Original Article

# Evaluation of Polymerase Chain Reaction for Detecting Coliform Bacteria in Drinking Water Sources

**Bahram Nasr Isfahani, Hossein Fazeli, Zeinab Babaie, Farkhondeh Poursina, Sharareh Moghim, Meisam Rouzbahani**

*From the Department of Microbiology and Virology, Isfahan University of Medical Sciences, Isfahan, Iran*

## Abstract

**Background:** Coliform bacteria are used as indicator organisms for detecting fecal pollution in water. Traditional methods including microbial culture tests in lactose-containing media and enzyme-based tests for the detection of β-galactosidase; however, these methods are time-consuming and less specific. The aim of this study was to evaluate polymerase chain reaction (PCR) for detecting coliform. **Materials and Methods:** Totally, 100 of water samples from Isfahan drinking water source were collected. Coliform bacteria and *Escherichia coli* were detected in drinking water using LacZ and LamB genes in PCR method performed in comparison with biochemical tests for all samples. **Results:** Using phenotyping, 80 coliform isolates were found. The results of the biochemical tests illustrated 78.7% coliform bacteria and 21.2% *E. coli*. PCR results for LacZ and LamB genes were 67.5% and 17.5%, respectively. **Conclusion:** The PCR method was shown to be an effective, sensitive, and rapid method for detecting coliform and *E. coli* in drinking water from the Isfahan drinking water sources.

**Keywords:** *Coliforms, LacZ, LamB, polymerase chain reaction*

## Introduction

Drinking water should be free from known pathogenic microorganisms and indicator bacteria, both signs of fecal water contamination.[1,2] Fecally contaminated drinking water is a major public health problem.[3] Coliform bacteria are general contaminants present in drinking water. Therefore, detecting them as indicators of human fecal contamination is very important for protection of public health.[4] Coliforms are aerobic and facultative anaerobic bacteria, Gram-negative, nonspore-forming and rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h when incubated at 37°C.[5]

Most coliforms are present in large numbers among the intestinal flora of humans and other warm-blooded animals and are therefore found in fecal wastes.[6] Conventional methods for detecting the microbial contamination of water are based on culturing water samples and diagnosing β-galactosidase using ortho-nitrophenyl-β-D-galactopyranoside.[7] These methods are time-consuming and give false positive results.[8] Polymerase chain reaction (PCR) has been suggested as a specific and reliable method for detecting coliforms in drinking water.[9]

In this study, the presence of coliforms in drinking water from Isfahan's refinery was evaluated by phenotypic and PCR by the specific amplification of the LacZ gene that encodes the β-Dgalactosidase enzyme and the LamB gene that codes maltose transport protein. These genes were selected because they are the basis of assays for detecting coliform bacteria and *Escherichia coli*, respectively.[10]

## Materials and Methods

### Sampling and sample preparation

Water samples were collected from Isfahan Refinery in aseptic conditions into 500 ml sterile container with propylene lids. Sodium thiosulfate was added to remove chlorine residual. The water samples were immediately examined for bacteriological (total coliform and *E. coli*) analyses in duplicate. Samples were passed through a 0.45 μm filter by a vacuum pump. To avoid possible contamination, analyses were conducted under a class two laminar flows.

**How to cite this article:** Isfahani BN, Fazeli H, Babaie Z, Poursina F, Moghim S, Rouzbahani M. Evaluation of Polymerase Chain Reaction for Detecting Coliform Bacteria in Drinking Water Sources. Adv Biomed Res 2017;6:130.

**Received:** January, 2015. **Accepted:** February, 2015.



***Address for correspondence:***
*Mrs. Zeinab Babaie, Department of Microbiology and Virology, Isfahan University of Medical Sciences, Isfahan, Iran.*
*E-mail: zeinab6877@yahoo.com*

**Access this article online**

**Website:** www.advbiores.net

**DOI:** 10.4103/2277-9175.216783

**Quick Response Code:**

Filters were transferred aseptically onto an eosin methylene blue agar medium containing 500 mg cyclohexamide to culture bacteria in the samples. This agar contains lactose and the dyes Eosin Y and methylene blue. Plates were incubated at 37°C for 18–24 h. The culture on the nutrient agar was analyzed by Gram-staining.

## Biochemical assays

The biochemical tests, oxidase production, methyl red, Vogues–Proskauer test, indole production, citrate test, motility test, and catalase production, were performed according to standard microbiological methods.

