substances from humans and animals are major contributors to microbial contamination in water sources, particularly the groundwater system. Disposal of untreated and improperly treated fecal and sewage contributes to the bacteriological contamination of water. In several countries, untreated sewage from towns and cities is directly disposed into open water bodies. Groundwater is a major water resource in India (Khurana & Sen 2008).

Increasing population, inadequate and unhygienic potable water supply are the foremost concerns in India. In India, only a few reports on the examination of bacterial fecal indicators have been studied. The level of fecal contaminants in the river Ganges revealed that microbial activity was abundant and varied seasonally, especially more during monsoon followed by summer and then winter (Baghel *et al.* 2005). The drinking water quality index of ground water in the inland region of southern Tamil Nadu was linked to numerous parameters. Fecal indicators indicated that the coliform bacteria level exceeded the guideline values (Ramesh *et al.* 2010). Research on the microbial contamination level in the west coast of India (Rodrigues *et al.* 2011) and rural and urban households of Karnataka (Mukhopadhyay *et al.* 2012) indicated higher microbial activity found in rural water supplies and a large proportion of isolates had multidrug resistance characteristics, which was alarming. Recently, a study conducted in Sikkim indicated possible fecal contamination of potable water in the community reservoir tanks and spring water used for household purposes. The authors suggested several monitoring and management options that should be followed by the state to maintain a hygienic drinking water supply chain (Singh *et al.* 2019).

Several approaches and monitoring devices have been developed by researchers for the detection and enumeration of indicator bacteria in the water supply. The conventional methods of detection are based on selective growth of indicator microbes on substrate-specific chromogenic media but suffer from long incubation periods, possibilities of contamination, inaccuracy, and lack of specificity. The modern polymerase chain reaction (PCR) based molecular techniques that detect microorganisms in the water sample are highly sensitive, specific and faster, thereby eliminating the need for long incubation time. It is widely used as a rapid detection tool in several countries to determine the quality and monitor their water systems (Fatemeh *et al.* 2014). The PCR-based method is not quantitative and hence cannot determine the extent of contamination. The drawbacks of the above methods warrant the need for an efficient and rapid method, namely the quantitative polymerase chain reaction (qPCR) technique.

Over a period, several genes specific to *E. coli* and total coliforms have been investigated as PCR primers or markers (Patel *et al.* 2011; Walker *et al.* 2017). The gene *lacZ* responsible for the production of enzyme *β*-D-galactosidase is used to detect total coliforms by PCR methods (Isfahani *et al.* 2017). The gene *uidA* encoding for*β*-D-glucuronidase enzyme is largely found in *E. coli* (Bej *et al.* 1991)with frequent activities observed in *Shigella* and *Yersinia* (Molina *et al.* 2015).The conventional PCR methods demand further electrophoresis techniques to detect the presence of the target gene, whereas qPCR can amplify the specific gene and confirm their presence using fluorescent signals developed during the amplification of DNA during the reaction/amplification time.

Different types of drinking water resources available to the general public were initially identified in Chennai, Tamil Nadu. Most of the population in Tamil Nadu depends upon the Government water supply, which goes through chlorination for sanitization. Lakes/ponds, community wells, groundwater and overhead tanks are the major resources available to the people in every city. The current study aims to quantify the load of coliforms and *E. coli* in these water sources through the rapid molecular qPCR technique and compare with the plating-based method.

2. MATERIALS AND METHODS

2.1. Sampling sites

Water samples were collected from lakes/ponds, community wells, groundwater and overhead tanks from different areas in and outside Chennai (Figure 1). A total of 39 water samples from 12 different sites were collected and analyzed for the presence of *E. coli* and coliform through detection of genes *uidA* and *lacZ* using qPCR.

2.2. Sampling procedure

Water samples were collected in 500 mL sterile, wide mouth plastic bottles (Tarsons Product Pvt. Ltd, India). All sample containers were washed in distilled water, dried and autoclaved prior to the use. The samples are collected from faucets as per USEPA guidelines on sampling for biological contaminants to strictly ensure the inside of the bottles and the cap were not contaminated, while leaving a one inch headspace in all the containers for air and mixing before analysis (USEPA 2005). The samples were then stored in the refrigerator at 4 °C for a maximum of 24 hours until further processing. The details of the samples and their sources are given in supplementary Table S1.

2.3. Comparison of methods

The efficiency of culture-based and molecular qPCR methods to detect *Escherichia coli* and total coliforms was studied using strains obtained from Microbial Type Culture Collection (MTCC). The representative microorganisms for microbial contamination in water, *Escherichia coli* (MTCC 1687) and coliforms belonging to genera Citrobacter (*Citrobacter freundii*; MTCC1658), Enterobacter (*Enterobacter aerogenes*; MTCC 6806) and Klebsiella (*Klebsiella pneumoniae*; MTCC 109) were used as a consortium in this study. The microorganisms were revived according to the manual of instructions provided by MTCC in their respective media and stored as glycerol stocks in a −80 °C deep freezer for further experiments.

