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RESEARCH TITLE

Isolating and identification of *Vibrio Fluvialis* from the water of the Shatt al-Kufa in Al Najaf province

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Abstract

Among Iraq's historically documented rivers, the Euphrates is thought to be the longest. To join the Tigris at what is known as Shatt al-Arab, which empties into the Arabian Gulf, the Euphrates flows over Syria and Iraq. A variety of sources, including surface water from natural reservoirs, groundwater, and rainfall water, were used to provide the drinking water. The current study was done on many water samples taken from different places of shatt Al kufa in al najaf city to explore the degree of contamination by *Vibrio fluvialis* . bacteria of Vibrio fluvialis were detected in 75 samples taken from the water of the Shatt al-Kufa Which was diagnosed according to microscopic, cultural and biochemical examinations, as belonging to the Vibrio fluvialis bacteria that were isolated and identified from the water of the Shatt al-Kufa. Ten of the 75 surface water samples were found to be positive for V. fluvialis in this study, indicating a relatively high prevalence.

Key Words: Vibrio Fluvialis, water, Al Najaf province.

Introduction

Initially known as group F and group EF-6 vibrios, Vibrio fluvialis was originally described in 1977 (Zheng et al., 2022). Researchers have discovered V. fluvialis in the stool of several infected patients with gastroenteritis, extra intestinal infections, and acute diarrheal illnesses. According to Ramamurthy et al. (2014), Vibrio fluvialis is a global organism that is primarily found in coastal habitats, such as oceans, estuaries, and salty waterways.

Because the cell rod has a straight to curved ratio, the microorganism has curved cell morphology. V. fluvialis can move at up to 100,000 rpm across liquids thanks to its polar flagellar motility. According to Zheng et al. (2017), these gram-negative species are short, slender anaerobes that form spirals and S shapes.

According to the U.S. Centers for Diseese Contorl and Prevention, from 1996 to 2010, the number of vibrio infections tripled. Although it is less prevalent than other vibrio species, Vibrio fluvialis can be easily spread by eating contaminated raw shellfish and spending too much time in brackish water (The Pew Charitable Trusts, 2013). Since *V.* fluvialis is important to know because it is among the top five causes of water-borne diseases in the US.

Warmer, 7% NaCl salt solutions are ideal for the growth of this harmful species. The growth of V. fluvialis is aided by the 23 degree Celsius temperature. An amensalism is a negative interaction between this water-borne virus and its host. If left unchecked, V. fluvialis creates substances that harm the host. Pathogenesis via seafood hosts and human eating make up the environmental contribution.

This microbe's cell physiology enables prolonged survival in the face of environmental stress and famine. In humans, V. fluvialis can occur both alone and in combination with other intestinal bacteria. The symptoms of vomiting, diarrhea, high fevers, moderate to severe dehydration, and abdominal pain have all been linked to V. fluvialis (Lockwood et al., 1981).

Aim the study : A complete survey of bacterial contaminants present in different places of Shatt Al-Kufa for the period from June 2023 to October 2023

2. Materials and procedures:

2.1. Culture Media :

Medium	Manufacturer	Origin
Blood agar base	Himedia	India
Thoisulfate–citrate–bile salts sucrose agar	Himedia	India
Nutrient-agar	Oxoid	UK
Motelity medieum semi- sol medium	Himedia	India
Kligler Iron Agar	Oxoid	India
Urease medium	Himedia	India
Peptone water medium	Himedia	India

2.2. Methods

2.2.1.Culture Media Preparation

As stated in (2.1), the manufacturer's instructions, which were affixed to the media's containers, were followed in the preparation of the media utilized in this study. All of the media were autoclaved for 15 minutes at 121˚C to sterilize them. Following sterilization, urea agar base was supplemented with 20% sterile urea solution, and blood agar base was supplemented with

5% human blood after the medium was cooled to 45˚C and then transferred into sterile Petri dishes. (MacFaddin, 2000).

Specimens Collection:

From June 2023 to October 2023, 75 clinical specimens were collected from water of shatt al kufa in Al–Najaf province. These specimens were taken from several locations within Shatt Al Kufa, when the water was 5 cm below the surface. 300 milliliters of water are obtained, and they are then sent straight to the laboratory. Isolated by take 20 ml from specimens mixed with 20 ml of alkaline peptone water.(Islam et al., 1994 and Eiliot et al., 2001)

Specimens Culture

The gathered specimens were spread out on each plate using a sterile loop after being inoculated on three different types of culture media: blood agar, thoisulfate–citrate–bile salts–sucrose agar, and nutrient agar. The plates were incubi.ted for twenty-four hours at 37˚C. Following an analysis of the plates for bacterial growth, a singl.e pure isolated colony was submitted for morphological examination using gram staining, and additional biochemical tests were performed to establish the isolates' identity.

Identification of bacterial isolates

Following the incubation period, the isolates were identified as follows using MacFaddin (2000):

Biochemical Tests:

Cata.lase test, Coagulase test, Oxid.ase test, Indole production test, Urease test, Kligler Iron Agar Test

Motility test

After piercing along the middle of the tube to roughly half of the medium's depth, the semisolid medium was infected, and it was then incubated for 24 hours at 37 ˚C. A positive result was defined as the stab line turbidity spreading throughout the medium (Collee et al., 1996).