## DNA extraction

Bacterial DNA was extracted by boiling. 4–5 colonies of bacterial dissolved in 500 µl sterile distilled water for 10 min, then stored in −20°C.[11]

## Polymerase chain reaction

Polymerase chain reaction amplification using the primers as shown in Table 1 was performed using a thermal cycler, and the amplification reaction in a final volume of 25 ml contained 2 µl extracted DNA and the 2.5 µl 10X buffer, 0.5 mM dNTPs, 2.5 units of Taq DNA Polimerase enzyme (cinnagen) and 1 µl of both primers (10 pmol/µl), and 17 µl double-distilled water. Totally, 35 cycles of amplification were performed in a thermal cycler under the following conditions: Initial denaturation at 95°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 10 min.

They yielded an amplified product 876 bp. A 554 bp sequence downstream from the sequence encoding the lambda attachment site peptide of LamB was amplified using two different 24-mer primers. The amplified products were electrophoresis on a 1.2% agarose gel. *E. coli* ATCC 25922 DNA and autoclaved deionized water were used as the positive and negative controls, respectively.

## Results

The study was conducted on 100 water samples in the laboratory. A total of 80 isolates that were Gram-negative rods were obtained based on Gram-staining and biochemical tests. The results of the PCR and the biochemical tests on all samples are shown in Tables 1-3.

Polymerase chain reaction analysis of 80 isolates obtained after biochemical analysis of water samples revealed that 68 of the organisms were positive for the LacZ gene; of the 17 organisms isolated from *E. coli*, 14 (17.5%) were positive for the LamB gene. Results of PCR for LacZ and LamB genes on these samples confirmed the occurrence of 876 bp and 554 bp bands. A similar band was found with the positive control's *E. coli* DNA that was used, but no amplification was observed with the negative control in which sterile deionized water was used instead of DNA.

**Table 1: Forward and reverse primers of lacZ and lamB gens**

| Gene | Primer set | Product size (bp) | Reference |
|---|---|---|---|
| LacZ | LZL: ATGAAAGCTGGCTAC AGGAAGGCC | 876 | [10] |
| | LZR: CACCATGCCGTGG GTTTCAATATT | | |
| LamB | LBL: GGATATTTCTGGTC CTGGTGCCGG | 554 | [10] |
| | LBR: ACTTGGTGCCGTTGTCG TTATCC | | |

**Table 2: Percentage of coliform bacteria isolated from water samples based on phenotyping**

| Variable | Total sample (100) | | | | |
|---|---|---|---|---|---|
| | *E. coli* | *Klebsiella* | *Citrobacter* | *Enterobacter* | *Serratia* |
| Percentage of coliform bacteria | 21.25 | 23.75 | 5 | 32.5 | 17.5 |

*E. coli: Escherichia coli*

**Table 3: Percentage of *E. coli* and coliform bacteria based on phenotypic and genotypic (PCR) tests**

| Bacteria | Number (%) | |
|---|---|---|
| | Biochemical tests | PCR |
| Coliform | 63 (78.8) | 54 (67.5) |
| *E. coli* | 17 (21.2) | 14 (17.5) |

PCR: Polymerase chain reaction, *E. coli: Escherichia coli*

## Discussion

Coliform bacteria and *E. coli* are used as indicators to measure the degree of pollution and sanitary quality of drinking water because testing for all known pathogens is a complicated and expensive process.[12] Forward and reverse primers of *lacZ* and *lamB* gens and product size.

The traditional methods of coliform detection like methods based on culture, have limitations, such as long incubation periods, interactions with other microorganisms, lack of specificity, lack of accuracy, and poor detection of slow-growing microorganisms.[13]

Identifying coliforms with molecular techniques is highly suggested as these methods allow for very specific and rapid detection[14] and can be used to correctly analyze the drinking water performance of the elimination of pathogen performance of the elimination of pathogens in drinking water and treatment of water used for drinking.[5] Three molecular-based methods are generally used: Immunological, PCR, and *in-situ* hybridization techniques. In the immunological method, various antibodies against coliform bacteria have been produced, but the use of this technique often shows low antibody specificity.

The PCR method can detect coliform bacteria using the LacZ gene (gene β-galactosidase) and *E. coli* bacteria using the LamB gene that codes the maltose transport protein.[10,15] In this study, 68 number of the organisms were positive for the LacZ gene and 14 (17.5%) of these were positive for the LamB gene.