2.3.1. Microbiological analysis

The assay was performed using a modified USEPA Method 1604 (USEPA 2002). HiCrome™ M-TEC agar and HiCrome™ chromogenic coliform agar (HiMedia Laboratories, India) are prepared under manufacturer's instruction and used for the detection of *E. coli* and total coliforms respectively. 100 ml of the water sample was filtered on a 0.45 μm pore size 47-mm diameter cellulose acetate filter (Millipore Corporation, USA) in a UV sterilized filtration unit (Tarsons Product Pvt. Ltd, India) and the filter was carefully transferred using

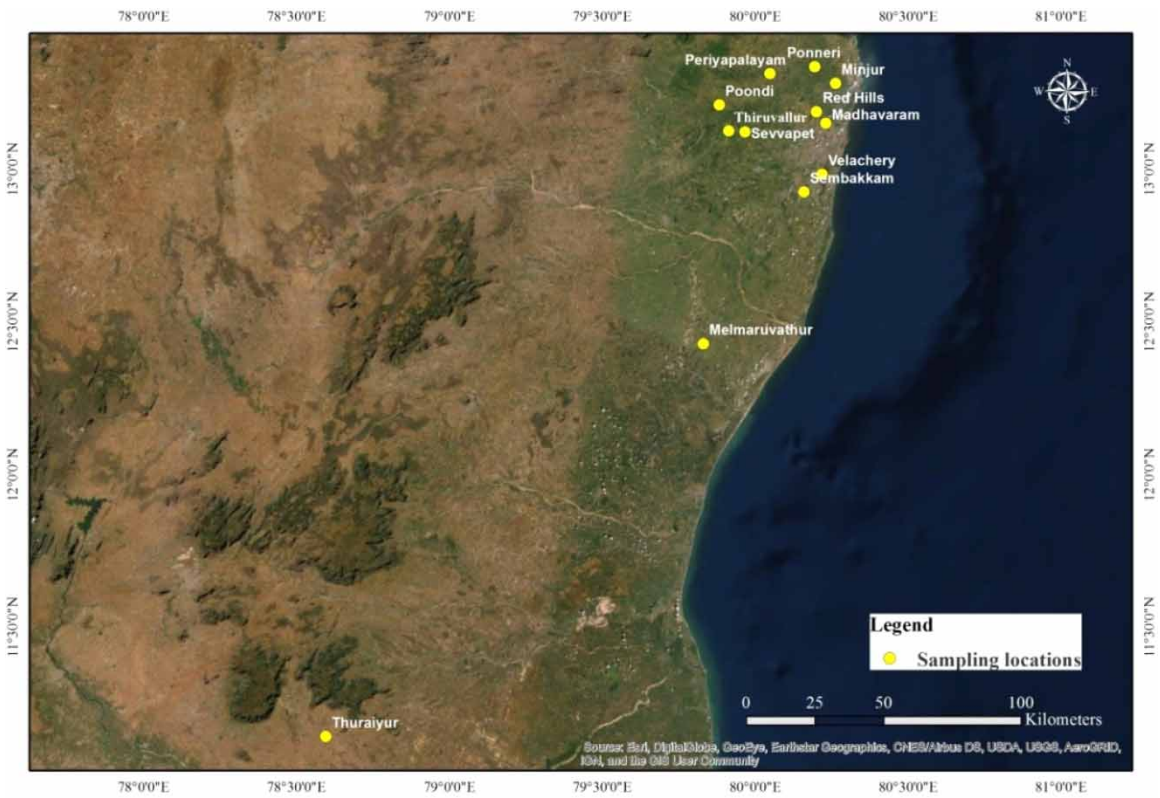


Figure 1 | Sampling locations across the city of Chennai and its suburbs, Tamil Nadu, South India.

flamed forceps on to the agar plates. The plates were incubated at 44.5 $\pm$0.2 °C and 37 °C for 24 hours. The performance of the plates was tested with positive and negative control strains *C.freundii*, *E. coli* and *P.aeruginosa*. Sterility control was included for all the experiments as mentioned (APHA 2005).

2.3.2. Molecular analysis

2.3.2.1. DNA isolation. The membrane filters (after filtration of samples) was transferred aseptically to a vial containing 1.5mlbuffer solution. The bacterial cells on the filter were collected by centrifugation at 13,000 rpm for 10 min. An optional upstream bead beating step using 0.1 mm diameter sterilized glass beads was included to enhance the recovery of cells from the filters. The genomic DNA (gDNA) was recovered from the bacterial pellet using the HiPurA™Bacterial Genomic DNA Purification Kit (HiMedia Laboratories, India) and the concentration of extracted gDNA yield was quantified by using NanoDrop™ 2000/2000c spectrophotometer (ThermoFisher Scientific, Massachusetts, USA). Finally, 50 µl of elution buffer was added for eluting DNA from the column and the extracted DNA is stored at −20 °C for further analysis. The gDNA standards were prepared by isolating genomic DNA from standard *E. coli* and coliforms (*C. freundii* MTCC1658, *E. aerogenes* MTCC 6806 and *K. pneumoniae* MTCC 109) purchased from culture collection. Performance characteristics of qPCR assays were determined from standard curves constructed using gDNA standard concentrations and the threshold cycle (C_T) values from qPCR.

2.3.2.2. qPCRprimers. The gene sequence for *uidA* targeting *E. coli* was acquired from NCBI (National Center for Biotechnology Information) and the corresponding gene FASTA sequence was uploaded in the Primer-BLAST to generate a specific primer for the template. A suitable primer with optimal concentration for qPCR was selected for this study. The degenerate primer of the *lacZ* (Martín *et al.* 2010) was designed using 22 different *lacZ* gene sequences of coliforms to generate the primer from the conserved region of all the sequences. The primers used are given in the Table 1.

