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RESEARCH ARTICLE

RAPID WATER POTABILITY TESTING BY PAPER-BASED PCR USING WHATMAN PAPER NO.1

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ABSTRACT

The analysis of potable water quality is important to protect consumers from water-borne or water-based illnesses caused by pathogens like microorganisms, viruses, and protozoa. Rapid identification is critical to ensure water safety. Various detection and identification methods exist; but, they are laborious and time-consuming, so potability confirmation takes longer. This study aims to develop the specific and fast detection of water contamination. We worked on a minimum sample preparation process. In this study, we've got developed a straightforward Paper-based PCR technique. The 16s rDNA primers were used for the detection of microorganism contaminants. LacZ primers were used for coliform detection, which causes serious unhealthiness and hence their detection is crucial for water potability. ITS primers were used for fungal detection. The unique thing about this study is Whatman paper no-1 was used as sample carrier material. We developed and validated the unique paper-based PCR method to detect microbes and coliforms. We evaluated this method for suitability in water potability testing using different water samples.

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INTRODUCTION

Water plays a vital function in human life. Water that physically seems colourless, odourless or even tasteless is not sufficient to decide it is safe for consumption. It is important to check the water is potable and safe to drink; it should be examined on the microbiological and chemical science levels to ensure it's potable and safe (Ihekoronye and Ngoddy, 1985) (11). There are 3 categories of test techniques for microbial water testing, namely qualitative, quantitative, and identification assessments. A closer observation at methods available for detection of organisms like total coliform and E. coli. using biochemical, immunological, genetic, and microscopic reveal that most of these methods have incubated at 37°C for 24 hours to obtain the results. The incubation period in available methods is time-consuming, so the testing method is developed to make it faster. Additionally, WHO guidance (2011) (31) points out that E. coli bacteria or heat resistant Coliform bacteria should not be observed in 100 ml of any samples of drinking water. The accuracy of the traditional microbial method, most probable number (MPN) technique in mentioned problems in detecting Coliform bacteria, genetic methods without culturing have been used to amplify specific sequences of DNA with PCR technique and replicated genome identification. Advantages of the PCR system include its high sensitivity (which is capable of identification of even one cell in 100 ml of water sample), specificity for the target microorganisms, high-speed testing from samples, and the ability to detect multiple bacteria simultaneously (Bej et al., 1991a; Bej et al., 1991b (3,4). Most paper-based biosensors use antigen-antibody interactions to detect the target analytics of interest in water, soil, urine, blood or saliva samples (7,12,13,22). Applications developed on paper-based PCR technology range from testing blood samples for infectious diseases and testing grains in agriculture to testing chemical contaminants in water and soil (7,12,13,17,21,22,). Hossain et al. (9) developed a paper-based microfluidic device to detect the presence/absence of bacteria. The bacteria in water samples are pre-concentrated using antibody-coated immune-magnetic nanoparticles and tested. The concentrated samples are then tested with the paper-based microfluidic device. Molina et al., 2015) (20) used a different strategy to identify bacteria with multiplex PCR. Oligonucleotide primers were designed to

detect and ensure the specificity for detecting E. coli and total coliforms in a single assay. These genetic methods provide results in very less time with accuracy and specificity. However, these genetic methods require high-end, costly instruments. Also, these methods require a time-consuming sample preparation process before testing.

The goal of this study was to optimise a direct paper-based PCR technique for the detection of bacteria, total coliforms and fungi from water samples without the DNA extraction method and sample preparation process. PCR-based detection method development and optimisation were done using the 16S rDNA gene, β-galactosidase gene, and ITS gene to establish the proof of concept for this technique. These are the most common genetic markers applied to study bacteria, coliform, and fungi. The 16S rDNA gene consists of variable and conserved areas. Prevalent primers target the conserved regions to detect the presence of microorganisms in a given pattern. In contrast, the variable regions are targeted for the identity of genus or species (Hassan, El Enany, & Rizk, 2014) (9). This current work is a proof of concept to establish direct amplification of 16S rDNA, ITS gene and coliform gene from a sample carried on a Whatman paper no 1 to check the presence of organisms without extracting genomic DNA.

