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Rapid Isolation and identification of *E. coli* bacteria from drinking water

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Abstract
Microbial quality of drinking water is commonly assessed on routine culture techniques which are time consuming and laborious. By the time the results are accessible, pathogenic organisms might have spread wide in the water distribution system causing severe life threatening diseases. Hence there is a major need of new methods to detect these constraints accurately in a substantially shorter period. In the present study we have been using Polymerase Chain Reaction as a tool to detect *Escherichia coli* (*E.coli)* due to its high sensitivity and specificity.

Keywords: Polymerase Chain Reaction, *Escherichia coli*, Most probable number, Lac-Z gene.

1. Introduction
Rapid and accurate identification of microorganism populations in various substances, such as water, air, soil and blood, is of great importance for clinical and environmental studies. Remarkable progress has been made and achieved in upgrading the speed and quality of various diagnostic methods in infectious cases including metagenomics. These advanced settings are not only expensive in nature reproducibility also a major concern. Though conventional methods are more reliable but are quite slow in detection and laborious to handle in contrast to rapid detection. Identification of water born diseases like "diarrhea" caused by the bacterium entero toxigenic *Escherichia coli* (ETEC) contamination in drinking water, often depends on conventional methods includes culturing, biochemical and more preferable by most probable number (MPN). Routine MPN method relatively accurate for a given test organism, but often this method did not achieve the standard due to the occurrence of pathogenic bacteria low in number and tend to incur large errors in sampling and enumeration [1]. In some instances, this method can completely fail to classify the given test organism species and thereby reduces the accuracy of the method. Evidently, routine testing methods for water qualities are not only outdated but also cannot afford enough fortification to public health. The use of PCR based techniques and nucleic acid probes have provided extremely sensitive detection methods for specific bacterial pathogens in environmental samples [2], specially to detect pathogenic *E.coli* [3] by immuno-capture PCR. But these procedures are very expensive, laborious and monospecific. Molecular methods which are require to accurately classify the organisms like *E.coli* are very important [4, 5]. Thus the present study tried to evaluate the identification of *E.coli* bacterium from potable water by Polymerase Chain Reaction using *E.coli* specific primers.

2. Methodology
2.1. *E.coli* bacteria isolation
25 ml of drinking water from various sources was collected in a sterile glass bottle and filtered through 0.45-µm-pore Cellulose Nitrate Filter paper, filter paper was placed on nutrient agar media and incubated at 37 ^{0}C for 24 hours, a small punctiform, color less colony was taken by inoculation loop and inoculated with 5 ml of Nutrient broth and incubated at 37 ^{0}C for 12 hours. After the incubation period 0.5 ml of broth was taken in a sterile 1.5ml eppendorf tube for DNA isolation.

2.2. DNA isolation

1.5 ml of broth was taken from each tube and labeled in micro centrifuge tube and centrifuged at 2000rpm for 2 min and the supernatant was discarded carefully without disturbed the pellet. To this pellet 467 µl TE (pH6.8) buffer, 30 µl of 10% SDS was added and vortexed for a while and incubate 1 hour at 37 °C. After incubation period an equal volume of phenol/chloroform/Isoamyle alcohol (PCI) was added and mixed well by inverting the tube until the phases are completely mixed and kept for a till phases are separated. The upper aqueous phase was transferred into a new tube and repeated the PCI step for two times and finally centrifuged at 5,000 RPM for 10 min at 4 ^{0}C. Upper aqueous phase was transferred to a fresh tube and 1/10 volume of sodium acetate and 0.6 volumes of isopropanol was added. The tube was again centrifuged at 5,000 RPM for 10 min at 4 ^{0}C and the supernatant was discarded carefully. 1 ml of 70% ethanol was added and centrifuged at 5,000 RPM for 10 min at 4 ^{0}C. Supernatant was discarded and tubes were dried at 37 ^{0}C for 1 hour. The pellet was suspended in 50-100 µl of TE buffer. The purity of the DNA was checked on 1% agarose gel.

2.3. PCR Detection of the Presence of *E. Coli* using specific Primers

From the isolated DNA, PCR amplification was carried for Lac-Z gene using *E.coli* specific primer sequence described by [6]. Briefly the PCR solution was consisted of an lx PCR amplification buffer (10x buffer containing 50 mM KCl, 100 mM Tris hydrochloride, 1.5 mM $MgCl_2$,) 10 pmol/ µl each forward and reverse primer, 10mM of dNTPs, and 1 µl template DNA, 1 U of Taq DNA polymerase, and nuclease free water. Isolated DNAs were initially denatured at 94°C for 5 min. Then a total of 35 PCR cycles was run under the following conditions: denaturation at 94 °C for 1 min, primer annealing at 63 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min.