2.3.2.3. qPCR amplification and detection. The qPCR method is a robust and efficient technique eliminating the need for any post analytical processing for enumeration and detection. The efficiency of the method and the

Table 1 | Primer sequences used for *uidA* and *lacZ* amplification

Bacteria/gene	Primer sequence	References
E. coli/*uidA*	FP-5′- CAAAGTCCCGCTAGTGCCTT -3′ RP-5′- GATCCATCGCAGCGTAATGC -3′	This study
Total coliforms/*lacZ*	FP-5′- CGCTACGGYCTGTAYGTSGT -3′ RP-5′- TCATCGGCACCATSCGTG -3′	Martín *et al.* (2010)

specificity of the primer were assessed with a log phase bacterial suspension of *E. coli* and coliforms at different dilutions. Briefly, 2 µl of genomic DNA isolated from the environmental samples was added to a 20 µl qPCR reaction mixture containing 10 µl of TB (Takara Bio) Green Premix Ex TaqII (2X) (TaKaRa Bio Inc, RR 820A), 0.4 µl of forward and reverse primer (0.4 µM) and the remaining reaction was made up with RNAse free sterile water. The ideal thermal cycling conditions for detection of *E. coli* were initial denaturation at 95 °C for 30sec, followed by 40 cycles of denaturation at 95 °C for 5 sec, primer annealing at 60 °C for 20 sec and extension at 72 °C for 20 sec followed by melt curve analysis. For detection of total coliforms, the conditions were followed as described elsewhere (Martín *et al.* 2010). A no template control (NTC) containing all the elements without the DNA template was included in all the experiments.

Various diagnostic parameters including amplification efficiency, limit of detection (LOD), specificity, sensitivity, positive and negative predictive values, positive and negative likelihood ratios were determined to test the efficiency of the qPCR analysis (Alberg *et al.* 2004; Caraguel *et al.* 2011; Nutz *et al.* 2011; Sedighi 2013; Gensberger *et al.* 2014a). The positive and negative samples were adjudicated in comparison with the culture method based on the diagnostic parameters (true positives and true negatives, false positive and false negatives)

To calculate the efficiency, linear regression of the value of C_T on the log of DNA concentration with the target sequence per reaction mixture. The linear regression equation obtained for *E. coli* is $y = -3.33x + 17.856$ and for coliforms it is $y = -3.243x + 19.857$ and are calculated from Table S2 and S3. The 'x' denotes the concentration of DNA in µg/µl and 'y' denotes the intercept which is C_T obtained from the qPCR experiment. The efficiency is calculated using the formula;

$$\%\ Amplification\ efficiency = 10^{((-1/slope)-1)} \times 100 \quad (1)$$

The acceptable values of slope are ($-3.67 \leq$ slope ≤ -3.1) and the amplification efficiency (87–110%) in both the standard curves of *Escherichia coli* and total coliforms to establish qPCR assay performance are provided in the USEPA method 1611 (USEPA 2013)

The limit of detection (LOD) of qPCR analysis was determined using C_T value of the non-template control (C_T (NTC)) according to the following formula (Caraguel *et al.* 2011). Samples were deemed as positive if their C_T values were lesser than C_T (LOD) and as negative/undetermined if C_T (sample) > C_T (LOD).

$$C_T(LOD) = C_T(NTC) - 3 \quad (2)$$

True positives are those where *E. coli* and coliforms are detected both in qPCR experiment and plate assay. True negatives are those which are undetected in both of the assays. If the samples were detected with *E. coli* and coliforms in qPCR but not in the plate assay are considered as false positives; on the other hand, false negatives are those undetected with *E. coli* and coliforms in qPCR but positive in the plate assay. Specificity, sensitivity, positive and negative predictive values, positive and negative likelihood ratio (LR^+ and LR^-) of the analysis were determined from the true positive and negatives, false positives and negatives using the following formulae.

$$Specificity = \frac{True\ negative}{True\ negative + False\ Positive} \quad (3)$$

$$Sensitivity = \frac{True\ Positive}{True\ positive + False\ negative} \quad (4)$$

$$Positive\ predictive\ values = \frac{True\ positive}{True\ positive + False\ positive} \tag{5}$$

$$Negative\ predictive\ values = \frac{True\ negative}{True\ negative + False\ negative} \tag{6}$$

$$LR^{+} = \frac{Sensitivity}{1 - Specificity} \tag{7}$$

$$LR^{-} = \frac{1 - Sensitivity}{Specificity} \tag{8}$$

The association between the culture-based and qPCR method was calculated by Fisher's exact tests (odds ratios and 95% confidence intervals) using positive and negative sources using GraphPad Prism version 6.0.