Cultures Used: *E. coli* (ATCC8739), *Staphylococcus aureus* (ATCC25923), *Candida albicans* (ATCC10231), *Salmonella typhi* (ATCC23564), *Klebsiella pneumoniae* (ATCC), *Pseudomonas aeruginosa* (ATCC9027) and *Bacillus subtilis* (ATCC6633) were procured from NCCS, Pune.

Media and Reagents: PCR reaction components, Taq polymerase, buffer and dNTPs were from Invitrogen. Whatman filter paper no. 1 was from GE Healthcare Life Sciences, 100bp ladder procured from GeNie, Luria Bertani (LB) agar components, MacConkey agar (MA) and Potato dextrose agar (PDA) were as procured from Himedia.

Method development

Bacterial Genomic DNA Extraction: Genomic DNA (gDNA) of E. coli was isolated using QIAamp DNA Mini Kit from Qiagen following the protocol recommended by the manufacturer. Additionally, isolated colonies of the selected strains mentioned above were picked up from LB and PDA agar plates. Isolated Colonies were re-suspended in 25µL of water in 2 distinct tubes. Kept these tubes in a boiling water bath to lyse the cells. The lysed cell suspension was spun down, and the supernatant containing the gDNA was used as a template DNA. Both the above methods extracted the gDNA. One was Qiagen mini kit, and another was cell suspension boiling, which was used as a PCR template.

Primer Used: 16s universal primers with the following sequences were synthesized (Sigma) for amplification of the 16S rDNA gene. Set: F2: 5'GTG TAG CGG TGA AAT GCG 3', R2: 5'ACG GGC GGT GTG TAC AA3' with amplicon size of 709 bp (Sauer, Gallo, Kesselova, Kolar, & Koukalova, 2005) (24). For fungal detection, ITS primers FP-5' TCC GTA GGT GAA CCT GCG G3'and RP-5' TCC TCC GCT TAT TGA TAT GC 3' (30) with amplicon size of 500bp and for coliform detection, primers LacZ3F-5" TTG AAA ATG GTC TGC TGC TG 3" and LacZ3R-5" TAT TGG CTT CAT CCA CCA CA 3"(6) with amplicon size of 234bp were used. Primers based on the lacZ gene have been used for the detection of coliforms because conventional coliform monitoring methods are based on the expression product (beta galactosidase) of this gene (4).

Annealing Temperature Optimization: The gradient PCR was set up in a thermal cycler. To evaluate the optimal annealing temperature for all primer sets mentioned in the above section, with an annealing temperature range of 55-58ºC whereas, other cycling hot start at 94˚C for 7min, denaturation at 94˚C for 30sec, annealing 55-58˚C for 1 min, elongation at 72˚C for 30sec, end the cycle like these 35 cycles were set then final elongation at 72˚C for 7min. I maintained these conditions. A single colony of *E. coli* and *S. aureus* and *Candida albicans* strain was suspended in 20µL of sterile water separately as a model organism. Colony suspension was spotted on pre-sterilised Whatman filter paper no.1 and processed as mentioned above. PCR reagent concentrations were used as 25µM of each dNTP, 0.25µM each primer, 0.5U Taq polymerase and 1X buffer to set up the PCR. The amplification was visualised using 2% agarose gel because LacZ amplicon size was 234 bp and ITS amplicon size was 500bp.

Robustness

Bacterial Culture Volume and Whatman paper size Optimization for Paper- Based PCR: The paper-based PCR method for amplifying the 16S rDNA gene, β-gal and ITS gene was established using Whatman filter paper no.1, optimising the lowest volume of culture sufficient to amplify the respective genes. The paper was dipped in 25µL of PCR master mix in a PCR tube, the volume of culture accommodated was 2, 3 and 5µL on 1mm×1mm, 3mm×3mm and 5mm×5mm size of the paper, respectively. *E. Coli. S. aureus* and *C. albicans* colony suspension were prepared in sterile water. Directly spotted this suspension on 3 independent pre-sterilised small strips of Whatman filter paper no.1. The strips were air dry in LAFU and were directly dipped in 25µL of PCR master mix, Carried out the detection and the amplification. Standard colony PCR process of the same culture was carried out along with this positive control.

