

## The first report of *Vibrio fluvialis* isolated from a clinical sample in Iran

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### ABSTRACT

**Background and Objectives:** *Vibrio fluvialis* is a Gram-negative, bacillus-shaped, curved bacterium known as an emerging pathogen. There are reports of outbreaks caused by this bacterium worldwide. Iran, especially Qom province, is an endemic region for gastrointestinal diseases caused by *Vibrio* species. So, the aim was to isolate *V. fluvialis* from clinical and environmental samples.

**Materials and Methods:** During six months, 363 clinical and surface water samples were evaluated. The samples were cultured on specific media, and all incubated for 24 hours at 37°C. Suspicious colonies were evaluated by Gram staining and biochemical tests. The BD Phoenix automated microbiology system was used for the final confirmation of the isolated bacteria. Evaluation of antibiotic resistance of isolated strains was also performed according to CLSI standard.

**Results:** Eight cases (2.2%) of *V. fluvialis*, including seven from surface water samples (87.5%) and one from clinical samples (12.5%), were isolated. Based on antimicrobial susceptibility testing, all *V. fluvialis* isolates were susceptible to amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, tetracycline, ceftazidime, and chloramphenicol. High-level resistance to ampicillin and amoxicillin/clavulanate was also observed. *V. fluvialis*-infected patient had a mild fever, watery diarrhea, vomiting, nausea, and abdominal cramps that were manifested after drinking contaminated water or eating contaminated vegetables. The patient's symptoms recovered without antibiotic therapy after four days, resulting in self-limiting disease.

**Conclusion:** The current study is the first human case of *V. fluvialis* infection isolated in Iran. Therefore, monitoring of water and food samples should be done routinely.

**Keywords:** *Vibrio fluvialis*; Gastroenteritis; Drug resistance; Microbial; Iran

### INTRODUCTION

*Vibrio* (*V.*) species are Gram-negative, bacillus-shaped and curved species that are closely related to other members of Enterobacteriaceae. They are motile using flagella and are widely distributed

in surface waters around the world, including natural constituents of freshwater, estuarine and marine environment. Infections caused by *Vibrio* species are classified into two types: cholera and non-cholera. Most of these infections are caused by eating uncooked seafood or drinking contaminated water, and

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also, person-to-person transmission has been reported (1, 2). Patterns of *Vibrio* spp. outbreaks indicate that they are widespread worldwide, and both cholera and cholera-like infections remain a health problem in developing regions, particularly in African and Asian countries, which can endanger the health of vulnerable people of society (3-5).

Of the more than 100 known species of *Vibrio*, 12 species cause infection in humans. *V. cholerae* is the most famous species of the genus that is the causative agent of cholera. The disease is characterized by abundant watery diarrhea, with rapidly depleting fluids and electrolytes (6). Non-cholera vibrios, including *V. parahaemolyticus*, *V. fluvialis*, *V. alginolyticus*, *V. vulnificus*, etc., can cause non-cholera infections ranging from self-limiting gastroenteritis to acute life-threatening septicemia and necrotic fasciitis (1, 4, 6).

Among these species, *V. fluvialis*, first identified in 1975 in a patient with diarrhea in Bahrain, is an emerging pathogen worldwide and has been noted due to the recent increase in diarrheal outbreaks and sporadic extraintestinal cases. The gastrointestinal symptoms caused by *V. fluvialis* strains are similar to those of cholera but frequently present with bloody diarrhea, indicating they are invasive bacteria. Other infections, including suppurative cholangitis, peritonitis, acute otitis, endophthalmitis, etc., have also been reported from these strains. Unfortunately, to date, little research has been done on the distribution and epidemiological features of *V. fluvialis*, especially in Iran, and it remains largely unknown (4, 7, 8). Considering that Qom is an endemic region of *V. cholerae* and in recent years some cases of non-agglutinable (NAG) strains have been isolated, our aim was to evaluate the isolation of *V. fluvialis* on clinical and environmental samples sent to the Reference Laboratory.