3. RESULTS

The presence of *E. coli* and coliforms in water samples is ascertained by conventional microbiological method using differential media. Purple to magenta colonies indicate *E. coli* and pink colonies indicate coliforms (Figure 2). Out of 39 samples, a higher number (CFU/ml) of *E. coli* or coliforms was detected in 31 different samples by qPCR compared to the culture method (Table S1)

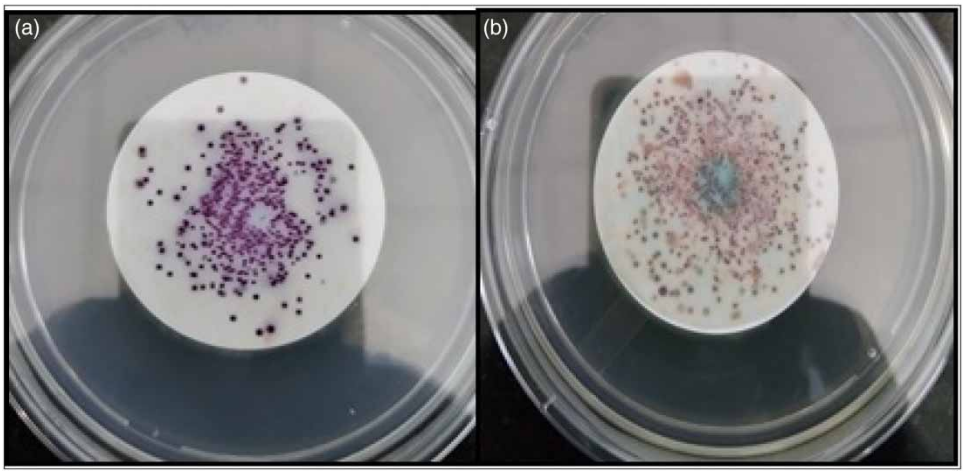


Figure 2 | (a) *E. coli* and (b) coliform colonies developed in HichromemTEC and Chromogenic coliform agar plates respectively.

The standard curve was linear for *E. coli* over 5 log units, namely from 10^5 to 10^1 CFU and for total coliforms; it was linear over 3 log units, from 10^4 to 1 CFU. In order to establish the analytical detection limit, genomic DNA of serially diluted *E. coli* and total coliforms were isolated and subjected to qPCR analysis (Table S2-S3). C_T values for NTC (No Template Control) were set at maximum cycles and hence the limit of detection was set as 37. The robustness of the qPCR assay along with its amplification efficiency was assessed using DNA of the target bacterial indicators (*E. coli* and coliforms), isolated from their mid-log phase cultures (Table 2). The amplification efficiency was calculated using the slope obtained from the linear regression equation. The r^2 values were $\geq$0.9 and a slope of −3.33 and −3.24, which were between the acceptable limits mentioned for a standard curve to determine the efficiency of the experiment (USEPA 2013). The percentage of amplification efficiency (AE %) for standard curve of *E. coli* is 99.6% and for total coliforms is 103.39%, which is well within the acceptable amplification efficiency (Bustin *et al.* 2009). High amplification efficiency denotes the reproducibility, the analytical sensitivity, and the robustness of the assay adopted.

3.1. Screening of water sources

Out of 39 samples analyzed from different water resources in the city through the molecular qPCR method, 50% in the OHT (overhead tank) samples tested positive for *E. coli* and 70% for the presence of coliform (Table 3). Lake samples were found to be positive for the target microorganisms, though the number of samples selected

Table 2 | Diagnostic parameters from qPCR analysis

Parameters	*E. coli*	Total coliforms
Amplification efficiency %	99.6%	103.4%
Slope	−3.33	−3.24
r^2 (correlation coefficient)	0.98	0.98
Sensitivity (%)	71.4	92.3
Specificity (%)	72.0	76.9
Odds Ratio	0.73	0.58
95% Confidence Interval	0.29–1.80	0.23–1.46
p value	0.64	0.35
Positive predictive values (%)	59.0	66.6
Negative predictive value (%)	81.8	95.2
Positive Likelihood Ratio (LR^+)	2.5	3.8
Negative Likelihood Ratio (LR^-)	0.40	0.10

Table 3 | Comparison of *E. coli* and coliform from various water sources using culture-based and qPCR methods

		Culture-based				qPCR			
Sample location	No. of samples	*E. coli*		Total coliforms		*E. coli*		Total coliforms	
		+	−	+	−	+	−	+	−
OHT	20	8	12	10	10	10	10	14	6
Wells	11	3	8	1	10	4	7	1	10
Lakes	3	2	1	1	2	2	1	1	2
Ground water	5	0	5	2	3	1	4	2	3

for the study was less. Well samples (36%) tested positive for *E. coli* and less than 10% for coliforms. Lake and ground water samples also tested positive for both the organisms, but the number of samples collected in the locations were less. The culture-based method indicated significant presence of the organisms in OHT and wells. The results indicate that the source of water and regular cleaning is important to prevent the growth of these organisms in OHT.

3.2. Diagnostic parameters

Multiple diagnostic parameters were determined for the molecular based assay, to understand its efficiency. The development of false positive and false negative signals was observed for both the indicator organisms in the qPCR experiment when compared to the culture-based technique. A non-significant association (*E. coli* Odds ratio: 0.73, 95% confidence interval: 0.29–1.8 and coliform Odds ratio: 0.58, 95% confidence interval: 0.23–1.46) was observed between the culture-based and qPCR method. False positive and false negative results from qPCR were registered for *E. coli* (17.9% and ~10.2%) and total coliforms (15.3% and 2.5%). There are a number of factors that can lead to varied results. The sensitivity and specificity of the qPCR ranged from 73 to 92% for detecting *E. coli* and total coliforms in water samples. Detection of total coliforms and *E. coli* retrieved positive predictive values of 66.67 and 59% respectively. The likelihood ratio for the detection of coliforms was better in comparison to *E. coli* detection.