Method Robustness Evaluation: The 16S rRNA gene is a housekeeping gene for all the bacteria, both for Gram-positive or Gram-negative. To ensure the robustness of the paper-based PCR to detect this gene, various Gram-positive and Gram-negative bacterial colony suspensions were prepared, and were spotted on pre-sterilized Whatman filter paper no.1 as mentioned earlier. The bacteria used for this study were *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi* and *Bacillus subtilis* sp. Each of these cultures were streaked on a sterile Luria Bertani agar plate to achieve a single isolated colony. A single colony of each of these cultures was picked up and suspended in 20µL of sterile water. 2 µL of each suspension was spotted on pre-sterilized independent Whatman filter paper no.1on 1mm×1mm size strips and the strips were air-dried in LAFU. These strips were dipped in the master mix and PCR condition was carried out as mentioned earlier in the case of annealing temperature optimization only change in annealing temperature was selected for all gene is 57˚C. The experiment was carried out with an F2/R2 primer set.

Master Mix volume variation: The paper-based PCR method for amplifying the 16S rDNA, β-gal and ITS gene was established using Whatman filter paper no.1. It was important

to optimise the master mix volume variation of PCR to check its effect on the amplicon. Colony suspension was prepared in sterile water. These suspensions were directly spotted on pre-sterilised small strips of Whatman filter paper 1in 2µL of volume. The strips were air dry in LAFU and were directly dipped in 25µL and 50 µL of PCR master mix. The gene amplification was done. Traditional colony PCR process of the same culture was also done along with this positive control.

Method robustness was checked by inter-day and intra-day experimental set up as well as analyst variation: On the first day, we carried out the same protocol set up two times, one was in the morning section with X person, and another was in the evening section with Y person. In this setup, intra-day and analyst variation is done. The same protocol set up the next day morning section with X person and compared result with first day morning section and next day morning section which did the same person (X person) but two different days like inter-day variation done and robustness was checked.

pH Variation: As the paper-based PCR method for amplifying the 16S rDNA, β-gal and ITS gene were established using different water pH for template preparation. The pH of template water accommodated was 4, 5, 6 and 7pH. As a model organism, a single colony of *S. aureus* and *E. coli* and *Candida albicans* strain were suspended in 20µL of 4,5,6 and 9 pH water separately. Different pH 2µL Colony suspension was spotted on pre-sterilised 1mm×1mm size Whatman filter paper no.1, The paper was directly dipped in 25µL of PCR master mix in a PCR tube. The amplification and the detection of the gene were carried out., it was important to check the different pH of the culture suitable to amplify the respective genes.

Specificity: Confirm the specificity of 16s rDNA primers to detect all bacteria using an established paper-based PCR method. The cultures used for this study were *Candida albicans* and *E. coli.*, *C. albicans* was used because *C. albicans* would not amplify the 16s rDNA region as it is absent in the genome. 2 µl of each suspension was spotted on sterile 1mm×1mm size Whatman no.1 paper and was allowed to air dry in sterile conditions. The paper was dipped to the master mix, and the PCR was carried out. The specificity of lacZ3 primers for detecting coliforms was evaluated using both coliforms (*E. Coli.)* and non-coliform (*S. typhi*.) cultures. As mentioned earlier, prospective bacterial suspensions were spotted on the paper, and the paper was dipped to the PCR master mix. The PCR process was carried out as mentioned above. The specificity of ITS primers for detecting fungi was assessed by using cultures of *Candida albicans* and *E. coli*. because of *E. coli*. genome doesn't carry ITS region and hence should not give amplification with ITS primers., For paper-based PCR, 2 µl of each suspension was spotted on sterile 1mm×1mm size Whatman no.1 paper and air dry in sterile conditions. The spotting cultures and PCR amplification were the same as mentioned earlier. After amplification, the amplified product was loaded on 2 % agarose gel with ethidium bromide, and the amplicon was visualised on a UV transilluminator.