3. Results

The lacZ gene primer pair specific for *E.coli* was used in PCR reactions to detect *E.coli* bacteria in water samples. PCR conditions and master mix were followed as described in the methodology. After PCR all the samples were run in 2% agarose gel electrophoresis and the presence of the 180 bp fragment confirmed presence of *E.coli* (Fig 1)

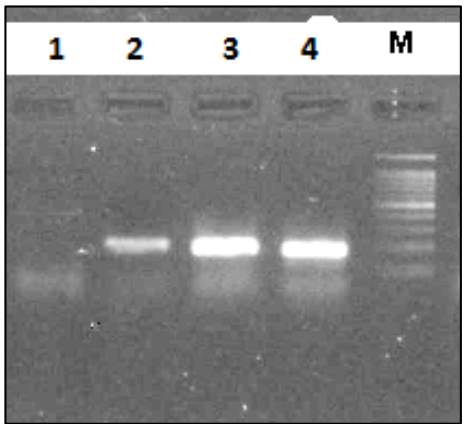

Fig 1: showing the amplified PCR products of isolated DNA from water samples
Samples 2-4 showing 180 bp product
Sample 1 – absence of 180bp product
M= DNA MARKER 100bp

4. Discussion

Water is routinely monitored for pathogenic microorganisms, conventional testing methods are not free from some disadvantages due to various reasons. First, the low inoculums size in water samples which leads to large errors in sampling and enumeration. Second, the routine culture techniques commonly used species-specific so time consuming procedures. Third, the difficulty in culturing [7, 8] because of their nutritional requirements. In order to overcome above issues, the present study for the rapid, sensitive and specific detection of *E.coli* bacteria in water resources, the polymerase chain reaction assay was used. In this present study primer pair specific to lacZ gene fragment for E. coli 80% (4 out of 5) of water samples were positive for the presence of *E.coli*. PCR and PCR based techniques used as molecular tools to detect the specific organism for quick confirmation. PCR based technology will give us accurate results within short time and low expensive compared to conventional methods. This technique is easy to perform and can be validated for industrial purposes for rapid sample analysis and minimal false results. By applying this method above issues can be avoided in real time and also pathogens can be detected accurately. In addition, the use of selective media containing inhibitory compounds to eliminate background bacteria may also be inhibited to environmentally stressed isolates [9]. The present study results proved that the PCR protocol allows the detection of bacteria in different types and sources of water samples.

5. Conclusion

The present study attempt successfully proved that PCR based gene amplification to detect *E.coli* which is used as an indicator organism for water contamination is a rapid, specific and sensitive approach.

6. Authors' Contribution

All authors participated in the research design and contributed to different parts of the research.

7. Conflict of Interest

The authors declare that there was no conflict of interest to publish this article.

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9. References

1 Fleisher JM. Conducting recreational water quality surveys: some problems and suggested remedies. Mar Pollut Bull.1990; 21(12):562-7.
2 Bej AK, Steffan RJ, DiCesare JL, Haf L, Atlas RM. Detection of *Escherichia coli* and *Shigella* spp. in water using the polymerase chain reaction and gene probes for uid. Appl Environ Microbiol. 1991; 57(4):1013-1017.
3 Ogunjimi AA, Choudary PV. Adsorption of endogenous polyphenols relieves the inhibition by fruit juices and fresh produce of immuno-PCR detection of *Escherichia coli* O157: H7. FEMS Immunol Med Microbiol. 1999; 23(3):213-20.
4 Straub TM, Dockendorff BP, Quiñonez-Díaz MD, Valdez CO, Shutthanandan JI, Tarasevich BJ *et al*. Automated methods for multiplexed pathogen detection. J Microbiol. Methods. 2005; 62:303-316.

5 Strauba TM, Chandlerb DP. Towards a unified system for detecting waterborne pathogens. J Microbiol. Methods. 2003; 53:185-197.
6 Shaban AB, Malkawi HI. Rapid detection of human enteric pathogens (viruses and bacteria) in water resources from Jordan using polymerase chain reaction (PCR). J Appl Sci Res. 2007; 3:1084-1093.
7 Byrd JJ, Xu H, Colwell R. Viable but nonculturable bacteria in drinking water. Appl. Environ. Microbiol. 1991; 57:875-878.
8 Ultee A, Souvatzi N, Maniadi K, Konig H. Identification of culturable and nonculturable bacterial population in ground water of a municipal water supply in Germany. J Appl. Microbiol. 2004; 96:560-568.
9 Arroyo G, Arroyo JA. Efficiency of different enrichment and isolation Procedures for the detection of Salmonella serotypes in edible offal. J Appl. Bacteriol. 1996; 78:281-289.