Vitek – 2 for Identification:

Gram-negative bacteria have been identified using GN identification cards. Using a Vitek-2 device (bioMérieux, France), The bacterial suspension was mixed with 2.5 milliliters of a 0.45% sodium chloride solution to reach the McFarland standard of 0.5. The time between inoculum preparation and card filling was never longer than 30 minutes. Since the GN identity card is a fully closed system, no chemicals need to be added. The card was placed on the cassette made specifically for the Vitek-2 system, put inside the device, autometically filled in a vacuam chamber, sealed, and then incubated at 35.5˚C. For a maximum of 8 hours, Using a fresh reading head, the card was automatically exposed to colorimetric measurement every fifteen minutes. Vitek-2, a database that enables kinetic organism identification starting 180 minutes after the incubation period begins, was used to examine the data (Guido and Pascale, 2005).

Testing the ability of bacterial isolates to tolerate acidity

Transferred 4-5 fresh colonies from TCBS medium Into a test tube containing 10 ml of basic peptone medium and incub.ated at 37 degrees for 18 hours. The peptone medium was prepared with different pH concentrations (4, 4.5,5.5,6.5 …..10.5) and distributed in sterile glass test tubes, 10 ml for each tube, in two replicates for each concentration. Then the test tubes were inoculated with 0.1 ml of the prepared bacterial suspension and left for 18 hours at 37C . leaving 2 tubes without inoculum as comparison treatments, then they were incubated at 37 degrees for 18 hours, and then they were grown on TCBS medium. (Woug et al 1998).

Testing the ability of bacterial isolates to tolerate salinity

The bacterial suspension was prepared as (2.2.7). Basic peptone water medium was prepared with different concentrations of sodium chloride(1%,2%, …….5%,6%) with two replicates for each concentration. Then the test tubes were inoculated with 0.1 ml of the prepared bacterial suspension and left for 18 hours at 37C , leaving 2 tubes without inoculum as comparison treatments, then they were incubated at 37 degrees for 18 hours, and then they were grown on TCBS medium. (Woug et al., 1998)

Results and Discussion:

Isolation and Ide.ntification of vibrio fluvialis:

The bacterial colonies appeared yellow in color on the TCBS medium. They also appeared microscopically in the form of rod-shaped, curved bacteria that were gram-negative and did not form spores. The results of the biochemical tests demonstrated that the bacteria produced an acid base on the Alkalin Acid (KIA) medium, positive for the oxidase test and negative for the indole and FOX tests. Pure, mobile, and hemolytic type when grown on blood culture media.

Ide.ntification with the automated VITEK-2:

GN-ID cards, which included 47 biochemical tests and one negative control well, were used to identify the automated VITEK-2. Therefore, it was at Shatt Al Kufa that this bacteria first appeared.

Testing the ability of bacterial isolates to tolerate acidity:

As for the bacteria's tolerance to different pH levels, the results appeared in Table (1) where the bacteria were able to grow at PH 5 to PH 9.5 , It could not grow at a concentration of 4 or at a concentration of 10.

Table -1: Tolerance of V .Fluvialis for different concentrations of PH

PH.	4	4.5	5	5.5	6	8	9	9.5	10
ISOLATE 1	-	-	+	+	+	+	+	+	-
ISOLATE 2	-	-	+	+	+	+	+	+	-

+ Turbidity - non turbidity (no growth)

Testing the ability of bacterial isolates to tolerate salinity

As for the bacteria's tolerance to different concentrations of NaCl, As show in table (2) where the bacteria cannot growth at 7% concentration of NaCl.

Table -2: Tolerance of V .Fluvialis for different concentrations of salt.

Salt NaCl	1%	%2	%3	%4	%5	%6	%7
ISOLATE 1	+	+	+	+	+	+	-
ISOLATE 2	+	+	+	+	+	+	-

+ Turbidity - non turbidity (no growth)

Discussion

Considered a harmful bacterium, Vibrio fluvialis has been linked to diarrhea outbreaks and isolated instances. Igbinosa and Okoh (2010) state that most vibrios, including the strains found in this investigation, are widely isolated in aquatic environments, mostly brackish and oceanic waters.

The incidence of Vibrio spp. is also more strongly associated with the decline of sanitary conditions and/or the scarcity of drinking water, according to Ramamurthy et al. (2014). Ten of

the 75 surface water samples in this investigation were positive for V. fluvialis, suggesting a rather high prevalence. This might have to do with sewage or excrement contaminating surface waters utilized for farming (Kahler et al., 2015).

The clinical isolation of V. fluvialis in the province of Al Najaf has not been studied, according to available scientific databases; however, Its isolation has been reported in other Iraqi provinces by a few different sources.In 2004, for example, Najdat Bahjat isolated V. fluvialis from Shatt Diala water.

The results shown in the table (2), show a clear difference from the results of Al-Fartusi 2002, which studied the tolerance of vibrio cholera to different degrees of pH, as its results showed that vibrio cholera isolated from clinical sources resisted high pH 4.5 and well grow, The reason why V. fluvialis cannot tolerate low pH is that they do not have genes that make them tolerate low pH, while in Vibrio cholera there are two types of genes(Pnp and gshB) on the large chromosome.(Merrell and Camilli 2002)

CONCLUSION

This is the first report of an isolated V. fluvialis infection in the province of Al-Najaf. It appears that this species is an emerging disease and may also be endemic in other parts of the province of Al Najaf, based on the identification of the bacteria from environmental samples. Consequently, it is advised that all patients with bloody and watery diarrhea have their V. fluvialis status assessed. Similarly to V. cholerae, food and water samples should be regularly monitored. Public health professionals worldwide must take into account the recommendation to use safe water for agriculture..

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