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Research Paper

Enrichment and Rapid Detection of Vibrio Cholerae From Water by Non-culture Method

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ABSTRACT

Background and Aim In the microbial contamination of food and water, identifying the trace amounts of contaminating bacteria has always been of researchers' interest and concern. The most frequent approach to resolve this problem is using culture-based methods to increase and enrich bacteria samples; accordingly, it extends the bacterial detection process to several hours or days. One of the smart strategies to solve this problem is the concentration of bacteria using physical methods. The present study aimed to enrich Vibrio cholerae as the most essential water-polluting germs. Accordingly, we used the filtration method and evaluated its function by culture method and two detection approaches of Adenosine Triphosphate (ATP) and PCR assay.

Methods & Materials A certain concentration of V. Cholerae was artificially added to a specified volume of sterile water. Then, the bacteria were extracted from the medium and filtered using 0.450 µm separable filters. Finally, the performance of the pre- and post-filtration processes was compared using bacterial cell culture (CFU), ATP, and PCR assay with the specific primers for the ompW gene of V. cholerae.

Ethical Considerations This article is a meta-analysis with no human or animal sample.

Results The present research results indicated that the applied method presented high efficiency and recovery performance. In other words, samples provided no positive response before filtration in both methods; however, after filtration in isolated and recovered samples, the presence of bacteria was detected in the ATP and PCR methods.

Conclusion In conclusion, the employed strategy can detect V. cholerae in non-culture and in the shortest time in contaminated water samples.

Extended Abstract

1. Introduction

In the microbial contamination of water and food, identifying small amounts of contaminating bacteria has always been a concern of researchers [4-7]. Accordingly, it is necessary to concentrate the bacteria and increase its number. The most prevalent approach to solve this problem is to grow bacteria to elevate their number, which increases the time of bacterial detection to several hours or even days [8, 9]. An effective and smart solution to solve this problem is to concentrate bacteria by physical and non-cultured methods [17-19]. The present study aimed to enrich Vibrio cholerae (V. cholerae), as the most crucial microbial

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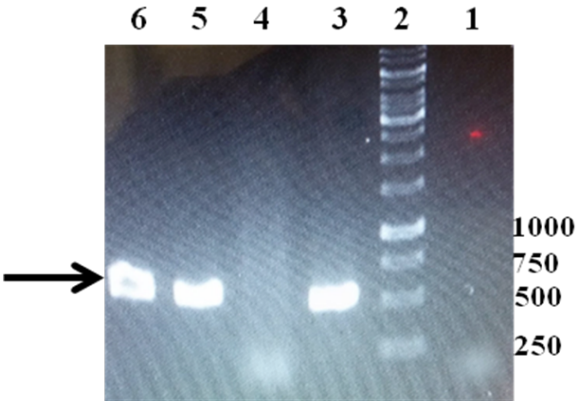

Figure 1. Evaluating filtration method using PCR and comparing pre-filtered sample (row 4) and filtered sample (row 3)

contaminant in water; thus, we used physical filtration and evaluated this yield by culture method and the diagnostic methods of ATP and PCR.

2. Materials and Methods

V. cholerae bacterium obtained from the reference laboratory (Bouali Hospital) was initially confirmed using specific tests (specific culture medium & molecular PCR methods). Then, a certain concentration of bacteria was artificially transferred in a certain volume of sterile water. Next, with the help of Watman's 0.45-micron filters, which contain detachable preservatives, bacteria were extracted from the environment and concentrated on the filter. Finally, the performance of the method after and before filtration using cell culture (determining & counting colony, CFU), ATP assay (using the leading Nuragen company kit & Hygina luminometer), and molecular PCR method (with specific primers of Vibrio cholerae ompW gene) were compared.

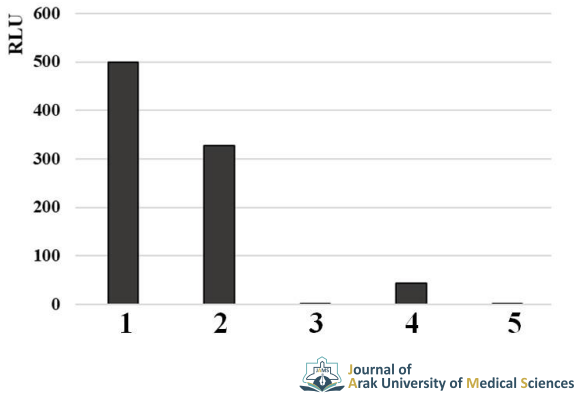

Figure 2. Determining filter method sensitivity using ATP test

Sample 1=10^6 CFU, sample 2=10^5CFU, sample 3=10^4 CFU, sample 4=10^3 CFU, sample 5=10^2CFU.