Evaluation of Paper based PCR method for the detection of contaminants in actual water samples: The application of the developed paper-based PCR method in different water sources tested the detection of bacteria and coliforms. The water sources used were purified water (i.e. Aqua guard, ALFA), packaged water, i.e. Bisleri and available water sources (i.e. tap water, borewell water, corporation water, drinking water in dormitory). In this paper, we compared two methods viz. Traditional Microbiological Method and Developed Paper-based PCR Method (Molecular Biological Method). In the Traditional Microbiological method, we followed the process mentioned by Miles & Misra (19) to detect the Colony Formation Unit per mL (CFU /mL) count. Samples were serially diluted up to10-6 and were spotted on LB agar, MA or PDA plates, as mentioned in the article by Miles & Misra (19). The CFU/mL in the case of each sample was calculated. As per microbial method detection, we got the cell counts, which are shown in the below table (table no.1); as per this count, we decided for detection of contaminants by paper-based PCR method, 2µL of respective water sample was spotted on 1mm x 1mm size of Whatman paper no 1 under sterile condition. Dried This paper in LAFU. Then the dried paper was dipped into a PCR master mix tube. PCR was carried out with each selected water source to detect 16S rDNA and LacZ3 genes. A set up negative control reaction also. After amplification, the amplified products were loaded on 2% agarose gel with ethidium bromide and visualised on a UV transilluminator. used the same method for milk samples contamination detection (1).

Sr No.	Water Sample	CFU/mL
1	Aqua guard water	Clear
2	Alfa water	Clear
3	Bisleri Water	Clear
4	Tap Water	6×10^3
5	Borewell Water	4.6×10^2
6	Corporation Water	9.6×10^3
7	Dormitory Water	2.33×10^2

Table no.1 Water sample contaminant CFU/mL Count

RESULTS AND DISCUSSION

Optimization of parameters for paper-based PCR method development and microbes detection: Universal primers were selected from the highly conserved region of the 16S rDNA sequence. Labelled as F2/R2. Amplify region, giving the amplicon of 709bp. Earlier, these primers were additionally employed by Matsuda et al. (Matsuda et al., 2011) (18). Suspicious blood infections and by Liu et al. (Liu et al., 2005) (14) for the bacterial infection in CSF. Universal primer set (F2/R2) was evaluated for their efficiency in amplifying the bacterial DNA. Primers of 16S rDNA were used by Sauer et al. (Sauer, Gallo, Kesselova, Kolar, & Koukalova, 2005) (24) to detect pathogenic bacteria causing prosthetic joint infections were synthesized. In that article, they have claimed that the primer pair is highly specific for many bacterial pathogens and can be used with various clinical specimens. This study aimed to establish the primer pair suitable for amplifying the 16S rDNA gene, using a paper-based PCR method developed to detect microbes from various water samples. It was needed to demonstrate the primer pair commonly used with a broad range of samples. The primer pairs were highly specific and accurate for the 16S rDNA gene. In the current study, the F2/R2 primer set was used in the PCR for amplification of the 16S rDNA gene from *S. aureus*. Strains, amplicon size of 709 bp was detectable on the agarose gel electrophoresis (2). The genomic DNA template PCR and colony suspension template PCR

(colony PCR) results were compared with the 100bp ladder, as shown in (Fig.1). Colony PCR gives better amplification than the genomic DNA template, so a further study used colony PCR as a positive control. Fig.1 concluded that amplification is not affected by the type of template. We used Whatman Paper as a template DNA carrier. Set up Each experiment was with a negative and positive control to reduce the false results.