## MATERIALS AND METHODS

The study was reviewed and approved by the Medical Ethics Committee of Qom University of Medical Sciences (Code No.: IR.MUQ.REC.1400.094).

**Sampling.** During six months (from March to September 2021), 340 patients, who had gastrointestinal symptoms and were referred to health centers of Qom, Iran, were enrolled. The stool samples were

collected using sterile swabs and were immediately sent to the Reference Laboratory of Cholera at Qom University of Medical Sciences in Cary Blair Transport media. One swab was cultured on Blood Agar and selective media such as Xylose Lysine Deoxycholate (XLD) Agar, Hektoen Enteric (HE) Agar, and Thiosulfate Citrate Bile-Salt Sucrose (TCBS) Agar; and all incubated for 24 hours at 37°C. For vibrios enrichment, another swab was also placed in Alkaline Peptone Water for 6-8 hours and then sub-cultured on TCBS. Twenty-three samples of surface waters used for agriculture (3 liters each) were also collected in sterile bottles from different parts of Qom province. Criteria for collecting water samples were areas of the province that have received more attention in recent years due to the isolation of *Vibrio* strains (NAG). The samples were filtered using 0.45-µm-pore-size cellulose nitrate filters (Advantec Toyo, Ltd., Tokyo) and then placed in Alkaline Peptone Water and sent to the Reference Laboratory of cholera and processed according to the above conditions. 2-3 mm in diameter, pure yellow, flattened colonies on TCBS media, and Gram-negative curved bacilli in Gram staining were evaluated by other tests. All media were purchased from Merck, Germany.

**Identification of the bacteria.** For initial diagnosis of suspected bacteria, a set of biochemical tests such as catalase, oxidase, Triple Sugar Iron (TSI) Agar, Kliger's Iron Agar (KIA), Sulfur-Indole-Motility (SIM), Bile Esculin Agar, Voges-Proskauer (VP), Lysine Decarboxylase, Arginine Dihydrolase, Ornithine Decarboxylase, Citrate, and ability to grow at different NaCl concentrations were performed (all media were purchased from Merck, Germany). The BD Phoenix automated microbiology system (BD Diagnostic Systems, Sparks, MD) was carried out to confirm the result of the biochemical tests. According to the manufacturer's instructions, strains of *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, and *K. pneumoniae* ATCC BAA-1705 were used as control strains. One strain of *V. cholerae* donated by the World Health Organization was also used as a positive control. The slide-agglutination technique by commercial antisera (Bahar Afshan CO., Iran) was also done for serogrouping of strains identified as *V. cholerae*. Clinical strains of *V. cholerae* O1 serotypes (Ogawa and Inaba), previously isolated and approved in our laboratory, were also used as positive controls

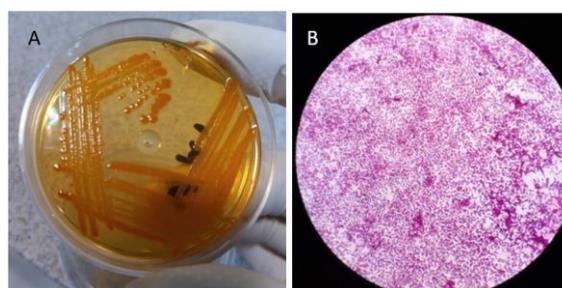
for the slide-agglutination technique.

**Antimicrobial susceptibility testing.** According to Clinical & Laboratory Standards Institute (CLSI) guidelines (9), the evaluation of antimicrobial susceptibility testing was performed using piperacillin (100 µg), ceftazidime (30 µg), cefoxitin (30 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), amoxicillin/clavulanate (20/10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), and chloramphenicol (30 µg) discs (Padtan Teb- Iran).

