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Original Article

Rapid detection of *Vibrio Cholerae* by Polymerase Chain Reaction based on nanotechnology method

Gholam Reza Herfehdoost[1], Mahdi Kamali[2], Hamid Reza Javadi[2], Davood Zolfagary[2], Asghar Emamgoli[2], Ali Choopani[3, 4*], Bahareh Ghasemi[4], Saeideh Hossaini[4]

Abstract

Quick identification of *Vibrio Cholerae* in epidemics is important, on the other hand; conventional methods are time-consuming and costly. The aim of this study was to develop a rapid, inexpensive and high sensitivity method for quick identification of *Vibrio Cholerae*. For this purpose we designed a PCR detection based on magnetic nanoparticles for identification of bacterial DNA by PCR Dynabead. So we used the biotinalated Probe for binding to DNA extracted from *Vibrio Cholerae* and other bacterial species (*Salmonella*, *Shigella*, *Pseudomonas*, *E.coli*) Using magnetic bead isolated with magnetic field, the Vibrio genome-specific primers (HlyA) for pathogen detection (PCR) was used. The results showed specific band was just for *Vibrio Cholera* (PCR positive); therefore designed probe was specific for *Vibrio Cholera*. According to the findings, this study is characterized the high sensitivity of PCR using biotin-containing probes for DNA of *Vibrio Cholera* in contrast to the Traditional methods.

1. Neuroscience research center, Baqiyatallah university of Medical Sciences, Tehran, Iran
2. Nanobiotecnology Research Center, Baqiyatallah University of Mnedical Sciences, Tehran, Iran
3. Applied Biotechnology Research Center, Baqiyatallah university of Medical Sciences, Tehran, Iran
4. Department of Biology, Payamnoor University, Tehran, Iran

* **Corresponding Author**
Ali Choopani
Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
E-mail: choopani.ali3266@gmail.com

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Introduction

Pathogenic species of the genus vibrio pose a considerable public health threat as the causative agents of both sporadic and epidemic human infections [1-3]. Cholera is a diarrheal illness in the intestinal tract caused by the Gram-negative bacterium *Vibrio cholerae* is a member of the family *vibrionaceae*, facultative anaerobe [1, 4, 5]. It is highly motile by a single polar flagellum, highly halophilic, usually rod-shaped, either straight or curved, and high sensitive to acid. They use fermentative or respir-atory metabolism and are heterotrophic [1, 3-5]. *Vibrio Cholerae* contains a 4.0 M bp genome, consisting of two circular chromosomes, there are a predicted 3,885 total genes [1, 6-8].

Early detection and initiation of treatment of these infections are very important, particularly for cholera and invasive Vibrio infections, because these may rapidly to death [2-4, 9]. Prevention of Vibrio infections requires a heightened awareness of these infections by clinical, laboratory technicians, and epidemiologists [2, 4]. To date, a number of methods for detection of *Vibrio Cholerae* in environmental have been developed [3, 10-13].

The classical detection methods include culture, morphological, biochemical and serological confirmation tests which generally take days to completed [1, 11, 14]. Current rapid detection methods include the use of immunomagnetic beads and nucleic acid-based methods such as PCR and DNA probe hybridization [3, 15-17]. Classical methods for DNA isolation are either column – based techniques or include precipitation and centrifugation steps with toxic organic solvents having the disadvantage that take many times, difficult to automate or not useful for downscaling to small sample volumes[1, 8, 11, 13, 18]. Through the magnetic bead technology these main limitations can be avoided [11, 12, 15, 17]. This makes the sample preparation fast and highly method for detection, hybrid capture using magnetic particles, which relies on selective isolation of target DNA by hybridization to oligonucleotide probes linked to magnetic nanoparticles, this would decrease the total detection time, increasing PCR sensitivity, and removing most of the inhibitors of the amplifications reaction and excess of non-target DNA [11, 12, 16, 17]. The purpose of this study was to develop a new method to isolate DNA target of *Vibrio Cholerae* from other nucleic acids. Therefore, a capture probe specific for Vibrio that was immobilized on magnetic beads and used for DNA extraction followed by PCR.

Materials and methods

Growth condition

Cholera strains were growth in L.B broth (Luria-Bertani Broth) or on LB agar at 37˚C and autoclaved for 18- 24 h. The strains were used in this study includes:

Bacteria strain	ATCC
Vibrio Cholerae	14035
Pseudomonas aeruginosa	27583
Shigella Flexner	29903
E. coli	25992
Klebsiella Pneumoniae	7881

Probe and Primer design

All primers and probes were used based on previously published (Table 1) which designed using standard sequence analysis software [3, 5, 7, 12, 14].

Table 1. Primer sequences

F Primer	5-GAGCCGGCATTCATCTGAAT-3- (hlyA)
R Primer	5-CTCAGTGGACTAATACGGTTCA-3 –(hlyA)

Capture probe design

The oligonucleotide probe sequence was selected from a highly conserved region of the *Vibrio Cholerae omp* gene. The capture probe was modificated with 6-12 chain of NH_2 in 5´ regions that named spacer. In 3´ regions of capturing probe attached biotin for flexibility [12].

Nanoparticles

In this study was used of Dynabead M-270 Carboxylic Acid and M-280 streptavidin. These are uniform, para-magnetic, polystyrene beads activated by Carboxylic acid functionality (M-270) or streptavidin covalently attached to their surface (M-280).

DNA purification

Bacteria genomic DNA was purified using the kit (Bi-oneer) following the manufacturer's instructions, and a phenol- chloroform based method, DNA purity and concentration were determined by absorbance at 260/280 nm. Alternatively, purified DNA was quantified on agarose gel and a gel-doc apparatus and nanodrup.