4. DISCUSSION

The association between the microbiological culture-based assay using specific chromogenic agar and molecular qPCR analysis for the detection of *E. coli* and total coliforms were compared. A total of 39 samples from various water sources (overhead tank, ground water, wells and lakes) available to the public were collected for the study and analyzed. The specific medium used in our study was able to detect 13 positive samples (8 OHT, 3 well and 2

lake samples) both *E. coli* and 14 positive samples (10 OHT, 1 well, 1 lake and 2 groundwater samples) in coliforms. This accounts for 35.8% of the samples containing pathogenic bacteria. The use of membrane filtration for screening the presence of *E. coli* and coliforms in water samples has been shown to possess an increased recovery rate of 90% with the use of a selective differential medium. The high number of positive OHT samples warrants the application of proper disinfection methods by the government, filtration, boiling of water before consumption, or regular cleaning of the tanks.

Similarly, qPCR retrieved 9 samples positive for both *E. coli* and coliforms. In this study, we have successfully used the *uidA* and *lacZ* genes as a target for the detection of *E. coli*-coliform bacteria respectively. These molecular methods are accurate and allow rapid detection of pathogenic bacteria in water sources with minimal limitations. The primer used to amplify the specific gene of pathogenic bacteria in qPCR generated a fluorescent signal on amplification of the target gene through the use of SYBR green q-PCR mix in the reaction. A gene fragment of 196 bp was obtained using the designed primer for *E. coli*. Different gene targets for detecting bacterial indicators in different water resources have also been reported. Potable water and riverine system samples of river Gomti were studied for the presence of *Enterobacter* using their conserved sequences of 16S rRNA and 23S rRNA genes in qPCR analysis, which showed very high specificity for the detection of *Enterobacter* (Patel *et al.* 2016). A SYBR green qPCR assay with *LT1* and *ST1* primers for Enterotoxigenic *E. coli* (Patel *et al.* 2011) and with *stx2* primers for Shiga toxin-producing *E. coli* (Ram *et al.* 2011) confirmed their presence in potable waters of a major city of northern India. Besides, several other bacterial pathogens (*Entamoeba histolytica* (16SrRNA primer), *Giardia lamblia* (16SrRNA primer), and *Salmonella* spp. (invasive A primer) have been detected in municipality water supplies (Shankar *et al.* 2019). High incidence of multidrug resistance and class 1 integrons of *E. coli* in the urban waters of river Yamuna water of India were also detected raising the concern for multidrug resistant (MDR) coliforms (Kaushik *et al.* 2019).

Alternatively, the real-time PCR technique has drawbacks including underestimation of pathogens due to losses in bacteria during sample filtration and nucleic acid extraction steps. A few of the samples were also found to be overestimated due to the inability to discriminate between live and dead forms of *E. coli* (Na *et al.* 2006). DNA is known to remain intact after cell death and may persist for a few days and up to 3 weeks after cell death. Therefore overestimation or false positive results may occur in using this technique (Nocker *et al.* 2007). Reports suggest that higher bacterial counts were observed in the qPCR method when compared to the culture method for the quantification of *Listeria monocytogenes* and *Enterobacteriaceae* in food samples (Takahashi *et al.* 2017). Persistence of dead fecal bacteria is still an indicator of water-borne disease, therefore qPCR could be viewed as the best assay for the detection of *E. coli* and coliforms. Negative and positive samples generated the odds of association between the two techniques, which was found to be insignificant (*E.coli* p-value: 0.64 and Coliform p-value: 0.35). The reference culture method also has drawbacks of its own as it underestimates the results of the qPCR method. Therefore, detection of *E. coli* and coliforms in complex environments requires the use of multiple gene primer probes. Multiple water quality indicator bacteria, larger sample size, and different assessment techniques should be used to obtain multiple lines of evidence on the occurrence of fecal contamination.

In order to study the efficiency and accuracy of the molecular method, several diagnostic parameters are to be determined. The drawbacks in the PCR could affect the sensitivity of the molecular detection method. The presence of inhibitors relative to the type of sample hinders both the culture and qPCR methods. The complexity of the sample influences the amplification efficiency and primer binding ability, often leading to false positive and false negative results (Schrader *et al.* 2012). Higher false positive (12%) and false negatives (13%) were assigned to *Enterobactericiae* detection in drinking water, where specific primers against *uidA* and 23SrRNA genes were employed for the detection of *E. coli* and Enterobactericiae. This was attributed to the drawbacks in the reference test, Colilert®-18 (Gensberger *et al.* 2014b). The sensitivity and specificity of qPCR was found to be on the lower end for the detection of *E. coli* (71.42 and 72%) and *Enterbactericiae* (92.31 and 76.92%). Similarly a lower positive predictive value for *E. coli* (59%) and higher value for *Enterobactericiae* (66.67%) was observed. Both the drinking and processed water samples showed lower sensitivity, specificity, negative and positive predictive values in qPCR analysis. Inefficient sample preparation was predicted as the reason for lower diagnostic parameters (Gensberger *et al.* 2014b). Both β-D-glucuronidase activity and *uidA* amplification were not found to effectively identify E. coli, which led to the design of alternative primers for detection, including *yaiO* (Molina *et al.* 2015), and *ybbW* (Walker *et al.* 2017). A qPCR assay based on *uidA* had lower sensitivity (71.42%) in comparison to 100% sensitivity using *ybb*W primers. The authors claim that none of the existing *uidA* qPCR assays

possesses complete inclusivity and exclusivity (specificity and sensitivity). This is due to the detection of *Shigella* along with *E. coli* and false positive results for non-*E.coli Escherichia* species (Walker *et al.* 2017). Most of the studies do not report the diagnostic parameters in comparison to a reference method, therefore the reliability of such results is to be questioned. Ours is one of the few studies which make a comparison between two methods.