## RESULTS

Mean  $\pm$  SD age of the patients was  $30.44 \pm 18.29$  years, and 58.3% and 41.8% of them were males and females, respectively. Based on laboratory findings (Table 1 and Fig. 1), eight cases (2.2%) of *V. fluvialis*, including seven from surface water samples (87.5%) and one from clinical samples (12.5%), were isolated. Also, seventeen (4.7%) isolates were detected as non-O1/ non-O139 *V. cholerae*, (ten clinical strains and seven environmental strains). Most isolated vibrios were identified during the summer. No isolation of *Salmonella* and *Shigella* species was also observed on XLD and HE media.

According to the evidence, the patient infected with



**Fig. 1.** A: View of *V. fluvialis* colonies isolated on TCBS after 24 hours; B: Microscopic image at 1000 $\times$  magnification.

*V. fluvialis* was a 19-year-old boy living in Qomroud village, east of Qom province (Fig. 2). He had symptoms of mild fever, watery diarrhea, vomiting, nausea, and abdominal cramps. The patient experienced diarrhea, nausea, and vomiting two to three times during the day. After examination and history-taking, the physician found no evidence of other systemic symptoms, and the patient became infected after drinking or consuming a contaminated source of water or vegetables (with an incubation period of ~12 to 15 hours). Because the patient had used both of them, the exact source of infection was not determined. The patient's symptoms recovered without antibiotic therapy after four days and specified self-limiting disease.

The clinical isolate of *V. fluvialis* was susceptible to amikacin, gentamicin, trimethoprim/sulfamethoxazole, chloramphenicol, ciprofloxacin, imipenem,

**Table 1.** Biochemical results obtained from isolated strains (22)

| Tests                   | Results                               |                                       |
|-------------------------|---------------------------------------|---------------------------------------|
|                         | <i>V. fluvialis</i>                   | <i>V. cholerae</i>                    |
| KIA                     | Alk/Acid                              | Alk/Acid                              |
| TSI                     | Acid/Acid, Gas-, H <sub>2</sub> S-    | Acid/Acid, Gas-, H <sub>2</sub> S-    |
| Catalase                | +                                     | +                                     |
| Oxidase                 | +                                     | +                                     |
| SIM                     | H <sub>2</sub> S-, Indole-, Motility+ | H <sub>2</sub> S-, Indole+, Motility+ |
| Bile Esculin            | -                                     | -                                     |
| Citrate                 | +                                     | +                                     |
| VP                      | -                                     | V                                     |
| Lysine decarboxylase    | -                                     | +                                     |
| Arginine dihydrolase    | +                                     | -                                     |
| Ornithine decarboxylase | -                                     | +                                     |
| Growth in NaCl (%)      |                                       |                                       |
| 0.0                     | -                                     | +                                     |
| 1.0                     | +                                     | +                                     |
| 6.5                     | +                                     | +                                     |

V: Classical *V. cholerae* strains are negative and El Tor strains are positive.



**Fig. 2.** Map of Qom province in Iran and location of Qomroud village.

ceftazidime, cefoxitin, and tetracycline. An intermediate susceptibility was also found to piperacillin. In addition, *V. fluvialis* strains isolated from environmental samples were susceptible to amikacin (100.0%), gentamicin (100.0%), trimethoprim/sulfamethoxazole (100.0%), chloramphenicol (100.0%), ciprofloxacin (100.0%), tetracycline (100.0%), ceftazidime (100.0%), cefoxitin (71.5%), and imipenem (28.6%). All clinical and environmental strains (100.0%) were resistant to ampicillin and amoxicillin/clavulanate. Also, 5 (71.5%) and 1 (14.3%) environmental strains were resistant to piperacillin and imipenem, respectively.

## DISCUSSION

*Vibrio fluvialis* is considered a pathogenic bacterium and has been associated with outbreaks and sporadic cases of diarrhea. Most of the vibrios, including the strains isolated in this study, are widely isolated in the aquatic milieu, mainly in the seas and brackish waters (10), which is consistent with the isolated areas in this report. Qom province is located ~120 km south of Tehran, the capital of Iran, and its soil is primarily sandy, but in the eastern areas of the province, which is close to the Namak Lake (or Salt Lake) of

Qom, they have saline soils and water sources with a percentage of salt. Therefore, there were suitable conditions for the growth of halophilic bacteria, including vibrios. However, Iran is an endemic region for *V. cholerae* (11), and it seems that the presence of other species is not far off.