Hybridization

The hybridization solution consisting of 200 µl of hybridization buffer (1x Binding & Washing buffer (B&W) consisting of 5 mM Tris-HCl (pH: 7.5), 0.5 mM EDETA, 1M NaCl and 1x hybridization solution.

PCR detection

PCR amplification was performed in a total reaction volume of 50 µl (Table 2).

Table 2. PCR Master Mix

Material	Concentration	Volume
PCR buffer 10x	10 x	5
dNTPs	2.5 mM	4
$MgCl_2$	50 mM	2
Primer 1	10 pm	2
Primer 2	10 pm	2
Taq DNA Pol	500 u	0.5
Template		33.5
Distilled Water		1

Results

We performed a comparative analysis to identified *Vibrio Cholerae* .The results of PCR between strain *Vibrio Cholerae* and mixer with other bacterial listed in material and method section and bacterial DNA singly show in figure 1, only the genome of *Vibrio Cholera*e is positive and other genome of bacterial not attached to probe. Columns 1 to 7 include: *Shigella Flexner, Pseudomonas aeroginosa, E.coli, ladder, Klebsiella Pneumonia, Vibrio Cholerae* and negative control, according the result of PCR the capture probe (biotinalated probe) only detect the *Vibrio Cholerae* genome and other bacterial is negative. Thus the probe specifically captures *Vibrio Cholerae* genome in mix another genome. For sensitivity of this study PCR with dilution titre of *Vibrio Cholerae* genome was done that show in figure 2.

The result of PCR prove that the dilution of 10 to 0.625 ng/µl is positive but the result PCR without the use of capturing probe is positive of 10 to 2.5 ng/µl.

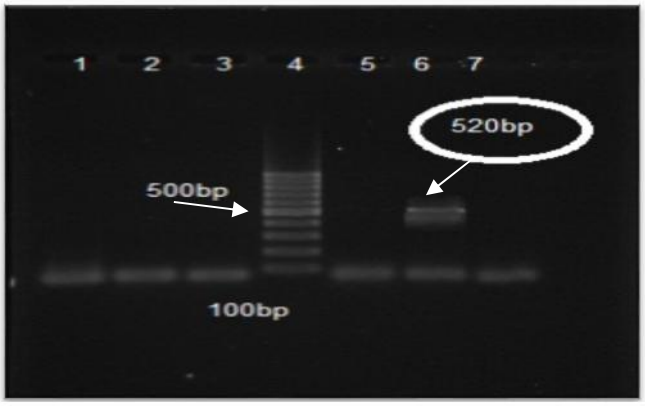

Figure 1. The result of PCR
Lane 1: *Shigella Flexner;* Lane 2: *Pseudomonas aeroginosa;* Lane 3: *E.coli;* Lane 4: DNA ladder*;* Lane 5: *Klebsiella Pneumonia;* Lane 6: *Vibrio Cholerae* and Lane 7: negative control.

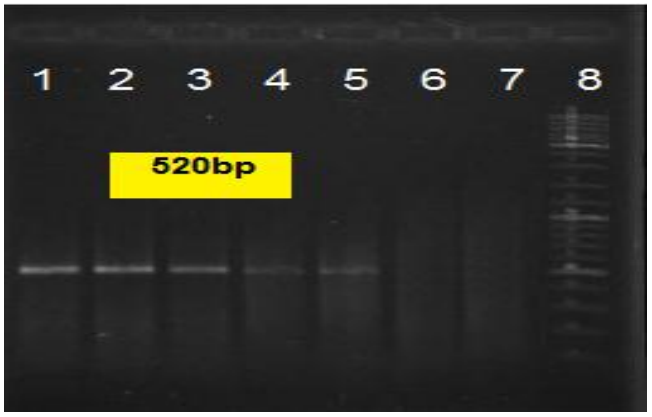

Figure 2. Lanes 1 to 6 including *Vibrio Cholerae* DNA serial dilution (ng/µl): 10, 5, 2.5, 1.25, 0.625, 0.312; Lane 7: Control; Lane 8: DNA ladder.

Discussion

LB and related media (SOC, Terrific Broth, 2xYT, etc) are used extensively in recombinant DNA work and other

molecular biology procedures and PCR primers have now been developed that allow for specific detection of a range of targets (species, serogroup, toxin, etc.) in any given sample [5]. Polymerase chain reaction is extensively used to aid (and replace) traditional microbiology, as it allows for rapid identification of bacterial species and detection of virulence genes [13]. The result of PCR based on nano-technology in compare with other methods, indicated more specialty and sensitivity and in term of time and coast is affordable and the earned results in diagnostic and detection are reliable. In common method the PCR technique was able to detect maximum 500 genome in ml but in this study detected about 100 genome in ml (0/625 ng/µl), thus the raise of sensitivity and specificity, decrease in time and coast are the important factors in detection and treatment. According to these properties, Amagliani *et al* used this method for detection of *listeria monocytogenes* in milk sample [16]. Also Thomson *et al* used a capture probe for detection of salmonella in water [17]. Dobryan *et al* used and developed Light Upon extension (LUX) real-time PCR assays by targeting species-specific poly-morphisms, that were successful in rapidly identification and differentiation of the major pathogenic Vibrio species [3, 12]. Low *et al*, worked on a thermo stabilized magne-togenosensing assay for DNA sequence-specific detection and quantification of *Vibrio Cholerae* [12]. This researches show the clinical applicability of the assay was successfully validated.

Conclusion

According to the results the nanotechnology is the Emerging science in medicine field. The use of magnetic nanoparticles is the reliable, expensive loss time and with specificity, sensitivity method.

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