The low positive predictive values for *E. coli* and coliforms (59 and 66.67% respectively) from the current study is due to the cross specificity with other microbes (*Shigella, Yersenia, Salmonella*) possessing target genes or due to the detection of genes from dead bacteria. Besides, PCR retrieves positive signal for strains that possess the gene sequence but negative for the corresponding gene. Negative predictive values were estimated to be 81.82 and 95.23% for *E. coli* and coliform respectively, which ascertains the probability of no indicator bacteria present in the samples. Both positive and negative predictive nature of tests depends on the existence of genes in the strains tested. On the contrary, likelihood ratio does not depend on the prevalence of genes in the strains, but is a true indicator of the test. A positive coliform qPCR test significantly enhances the probability of its presence (LR^+: 3.83) in comparison to low prediction of *E. coli* (LR^+: 2.53). All the diagnostic parameters are predicted in comparison to the culture based method, therefore the limitations of the technique will affect the outcome. These low values arise due to signals from DNA of viable and dead cells, possible presence of chemical inhibitors in environmental samples (Wolffs *et al.* 2005), inefficient sensitivity/specificity with the current *uidA*/*lacZ* primers and sometimes due to contamination or improper handling of experiments. An insignificant association between the two techniques justifies the low predictability of the indicator organisms. A higher false positive ratio is acceptable only if the results of the study are screened further through confirmatory assays. The chances of reducing the rate of false positives in qPCR detection can also be achieved through proper sample preparation and culture enrichment methods such as immuno-magnetic separation (Nogva *et al.* 2000) of cell and free DNA and floatation (Wolffs *et al.* 2004). Propidiummonoazide coupled with DNA of *E. coli* inhibited PCR amplification of DNA derived from killed cells (Deshmukh *et al.* 2020). In few cases, the samples collected from environment were treated with sodium thiosulfate for removal of chlorine residues to increase the detection efficiency through qPCR (Isfahani *et al.* 2017).

The limitations involved in the traditional culture based microbiological method are their long incubation time for results and labor intensiveness. The method lacks sensitivity, specificity over molecular tools for enumeration of pathogens, which are often the reasons to promote the latter for detection. The culture-based methods in chromogenic agar plates have constraints that include interaction and growth of other microorganisms, inefficiency to identify and detect VBNC (viable but non-culturable) bacteria in the medium due to their low metabolic activity (Park *et al.* 2011). Moreover, the recovery rate of microbes in the chromogenic agar can change based on the method of concentrating the microorganisms in the sample and a change in colony specification might arise which needs further confirmation steps.

The majority of people in the city of Chennai, Tamil Nadu, depend on the water sources mentioned in the study. Overhead Tanks are one of the major sources of water supply to the society. The tanks are built around the city for storage and supply of water from time to time. The study reports 50 and 70% of OHT samples were detected with *E. coli* and coliform respectively, which indicates sanitation measures for controlling the spread of disease through these water supplies are not adequately taken care. The disinfection strategies of basic chlorination within permissible limits and membrane filtration should be adopted, especially during the monsoon season (July to September). It is recommended that monitoring through sampling of these resources be done twice in a year. Sewage run off into wells and ponds needs to be addressed to prevent the contamination of water bodies. Consumer or public education is the foremost criterion that is required to reduce the development of waterborne diseases.

5. CONCLUSION

The presence of *E. coli*-coliform in water resources from different locations in and around the city of Chennai (South India) was identified using molecular qPCR method and compared with the plating method, which is slow and time consuming. It is significant to monitor bacteriological indicators in OHT, which is one of the major sites of contamination, as identified in the present study. Measures to device the contamination through point of care testing equipment are essential to promote appropriate preventive measures to the community. The rapid molecular testing is compared with the standard microbiology technique to determine the specificity

and efficiency of the study. Large-scale validation studies with substantial and extensive data points from different sources will be required to dictate the efficacy of the molecular method. Furthermore, a focus on advanced qPCR detection such as multiplex PCR and ddPCR (Digital Droplet PCR) for multiple target detection using meticulous design of primers in the environmental water samples is needed. Finally, the results of the current research are pertinent to untreated water resources available to the community; and it could be different for other water types.

ACKNOWLEDGEMENTS

The authors thank Hermes i Tickets Private Limited for the financial support.

CONFLICT OF INTEREST

The authors of the manuscript confirm that there are no known conflicts associated with this publication.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Alberg, A. J., Park, J. W., Hager, B. W., Brock, M. V. & Diener-West, M. 2004 The use of 'overall accuracy' to evaluate the validity of screening or diagnostic tests. *Journal of General Internal Medicine* **19**, 460–465. https://doi.org/10.1111/j.1525-1497.2004.30091.x

APHA/AWWA/WEF 2005 Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC.