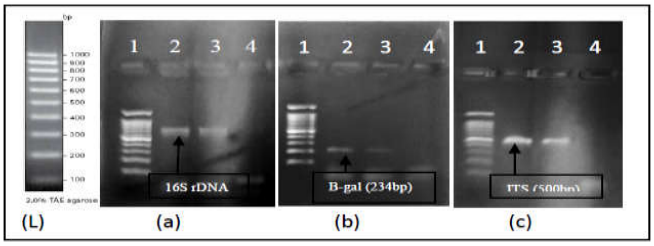

Fig.1. Genomic DNA and Colony PCR testing for method development

a) 16s r-DNA: Lane 1- 100bp Ladder, Lane 2- Genomic DNA PCR, Lane 3- Colony PCR, Lane 4- Negative Control.
b) β-galactosidase: Lane 1- 100bp Ladder, Lane 2- Genomic DNA PCR, Lane 3- Colony PCR, Lane 4- Negative Control.
c) ITS: Lane 1- 100bp Ladder, Lane 2- Genomic DNA PCR, Lane 3- Colony PCR, Lane 4- Negative Control.

Annealing temperature optimization for microbes detection by the 16S rDNA, LacZ3 and ITS gene: For any PCR reaction, primer annealing temperature Plays a key role in the best magnification and to avoid non-specific binding. Therefore, for Developed PCR reaction, optimization is very important Suitable for all three annealing temperatures Primer set. The recommended annealing temperature for the 16S rDNA universal primer used in this study was 55 °C, LacZ3 was 57 °C, and the ITS gene was 55°C. To better optimize this annealing temperature Amplification, gradient PCR is set to range 56, 57 and 58 °C. The annealing temperature for all three primers is 57 °C (Fig.2). Standard colony PCR without Whatman filter paper as a positive control.

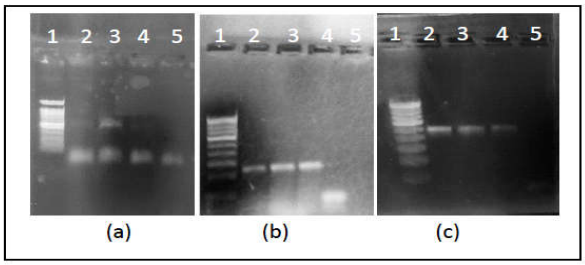

Fig.2 Annealing Temperature Optimization

a) 16s r-DNA amplification using F2/R2 primers (*S. aureus* CS): Lane 1- 100bp Ladder, Lane 2- 55˚C, Lane 3- 57˚C, Lane 4- 58˚C, Lane 5-Negative Control. b) β-galactosidase amplification using LacZ primers (*E. Coli*. CS): Lane 1- 100bp Ladder, Lane 2- 55˚C, Lane 3- 57˚C, Lane 4- 58˚C, Lane 5- Negative Control c) ITS amplification using ITS primers (*C. albicans* CS): Lane 1- 100bp Ladder, Lane 2- 55˚C, Lane 3- 57˚C, Lane 4- 58˚C, Lane 5- Negative Control. (L): 100bp ladder.

Paper size and Culture volume Optimization: To evaluate the minimum possible volume of culture and the smallest size of paper that can give the best result, the paper dimension and the volume of culture was varied and set up the PCR reaction was. The amplification of 16S rDNA, β-gal and ITS genes could be achieved in (Fig.3) with as lowest volume of culture on 1mm×1mm size paper, using the PCR conditions optimized so far. Standard colony PCR reaction without Whatman filter paper no.1 served as a positive control. Bacterial colony suspension 2 µl of volume was chosen as the optimal volume. On 1mm×1mm size, Whatman no.1 paper Two µl volume to be spotted, and used the same was in all future experiments. We tried here 2 µl volume of colony suspension spotted on 1mm×1mm size Whatman paper no-1, 3 µl volume of colony suspension spotted on 3mm×3mm size of Whatman paper no-1 and 5 µl volume of colony suspension spotted on 5mm×5mm size of Whatman paper no.1. Larger size paper may create the problem of absorption of master mix from the tube, which hampers the reaction results. Earlier studies by Lokur et al. (2) have also shown culture volume optimization in the paper-based PCR method to amplify the 16srDNA gene.

