

Short Communication

The prevalence of *Vibrio cholerae* among patients with diarrheal infections in the flood-affected population of Pol-e Dokhtar city in 2019

Zahra Babaei¹, Nikou Bahrami², Sahar khalili³, Iman Pouladi^{4*}

¹Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.

²Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Iran.

³Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

⁴Student Research Committee, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

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Abstract

Background: *Cholera* is an acute diarrheal intestinal infection caused by *Vibrio cholerae*. *Cholera* is defined as endemic in Iran, thus rapid detection in the early stage of infection is crucial, especially during the disease outbreaks.

Materials and Methods: We collected 172 rectal swab samples from patients with diarrheal infections, and sterile beakers were used to collect water samples from some regions of Pol-e Dokhtar city. The collected specimens were transported to the laboratory using Cary-Blair transport medium and were then grown and incubated on alkaline peptone water (APW) and thiosulfate citrate bile salts sucrose (TCBS) agar. Then, all colonies that grew in Kligler Iron Agar (KIA) were cultured. **Results:** In this study, colonies were grown on APW and TCBS media, and were then transferred to the KIA. None of the colonies were shown to be belonged to *V. cholerae* strains. Thus, all collected samples were negative for *V. cholerae* in all patients as well as the drinking water in most regions of the city. **Conclusion:** The findings of this study suggest that *V. cholerae* was not the major cause of severe diarrheal infections in a flood-hit population of Pol-e Dokhtar city, and therefore other bacterial and viral agents should be responsible for severe diarrheal illnesses in this population.

Keywords: *Vibrio cholerae*, diarrheal infections, flood-affected population, Pol-e Dokhtar city

*Corresponding Author: Iman Pouladi. Tel: (+98) 66- 33120133; Email: Imanpouladi96a@gmail.com

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Introduction

Vibrio is a ubiquitous genus of marine bacteria with a wide variety of organisms commonly found in marine surface waters throughout the world. It is composed of 36 species, of which 8 are pathogenic to humans. The genus *Vibrio* is a member of the Vibrionaceae family [1]. *Cholera* is one of the world's most common infectious diseases caused by drinking water or eating food contaminated with *V. cholerae*. The *cholera* is caused by the bacteria *V. cholerae* and is originated in Southeast Asia [2, 3, 4]. This disease as the cause of severe acute diarrhea can severely affect

the health and overall economic growth of the affected communities for centuries [5, 6]. This bacterium as a facultative pathogen in humans has various biotypes and serotypes, originally inhabiting the marine environments in the slope of the mouth of bays and rivers [5]. To date, more than 200 different O-antigen serotypes of this pathogen have been identified. However, only two serotypes of O1 and O139 are responsible for *cholera* epidemics and pandemics all around the world. These two serotypes can rapidly lead to dehydration and death [7, 8]. O1 serotype is subdivided into Inaba, Ogawa and Hikojima serotypes

based on the composition of antigens A, B, and C in the lipopolysaccharide structure; and subdivided into two biotypes of classical and El Tor, which is the major cause of classical *cholera* [4]. This medical condition is a major health problem in developing countries, such as Iran. Seven *cholera* pandemics have been reported during which *V. cholerae* O1 El Tor biotype has spread to many countries of Asia [7, 9]. Iran has been introduced as one of the endemic countries for *cholera* outbreaks in recent years [1]. Early detection of the disease and identification of associated causal factors in water and food samples remarkably contribute to the prevention of an outbreak. Therefore, nowadays, there are many microbiological methods, such as cultivation, serotyping of microorganisms through agglutination, biochemical and biological methods, toxin assay, Enzyme-Linked Immunosorbent Assay (ELISA), and immunofluorescence assay for detection of *V. cholerae* [5, 10]. In Iran, according to WHO guidelines, direct slide observation, Cary-Blair transport medium, and Thiosulphate-Citrate-bile Salt-Sucrose agar medium are used to detect *V. cholerae* [4]. The present study aimed to evaluate the prevalence of *V. cholerae* in diarrheal patients in flood-affected Pol-e Dokhtar city.