Baghel, V. S., Gopal, K., Dwivedi, S. & Tripathi, R. D. 2005 Bacterial indicators of faecal contamination of the Gangetic river system right at its source. *Ecological Indicators* **5**, 49–56. https://doi.org/10.1016/j.ecolind.2004.09.002.

Bej, A. K., DiCesare, J. L., Haff, L. & Atlas, R. M. 1991 Erratum: detection of *Escherichia coli* and Shigella spp. in water by using the polymerase chain reaction and gene probes for uid (applied and environmental microbiology 57 (1013–1017)). *Applied and Environmental Microbiology* **57** (8), 4. https://doi.org/10.1128/AEM.57.8.2445-2445.1991.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J. & Wittwer, C. T. 2009 The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* **55**, 611–622. https://doi.org/10.1373/clinchem.2008.112797.

Caraguel, C. G. B., Stryhn, H., Gagné, N., Dohoo, I. R. & Hammell, K. L. 2011 Selection of a cutoff value for real-time polymerase chain reaction results to fit a diagnostic purpose: analytical and epidemiologic approaches. *Journal of Veterinary Diagnostic Investigation* **23** (1), 2–15. https://doi.org/10.1177/104063871102300102.

Cotruvo, J. A. 2017 2017 WHO guidelines for drinking water quality: first addendum to the fourth edition. *American Water Works Association* **109**, 44–51.

Deshmukh, R., Bhand, S. & Roy, U. 2020 A novel method for rapid and sensitive detection of viable *Escherichia coli* cells using UV-induced PMA-coupled quantitative PCR. *Brazilian Journal of Microbiology* **51**, 773–778.

Fatemeh, D., Reza, Z. M., Mohammad, A., Salomeh, K., Reza, A. G., Hossein, S., Maryam, S., Azam, A., Mana, S., Negin, N., Reza, K. A. & Saeed, F. 2014 Rapid detection of coliforms in drinking water of Arak city using multiplex PCR method in comparison with the standard method of culture (Most probabl number). *Asian Pacific Journal of Tropical Biomedicine* **4**, 404–409. https://doi.org/10.12980/APJTB.4.2014C896.

Gensberger, E. T., Polt, M., Konrad-Köszler, M., Kinner, P., Sessitsch, A. & Kostić, T. 2014a Evaluation of quantitative PCR combined with PMA treatment for molecular assessment of microbial water quality. *Water Research* **67**, 367–376. https://doi.org/10.1016/j.watres.2014.09.022.

Gensberger, E. T., Polt, M., Konrad-Köszler, M., Kinner, P., Sessitsch, A. & Kostić, T. 2014b Evaluation of quantitative PCR combined with PMA treatment for molecular assessment of microbial water quality. *Water Research* **67**, 367–376.

Isfahani, B., Fazeli, H., Babaie, Z., Poursina, F., Moghim, S. & Rouzbahani, M. 2017 Evaluation of polymerase chain reaction for detecting coliform bacteria in drinking water sources. *Advanced Biomedical Research* **6**, 130. https://doi.org/10.4103/2277-9175.216783.

Kaushik, M., Khare, N., Kumar, S. & Gulati, P. 2019 High prevalence of antibiotic resistance and integrons in *Escherichia coli* isolated from urban river water, India. *Microbial Drug Resistance* **25**, 359–370.

Khurana, I. & Sen, R. 2008 Drinking water quality in rural India: issues and approaches. *WaterAid India* **89** (3), 1–23.

Maheux, A. F., Boudreau, D. K., Bisson, M. A., Dion-Dupont, V., Bouchard, S., Nkuranga, M., Bergeron, M. G. & Rodriguez, M. J. 2014 Molecular method for detection of total coliforms in drinking water samples. *Applied and Environmental Microbiology* **80**, 4074–4084. https://doi.org/10.1128/AEM.00546-14.

Martín, M. C., Martínez, N., del Rio, B., Ladero, V., Fernández, M. & Alvarez, M. A. 2010 A novel real-time polymerase chain reaction-based method for the detection and quantification of lactose-fermenting Enterobacteriaceae in the dairy and other food industries. *Journal of Dairy Science* **93**, 860–867. https://doi.org/10.3168/jds.2009-2481.

Molina, F., López-Acedo, E., Tabla, R., Roa, I., Gómez, A. & Rebollo, J. E. 2015 Improved detection of *Escherichia coli* and coliform bacteria by multiplex PCR. *BMC Biotechnology* **15**, 1–9. https://doi.org/10.1186/s12896-015-0168-2.

Mukhopadhyay, C., Vishwanath, S., Eshwara, V. K., Shankaranarayana, S. A. & Sagir, A. 2012 Microbial quality of well water from rural and urban households in Karnataka, India: a cross-sectional study. *Journal of Infection and Public Health* **5**, 257–262. https://doi.org/10.1016/j.jiph.2012.03.004.

Na, S. H., Miyanaga, K., Unno, H. & Tanji, Y. 2006 The survival response of *Escherichia coli* K12 in a natural environment. *Applied Microbiology and Biotechnology* **72**, 386–392.