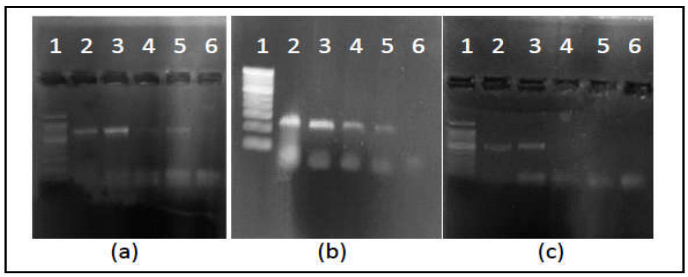

Fig.3 Paper size and culture volume Optimization

a) 16s r-DNA: Lane 1- 100bp Ladder, Lane 2- Colony PCR, Lane 3- 1mm×1mm size ppr with 2µL CS, Lane 4- 3mm×3mm size ppr with 3µL CS, Lane 5-5mm×5mm size ppr with 5µL CS, Lane 6- Negative control b) β-galactosidase Lane 1- 100bp Ladder, Lane 2- Colony PCR, Lane 3- 1mm×1mm size ppr with 2µL CS, Lane 4- 3mm×3mm size ppr with 3µL CS. Lane 5-5mm×5mm size ppr with 5µL CS, Lane 6- Negative control. c) ITS Lane 1- 100bp Ladder, Lane 2- Colony PCR, Lane 3- 1mm×1mm size ppr with 2µL CS, Lane 4- 3mm×3mm size ppr with 3µL CS, Lane 5-5mm×5mm size ppr with 5µL CS, Lane 6- Negative control.

Master Mix Volume Variation: Since in PCR master mix volume was used for the paper-based PCR reaction was limited, it was important to evaluate the impact of the volume of the master mix on the quality of the amplicon. Hence, the set up two similar reactions having 25 µL and 50 µL master mix and the (2 µl) of colony suspension was spotted on filter paper and dipped in the master mix for amplification of each gene. 10 µl of amplicon from each reaction was loaded on a 2 % agarose gel. Both the PCR reactions gave strong bands (Fig.4). It means, master mix volume variation does not matter. Also, the same quantity of colony suspension can result in a large volume of master mix.

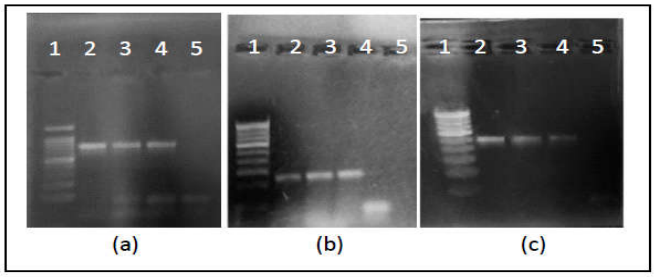

Fig. 4. PCR Master mix volume Variation

16s r-DNA: Lane 1- 100bp Ladder, Lane 2- Colony PCR, Lane 3-25µL master mix volume, Lane 4- 50µL master mix volume, Lane 5- Negative Control. b) β-galactosidase Lane 1- 100bp Ladder, Lane 2- Colony PCR, Lane 3-25µL master mix volume. Lane 4- 50µL master mix volume, Lane 5- Negative Control. c) ITS: Lane 1- 100bp Ladder, Lane 2- Colony PCR, Lane 3-25µL master mix volume. Lane 4- 50µL master mix volume, Lane 5- Negative Control.

pH Variation: PCR is a very sensitive method. We need to check all types of parameters for the method development process. Many kinds of literature are available, where pH and another internal sample source or media component impacted the PCR amplification process. Hence here, we evaluate the impact of pH variation in the developed paper-based PCR method. We used 4, 5, 6 and 9 pH water sources (Fig.5) and spiked the culture in these pH water. Other PCR conditions remained the same. Here covered all acidic, alkaline and

neutral pH. Already all the above parameters were evaluated with neutral pH-7.