Methods

Clinical sample collection. In this study, 172 clinical rectal swab specimens were collected using sterile swabs stained with the stool of flood-affected patients with severe diarrheal infection in spring 2019 in Pol-e Dokhtar city. All specimens were transported on the Cary-Blair transport medium. Also, sterile beakers were used to collect water samples. Water samples were collected from different regions across Pol-e Dokhtar and were then sent to microbiological laboratories for culture and examination.

Primary isolation of clinical specimens and phenotypic identification. In the microbiology laboratory, clinical rectal swab samples were first cultured on the first (thiosulfate-citrate-bile salts-sucrose) TCBS I agar, incubated for 2 h at 2 °C, and then the rectal swabs were placed in alkaline peptide water (APW) medium. After 6–8 hrs of incubation at 35–37 °C, samples were harvested from the uppermost surface of the APW medium and cultured in TCBS II agar. Suspect *V. cholerae* appears as yellow colonies on the medium, and suspected colonies were then gram

stained. Afterward, other tests such as catalase, Kligler Iron Agar culturing, lysine iron agar, filamentous test, and oxidase were performed.

Results

The present study was conducted on 172 patients with severe diarrheal infections, of whom 92 cases (53.49%) were male and 80 cases (49.51%) were female. The patients recruited into this study were aged from 6 months to 3 years. The average age of the patients was 25.5 years.

According to the results of tests, none of the patients were affected with *cholera*, and all water samples were indicated to be negative for *V. cholerae*.

Discussion

During a flood that occurred in Pol-e Dokhtar city in spring 2019, all sewage Pipes were damaged in the city, towns, and villages and the surface water and drinking water of all areas were reduced together. Moreover, the level of drinking water hygiene decreased, and due to the increasing prevalence of diarrheal infections in the area, the risk of contamination of drinking water with microbial agents was increased in all flood-affected areas. Therefore, 172 samples from people suspected *cholera* infection were collected and examined.

Since *cholera* has been an endemic disease in Iran, and this city is one of the main focus of *cholera* virus, rapid and accurate detection of this pathogen is crucial in *cholera* epidemics [4]. Identification of the organism causing *cholera* is the initial step in the diagnosis of the disease because they are the only toxigenic pathogens associated with watery diarrhea and related epidemics. Common microbiological methods used to identify *V. cholerae* include bacterial culture, biochemical and immunological methods [4, 5, 11]. Although molecular methods have high sensitivity and specificity, they usually require an advanced device and sometimes are costly, as employed in the present study due to a large number of collected samples. In contrast, common microbiological and immunological methods, such as latex agglutination test have high sensitivity and specificity, affordable, easy, and interpretation and high response rate. We

examined a total of 172 clinical samples using TCBS medium and biochemical tests, and found that none of the patients had *cholera*, and all tests were negative for *V. cholera*. Taramsari et al. examined 161 samples in two outbreaks of *cholera* in Gilan province. They showed that 142 cases were positive for *cholera*, of whom 140 cases were caused by Ogawa serotype and 2 cases were Nan Agglutinating Groups [2]. Bakhshi et al. isolated 20 samples of *V.cholerae* serotype O1 with El Tor biotype in the northwest of the country, with 11 cases reported using purified piped water and 9 cases reported using natural well water [12]. Lack of samples which was positive for *V. cholera* can be attributed to the consumption of healthy plain water and food by the study participants. Rahbar et al. 2005 investigated 1118 patients with *cholera* during the outbreak that occurred in 2005 in 26 provinces of the country. They revealed that 1104 isolates of *V.cholerae* were serotype Inaba and 14 isolates were serotype, Ogawa. They also showed that the most likely source of *cholera* was eating raw vegetables irrigated with sewage [3]. Studies in other parts of the country show that all isolated strains in Hamadan in 1998, Zahedan in 2003, and Zabul in 2005 had El Tor biotypes and Ogawa serotypes [2]. In contrast to the mentioned studies, we did not detect *V.cholerae* strains from all studied samples.

Conclusion

The findings of our study suggest that *V. cholera* was not the cause of severe acute diarrheal infections in patients exposed to floodwater in Pol-e Dokhtar city, and other bacterial pathogens, such as *E. coli*, *Shigella*, etc. were responsible for severe acute diarrhea among these individuals.

Conflicts of Interest

There is no conflict of interest among authors.

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