3. Results

To confirm the filtration method, V. cholerae was filtered 3 times with different concentrations. The present research results indicated that the physical filtration method in concentrating V. cholerae bacteria presents high efficiency and recycling performance. The sample, before filtration, provided no positive result in the ATP and PCR assay methods; however, after filtration, the presence of bacteria in both methods was observed and proven in isolated and recycled samples (Figures 1 & 2). The sensitivity of the filter method was also evaluated by PCR test, i.e. estimated according to Figure 3 and by comparing Figures 3A and B; accordingly, by revealing the PCR reaction results of concentrated

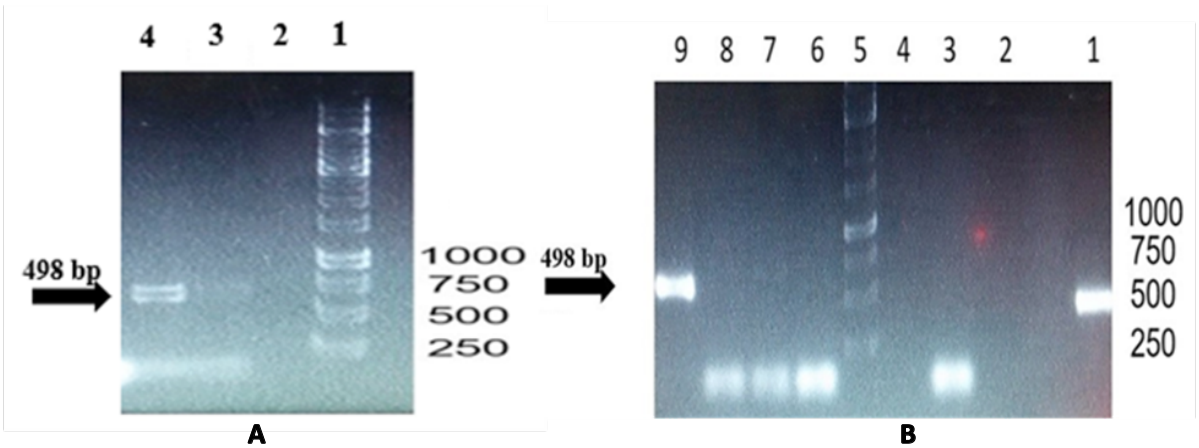

Figure 3. Determining the sensitivity of the filter method using ATP test for samples

A: After; and B: Before the filtration of contaminated samples.

and non-concentrated samples, the sensitivity of CFU 10^1 filter technique was estimated.

4. Discussion and Conclusion

According to the obtained data, the filtration method in concentrating V. cholerae can be introduced as reliable and practical in removing contaminants, concentrating, and isolating bacteria. The main problem of the filter-based concentration method is the recycling of bacteria from the filter. With the method used, the bacterial recycling efficiency reached 100% (Figure 1). The same efficiency was observed in previous investigations. For example, in 1996, Hug et al. used the filter method to remove V. cholerae contamination from contaminated water, which also achieved 100% filtration separation efficiency [17]. Other methods of concentrating bacteria include the adsorption approach using magnetic nanoparticles Immunomagnetic Separation (IMS). Accordingly, in 2001, Hudson et al. could use this method to separate Listeria bacteria from meat samples and in <24 hours, the bacteria were isolated and identified by PCR [23].

The current research findings suggested that using a physical concentration strategy with filtration, V. cholerae can be detected in contaminated water samples in the shortest time without the need for culture.

Ethical Considerations

Compliance with ethical guidelines

This article is a meta-analysis with no human or animal sample.

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Authors' contributions

Conceptualization, methodology, writing – original draft, and writing – review & editing: Mehdi Zeinoddini; Investigation: Abolfazl Moradi.

Conflicts of interest

The authors disclosed no conflicts of interest.

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