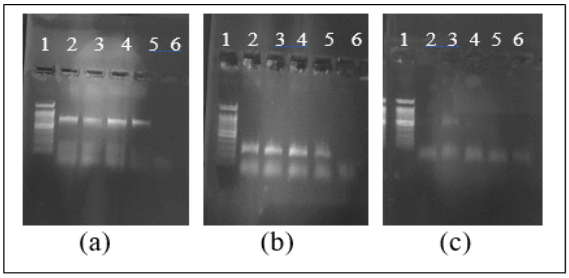

Fig. 5. pH Variation

a) 16s r-DNA: Lane 1- 100bp Ladder, Lane 2- pH 4, Lane 3- pH 5, Lane 4- pH 6, Lane 5- pH 9Lane 6- Negative Control. b) β-galactosidase Lane 1- 100bp Ladder, Lane 2- pH 4, Lane 3- pH 5, Lane 4- pH 6, Lane 5- pH 9Lane 6- Negative Control. c) ITS: Lane 1- 100bp Ladder, Lane 2- pH 4, Lane 3- pH 5, Lane 4- pH 6, Lane 5- pH 9Lane 6- Negative Control.

Specificity: The specificity of 16s rDNA, LacZ3 and ITS primers for detecting bacteria, coliforms and fungi was performed using a paper-based PCR method wherein bacteria *S. aureus* and *Candida albicans*, *E. coli* and *S. typhi* were tested. For fungal detection, *Candida albicans* and *E.Coli* were tested. The ITS primers use conserved regions of the 18s, 5.8s and 28s rRNA genes to amplify the noncoding regions between them (30). As expected, reactions showed a strong band indicating the presence of 16s rDNA, LacZ and ITS gene (Fig.6, lanes 2-4). This indicates the specificity of the developed paper-based method is very high. As per ICH guidelines, it is important to establish the specificity of the newly developed method for the desired result (28). The specificity of 16S rDNA, LacZ3, and ITS primers for detecting bacteria, coliforms, and fungi was established. The efficiency of the developed paper-based PCR method was checked by using the method to detect contaminating microbes in different water samples. These water samples were also tested using traditional microbiological methods to detect contaminants. APJ et al. same process used for milk samples (1). Several methods are available for counting viable bacterial cells in milk (29). Different ISO methods are also available to quantify probiotics and fermenting microbes employed in the dairy industry (5).

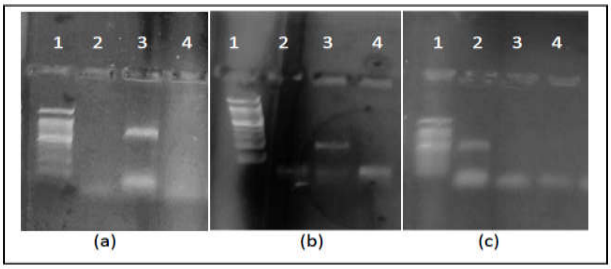

Fig. 6. Specificity

(a)16s r-DNA: Lane 1- 100bp Ladder, Lane 2- 16s rDNA with C. albicans culture, Lane 3- 16s rDNA with *S. aureus* culture, Lane 4-Negative Control. b) β-galactosidase: Lane 1- 100bp Ladder, Lane 2- β-gal with *S. typhi,* Lane 3- *β-gal* with *E. Coli.,* Lane 4-Negative Control. c) ITS: Lane 1- 100bp Ladder, Lane 2- ITS with *E. Coli*., Lane 3- ITS with *C. albicans*, Lane 4- Negative Control.

Actual Water samples used for paper based PCR method: When the reaction was tested using different actual water samples, the results observed were the same as those with the standard PCR reactions. A 16s rDNA and coliforms gene band were observed when reaction samples were run on a 2% agarose gel and visualized under UV transilluminator (Fig.7). the image proves that the components of waters (variation of salt and minerals concentration.) do not interfere with any components of the PCR mix. Hence, the paper-based PCR method can also detect 16s rDNA and coliforms in any water sample. As mentioned in the materials and methods section, 2 µL of water samples were directly spotted on the pre-sterilized Whatman filter paper and allowed to dry. These paper squares were directly dipped in a PCR master mix to detect bacterial contamination by amplifying the 16S rDNA gene and coliform contamination by amplifying the LacZ gene. As seen in Fig. 7, except aqua guard water samples, all others could detect the presence of bacteria and coliforms as both the genes got amplified, showing the respective amplicons on the gel. Interestingly, these results perfectly matched the data obtained through microbial analysis (CFU/mL), as mentioned in Table 1. PCR method was proved more sensitive and accurate, robust method in results (Fig.7). If we observed and compare the results in Table no.1 and Fig.7, it shows Aqua guard, Alfa and Bisleri water shows clear results on the microbial plate after 18 hrs. Incubation. Developed PCR shows the perfect size, specific amplicon band in molecular method within 2-3hrs. It means this advanced method is perfectly validated with microbial method comparison. The article mentioned many water contaminant detection methods in identifying future drinking water contaminants (1999)(10). Still, all are very time-consuming and traditional microbial, culture-based, assays and activity-based detection methods, which are not much accurate and specific as compared to advanced PCR methods.