In one study in Baghdad city of 300 samples, 270 were positive for the fecal and total coliform with routine diagnosis methods, in the same time (200) sample were positive for *E. coli*. The PCR amplification assay detected the presence of bacteria in 250 of 300 water samples depending on the LacZ genes.[16] Another study in Egypt reported 90% of the collected water samples were positive for coliform.[17]

The *E. coli* genes dct A, uidA, dcuB, frdA, dcuS, and dcuR were modified for use as in the noncultivation-based molecular method to detect *E. coli* populations in water samples without the need for pure and identified tests.[18] None of these molecular methods however have been standardized for routine usage.[7] Other primer sets considered for two different regions have been proposed for the detection of *E. coli*, one of them coding for an outer-membrane protein (phoE gene)[19] and the other coding DNA sequences for the V3 and V6 regions of the 16S rRNA genes of pathogenic and nonpathogenic strains of *E. coli*. These primer sets allow the specific detection of not only *E. coli*, but also *Shigella* species when the recommended sequences are amplified.[20]

## Conclusion

The PCR technique is specific and consistent in the clear detection of coliforms and can, therefore, be popularized for routine laboratory assays. Following the isolation of coliform bacteria in drinking water from the refinery of Isfahan, regulations and overseeing various parts to minimize water-borne diseases are recommended.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

1. Fatemeh D, Reza ZM, Mohammad A, Salomeh K, Reza AG, Hossein S, *et al.* Rapid detection of coliforms in drinking water of Arak city using multiplex PCR method in comparison with the standard method of culture (Most Probably Number). Asian Pac J Trop Biomed 2014;4:404-9.
2. Stepenuck KF, Wolfson LG, Liukkonen BW, Iles JM, Grant TS. Volunteer monitoring of *E. coli* in streams of the upper Midwestern United States: A comparison of methods. Environ Monit Assess 2011;174:625-33.
3. Fatahi-Bafghi M. Phenotypic and molecular identification of nocardia in brain abscess. Adv Biomed Res 2017;6:49.
4. Awwa A. Standard Methods for the Examination of Water and Wastewater. Washington, DC: American Public Health Association; 1998. p. 20.
5. Rompré A, Servais P, Baudart J, de-Roubin MR, Laurent P. Detection and enumeration of coliforms in drinking water: Current methods and emerging approaches. J Microbiol Methods 2002;49:31-54.
6. McFeters GA, Cameron SC, LeChevallier MW. Influence of diluents, media, and membrane filters on detection fo injured waterborne coliform bacteria. Appl Environ Microbiol 1982;43:97-103.
7. Tharannum S, Sunitha S, Nithya J, Chandini M, Vanitha J, Manjula T, *et al*. Molecular confirmation of the presence of coliforms in drinking water using polymerase chain reaction. Kathmandu Univ J Sci Eng Technol 2009;5:130-6.
8. Kämpfer P, Nienhüser A, Packroff G, Wernicke F, Mehling A, Nixdorf K, *et al.* Molecular identification of coliform bacteria isolated from drinking water reservoirs with traditional methods and the Colilert-18 system. Int J Hyg Environ Health 2008;211:374-84.
9. Clifford RJ, Milillo M, Prestwood J, Quintero R, Zurawski DV, Kwak YI, *et al.* Detection of bacterial 16S rRNA and identification of four clinically important bacteria by real-time PCR. PLoS One 2012;7:e48558.
10. Bej AK, Steffan RJ, DiCesare J, Haff L, Atlas RM. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl Environ Microbiol 1990;56:307-14.
11. Ahmed OB, Asghar AH, Elhassan MM. Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. Afr J Microbiol Res 2014;8:598-602.
12. Spellman FR, Drinan JE. The Drinking Water Handbook. McNeese State University: CRC Press; 2012.
13. Tantawiwat S, Tansuphasiri U, Wongwit W, Wongchotigul V, Kitayaporn D. Development of multiplex PCR for the detection of total coliform bacteria for *Escherichia coli* and *Clostridium* perfringens in drinking water. Southeast Asian J Trop Med Public Health 2005;36:162-9.
14. Van Belkum A. DNA fingerprinting of medically important microorganisms by use of PCR. Clin Microbiol Rev 1994;7:174-84.
15. Locas A, Barthe C, Margolin AB, Payment P. Groundwater microbiological quality in Canadian drinking water municipal wells. Can J Microbiol 2008;54:472-8.
16. Al-Thwani AN. Monitoring of drinking water quality in Baghdad city by using polymerase chain reaction (PCR). J Babylon Univ Pure Appl Sci 2014;3.
17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-Second Informational Supplement. CLSI Document M100-S22. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2012.
18. Abo-Amer AE, Soltan el-SM, Abu-Gharbia MA. Molecular approach and bacterial quality of drinking water of urban and rural communities in Egypt. Acta Microbiol Immunol Hung 2008;55:311-26.
19. Spierings G, Ockhuijsen C, Hofstra H, Tommassen J. Polymerase chain reaction for the specific detection of *Escherichia coli*/*Shigella*. Res Microbiol 1993;144:557-64.
20. Tsen HY, Lin CK, Chi WR. Development and use of 16S rRNA gene targeted PCR primers for the identification of *Escherichia coli* cells in water. J Appl Microbiol 1998;85:554-60.