Nocker, A., Sossa, K. E. & Camper, A. K. 2007 Molecular monitoring of disinfection efficacy using propidium monoazide in combination with quantitative PCR. *Journal of Microbiological Methods* **70**, 252–260.

Nogva, H. K., Bergh, A., Holck, A. & Rudi, K. 2000 Application of the 5′-Nuclease PCR assay in evaluation and development of methods for quantitative detection of *Campylobacter jejuni*. *Applied and Environmental Microbiology* **66**, 4029–4036.

Nutz, S., Döll, K. & Karlovsky, P. 2011 Determination of the LOQ in real-time PCR by receiver operating characteristic curve analysis: application to qPCR assays for *Fusarium verticillioides* and *F. proliferatum*. *Analytical and Bioanalytical Chemistry* **401**, 717–726. https://doi.org/10.1007/s00216-011-5089-x.

Park, S. H., Hanning, I., Jarquin, R., Moore, P., Donoghue, D. J., Donoghue, A. M. & Ricke, S. C. 2011 Multiplex PCR assay for the detection and quantification of Campylobacter spp., *Escherichia coli* O157: H7, and Salmonella serotypes in water samples. *FEMS Microbiology Letters* **316**, 7–15.

Patel, C. B., Vajpayee, P., Singh, G., Upadhyay, R. S. & Shanker, R. 2011 Contamination of potable water by enterotoxigenic *Escherichia coli*: qPCR based culture-free detection and quantification. *Ecotoxicology and Environmental Safety* **74**, 2292–2298. https://doi.org/10.1016/j.ecoenv.2011.07.022.

Patel, C. B., Shanker, R., Gupta, V. K. & Upadhyay, R. S. 2016 Q-PCR based culture-independent enumeration and detection of Enterobacter: an emerging environmental human pathogen in riverine systems and potable water. *Frontiers in Microbiology* **7**, 172.

Ram, S., Vajpayee, P., Dwivedi, P. D. & Shanker, R. 2011 Culture-free detection and enumeration of STEC in water. *Ecotoxicology and Environmental Safety* **74**, 551–557.

Ramesh, S., Sukumaran, N., Murugesan, A. G. & Rajan, M. P. 2010 An innovative approach of drinking water quality index – a case study from southern Tamil Nadu, India. *Ecological Indicators* **10**, 857–868.

Rodrigues, V., Ramaiah, N., Kakti, S. & Samant, D. 2011 Long-term variations in abundance and distribution of sewage pollution indicator and human pathogenic bacteria along the central west coast of India. *Ecological Indicators* **11**, 318–327. https://doi.org/10.1016/j.ecolind.2010.05.010.

Schrader, C., Schielke, A., Ellerbroek, L. & Johne, R. 2012 PCR inhibitors–occurrence, properties and removal. *Journal of Applied Microbiology* **113**, 1014–1026.

Sedighi, I. 2013 Interpretation of diagnostic tests: likelihood ratio vs. predictive value. *Iranian Journal of Pediatrics* **23**, 717.

Shankar, P., Mishra, J., Bharti, V., Parashar, D. & Singh, S. 2019 Multiplex PCR assay for simultaneous detection and differentiation of *Entamoeba histolytica*, *Giardia lamblia*, and Salmonella spp. in the municipality-supplied drinking water. *Journal of Laboratory Physicians* **11**, 275.

Singh, A. K., Das, S., Singh, S., Pradhan, N., Gajamer, V. R., Kumar, S., Lepcha, Y. D. & Tiwari, H. K. 2019 Physicochemical parameters and alarming coliform count of the potable water of Eastern Himalayan state Sikkim: an indication of severe fecal contamination and immediate health risk. *Frontiers in Cell and Developmental Biology* **7**, 174. https://doi.org/10.3389/fpubh.2019.00174

Takahashi, H., Gao, Y., Miya, S., Kuda, T. & Kimura, B. 2017 Discrimination of live and dead cells of *Escherichia coli* using propidium monoazide after sodium dodecyl sulfate treatment. *Food Control* **71**, 79–82.

USEPA 2002 Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique. U.S. Environmental Protection Agency, EPA-821-R-02-024, Office of Water, Washington, DC.

USEPA 2005 *Quick Guide To Drinking Water Sample Collection 20*. US EPA, Washington, DC.

USEPA 2013 *Method B: Bacteroidales in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay 111*. US EPA, Washington, DC.

Walker, D. I., McQuillan, J., Taiwo, M., Parks, R., Stenton, C. A., Morgan, H., Mowlem, M. C. & Lees, D. N. 2017 A highly specific *Escherichia coli* qPCR and its comparison with existing methods for environmental waters. *Water Research* **126**, 101–110. https://doi.org/10.1016/j.watres.2017.08.032.

Wolffs, P., Knutsson, R., Norling, B. & Rådström, P. 2004 Rapid quantification of *Yersinia enterocolitica* in pork samples by a novel sample preparation method, flotation, prior to real-time PCR. *Journal of Clinical Microbiology* **42**, 1042–1047.

Wolffs, P., Norling, B. & Rådström, P. 2005 Risk assessment of false-positive quantitative real-time PCR results in food, due to detection of DNA originating from dead cells. *Journal of Microbiological Methods* **60**, 315–323.

First received 25 October 2021; accepted in revised form 7 February 2022. Available online 22 February 2022