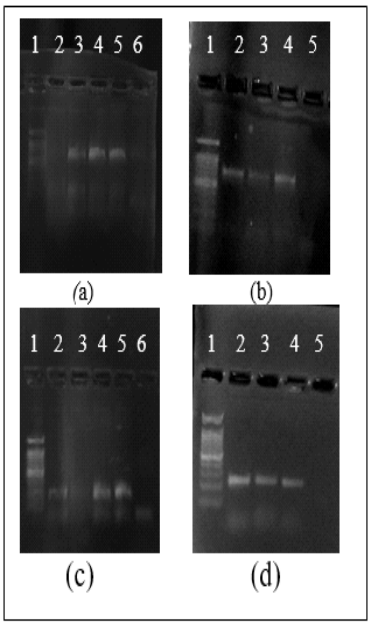

Fig.7 Water sample testing as a media

a)16s r-DNA: Lane 1- 100bp Ladder, Lane 2- Aqua guard water, Lane 3- Alfa water, Lane 4- Dormitory water, Lane 5- Bisleri water, Lane 6 - Negative Control.
(b)16s r-DNA: Lane 1- 100bp Ladder, Lane2- Tap water, Lane 3-Borewell water, Lane 4- Corporation water, Lane 5- Negative Control.
c) β-galactosidase Lane 1- 100bp Ladder, Lane 2- Aqua guard water, Lane 3- Alfa water, Lane 4- Dormitory water, Lane 5- Bisleri water, Lane - Negative Control.
d) β-galactosidase Lane 1- 100bp Ladder, Lane2- Tap water, Lane 3-Borewell water, Lane 4- Corporation water, Lane 5- Negative Control.

CONCLUSION

In summary, we've developed a unique technique referred to as the paper base PCR technique to detect coliforms and bacterial contamination in water samples. The method is easy to handle and simple to test the water samples. Currently, for testing time is 2 to 3hrs, the method can detect as low as 200 CFU/mL in 2

hrs and higher concentrations. Also, the utilization of positive and negative controls altogether our experiments excluded the likelihood of false-positive and false negatives, which can cause interpretation of the results. To enhance our concept and reduce the number of preparatory steps required for PCR. Including DNA template preparation. The performance of the developed method is checked and verified under different kinds of water samples containing interfering bacteria and other contaminants. This method would eventually be very easy to collect the samples from a remote area that can be transported in a pocket. No specific conditions are required. No specific temperature is required for the sample transportation process. A non-technical person can also collect the samples. The sample carrier Whatman paper can even be disposed of simply when finishing the test. The present technique platform is custom-made and integrated with more developments in detecting alternative microorganisms and pathogens and used for water samples and plenty of alternative products (wine, juices, etc.) and the food business. A serious application in pathological sample assortment.

Abbreviations

PCR: Polymerase chain reaction; **LB:** Luria–Bertani; **MA:** MacConkey agar; **PDA:** Potato dextrose agar; **LAFU:** Laminar air flow unit; **g.DNA**: Genomic DNA; **β-gal:** Beta galactocidase; **ITS:** Internal transcribed spacer

Units of measurement

Gm-Gram, µL- micro litter, mL- milli litter, °C- degree Celsius, CFU- colony forming unit

Authors' contributions

APJ performed all the experiments and compiled the data; BER Ph.D. guide. AD conceived and planned the study. All authors have read and approved the final manuscript.

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