The prevalence of *Vibrio* spp. is also more related to the breakdown of sanitary conditions and/or due to scarcity of drinking water (7). In this study, seven of the twenty-three surface water samples were positive for *V. fluvialis*, indicating a relatively high prevalence. This could be related to the contamination of surface waters used in agriculture with feces or sewage (12, 13).

The patient under study was infected through unsafe water or contaminated vegetables, so the exact source of the infection was unknown. The reasons for the infection with *Vibrio* species may be related to the consumption of vegetables irrigated with the above-mentioned water sources in some areas of Qom. In addition, the lack of drinking water in some rural parts of the province and the use of alternative water sources are other reasons, because this province is located in the hot and dry region of Iran and some areas are facing water shortages.

According to published scientific databases, no study has been reported on the clinical isolation of

*V. fluvialis* in Iran, but there are few reports on its isolation from different sources in our country and other parts of the world. For example, Hosseini et al. isolated *V. fluvialis* from shrimps caught off the south coast of Iran (14). Amalina et al. in a study in Malaysia, reported an isolation rate of *V. fluvialis* of 0.3% in cultured groupers (15). Another study in Kolkata, India, identified 131 *V. fluvialis* strains out of 400 NAG *Vibrio* species among patients with diarrhea (16). Other reports of bacterial isolation have been published from other countries, including Romania (17).

The infection developed in the patient under study in the summer, which could reaffirm a seasonal prevalence of the bacterium. According to studies, a seasonal association of *Vibrio* species has been reported. Temperature, for example, is important for the growth rate of these bacteria, as *V. fluvialis* survives in water at temperatures between 9 and 31°C, but grows when the water temperature rises >18°C (10). However, Qom province experiences a temperature of about 40°C on some summer days, so infection with *Vibrio* strains is predictable.

Our patient recovered after four days without antibiotic therapy, indicating the disease was self-limited. While most non-cholerae bacteria can usually lead to mild and self-limiting gastroenteritis (6), it should not be forgotten that *V. fluvialis* is an important cause of cholera-like bloody diarrhea and extraintestinal infections in patients, particularly those with immunocompromised states and/or with a poor health level (10).

Although most of the isolates in this study were susceptible to the antibiotics, resistance was observed in some strains of *V. fluvialis* similar to other intestinal pathogens (18). Bacteria had high levels of resistance to ampicillin, amoxicillin/clavulanate, and piperacillin. Not many studies have been performed on the antibiotic resistance of *V. fluvialis*. In the study of Ahmed et al. in a strain of *V. fluvialis* isolated from a 6-month-old hospitalized infant, resistance to chloramphenicol, streptomycin, spectinomycin, ampicillin, cotrimoxazole, furazolidone, nalidixic acid, and gentamicin was detected (19). In another study in Italy, resistance of *V. fluvialis* to ampicillin, cefalotin, carbenicillin, kanamycin, and sulfadiazine/trimethoprim was reported (20). A study by Srinivasan found that efflux pumps play an important role in the resistance of quinolone compounds such as nalidixic acid and ciprofloxacin in *V. fluvialis* (21).

This research had some limitations. For example,

due to financial constraints, bacterial isolation sites were not evaluated by geographical conditions or other environmental parameters. In addition, molecular evaluation of bacterial pathogenic genes was not performed.

## CONCLUSION

The current study is the first clinical report of *V. fluvialis* infection isolated in Iran. Due to the identification of the bacteria from clinical and environmental samples, it seems that this species is an emerging pathogen and may appear to be endemic in other regions of Iran as well. Therefore, it is suggested that *V. fluvialis* be evaluated for all patients with watery and bloody diarrhea. In addition, monitoring of water and food samples should routinely be done similar to *V. cholerae*. The use of safe water for agriculture is also recommended, which must be considered by public health officials around the world.

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