

Study of *Vibrio Cholerae* with its Virulence Factors Isolated from Diarrheal Patients in Babylon Province

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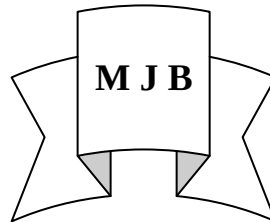
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Abstract

Stool specimens were collected from (136) patients with watery diarrhea aged (from 5 months to 80 years) prior administration to antimicrobial agents who were referred to Hilla teaching hospital (Emergency department) during a period from (September to December 2008).

This study found that 44 (32.35%) suspected isolates of *Vibrio cholerae* 23(52.3%) male and 21 (47.7%) female. All isolates were proven to be *Vibrio cholerae* in central public health laboratory in Baghdad. Regarding to the serotyping of *Vibrio cholerae* this study found that all isolates have only *Inaba* serotype. The virulence factors were detected in all isolates was produce siderophores and colonization factor antigens.

الخلاصة

من خلال هذه الدراسة تم جمع عينات خروج من (136) مريض يعانون من إسهال مائي تتراوح أعمارهم بين (٥ أشهر إلى ٨٠ سنة) ممن أحيلوا إلى مستشفى الحلة التعليمي (الطوارئ) للفترة من (شهر أيلول حتى شهر كانون الأول ٢٠٠٨) وقد حرصنا على اخذ العينات قبل تعاطي المرضى لأي مضاد حيوي.

وقد تم عزل ٤٤ عزلة كوليرا مشتبه بها من بينهم (٢٣(٥٢,٣%) ذكر و (٢١(٤٧,٧%) أنثى}. جميع العزلات أكدت صحتها في مختبر الصحة المركزي في بغداد. فيما يخص الأنماط المصلية لهذه العزلات فقد وجد إن كل العزلات قد أظهرت النمط المصلي (*Inaba*) فقط، أما عوامل الضراوة لهذه البكتيريا فقط ظهرت جميع العزلات لها القابلية على إنتاج السايذروفورات وتمتلك عوامل الاستيطان.

Introduction

V*ibrio cholerae*, members of the genus *Vibrio* are defined as Gram-negative, asporogenous rods that are straight or have a single, rigid curve. They are motile; most have a single polar flagellum, when grown in liquid medium. Most produce oxidase and catalase, and ferment glucose without producing gas [1].

Differences in the sugar composition of the heat-stable surface somatic "O" antigen are the basis of the serological classification of *V. cholerae* first described by [2]; currently the organism is classified into 206 O serogroups [3, 4].

Cholera is an acute enteric infection caused by the ingestion of bacterium *Vibrio cholerae* present in faecally contaminated water or food [5].

It is an enzymatic process causing a brief, acute onset diarrhoeal illness, with recurrent vomiting and stools that resemble rice water. This leads to acute rapid dehydration with fluid and electrolyte loss, ending with acidosis and hypovolaemic shock, which is usually fatal if untreated [6, 7].

Cholera spreads mainly via drinking water supplies contaminated by human excreta, especially from sub clinical carriers and mild cases [8]. It differs from other enteric diseases in its

clinical course (a very short incubation period) and epidemic pattern rapidly spreading to different countries and disappearing rapidly when outbreaks subside [9].

Cholera is endemic in Iraq, WHO estimates that up to 600 cases of cholera occur in Iraq annually. Following the cholera outbreak in 2007, the Ministry of Health, with the support of WHO has established 950 surveillance sites in Iraq. These sites report every two weeks on acute watery diarrhea and cholera cases. In addition, a Cholera Preparedness and Response Plan were developed and the laboratory and testing capacity of the Ministry enhanced at the governorate and central levels [10].

Pathogenicity for humans, and virulence factors:

The major features of the pathogenesis of cholera are well established. Infection due to *V. cholerae* begins with the ingestion of contaminated water or food. After passage through the acid barrier of the stomach, the organism colonizes the epithelium of the small intestine by means of the toxin-coregulated pili [11] and possibly other colonization factors such as the different haemagglutinins, accessory colonization factor, and core-encoded pilus, all of which are thought to play a role. Cholera enterotoxin produced by the adherent vibrios is secreted across the bacterial outer membrane into the extracellular environment and disrupts ion transport by intestinal epithelial cells. The subsequent loss of water and electrolytes leads to the severe diarrhea characteristic of cholera. The existence of cholera enterotoxin (CT) was first suggested by Robert Koch in 1884. Subsequent purification and structural analysis of the toxin showed it to consist of an A subunit and 5 smaller identical B subunits [12]. The A subunit possesses a specific enzymatic

function and acts intracellularly, raising the cellular level of cAMP and thereby changing the net absorptive tendency of the small intestine to one of net secretion. The B subunit serves to bind the toxin to the eukaryotic cell receptor, ganglioside GM1. The binding of CT to epithelial cells is enhanced by neuraminidase. Although numerous researchers have been reported, the aim of this study is to isolation and identification of *V. cholerae*, study of some virulence factors; siderophores and colonization factor antigen, and study of serotype for the isolates.

Patients and Methods

Patients:

One hundred thirty six stool specimens were collected from patients with watery diarrhea aged (from 5 months to 80 years) prior administration to antimicrobial agents who were referred to Hilla teaching hospital during a period from (August to November 2008). All patients involved in this study were distributed according to their age and sex.

Cholera diagnosis

In this study the diagnosis, virulence factors and serotyping study were studied and describe in figure (1).

Methods

Stool specimens were collected and transported with Cary and Blair transport medium to laboratory and enrichment in APW (alkaline peptone water) for 6-8 hrs, with incubation in 37°C then culturing on T.C.B.S. (Thiosulfate Citrate Bile salts Sucrose agar). Colonies suspicious for *V. cholerae* will appear on TCBS agar as yellow, shiny colonies. All identified isolated were confirmed in Central Public Health Laboratory in Baghdad.

Isolation and identification

The colonies suspicious were identified biochemically according to [21].

1. Identification by biochemical tests:

Oxidase test:

This test was done by place 2 to 3 drops of oxidase reagent on a piece of filter paper in a Petri dish. Smear the culture across the wet paper. In a positive reaction, the bacterial growth becomes dark purple immediately [13].

String test:

The string test done by using fresh growth from nonselective agar and it performed on a glass microscope slide by suspending 18- to 24- hour growth culture in a drop of 0.5% aqueous solution of sodium deoxycholate. We detect the bacterial cells will be lysed by the sodium deoxycholate, the suspension will lose turbidity. A

mucoid “string” is formed when an inoculating loop is drawn slowly away from the suspension [14].

2. Identification by serologically of *V. cholerae*:

The strains that were identified as *V. cholerae* on the basis of biochemical tests which agglutinate with “O” antiserum to give two serotype (Ogawa and Inaba, and Hikojimma).

antigenic factors allow further differentiation into two major serotypes—Ogawa and Inaba. Strains of the Ogawa serotype are said to express the A and B antigens and a small amount of C antigen, whereas Inaba strains express only the A and C antigens [15].

We identified the Serotype by using antiserum with specific O antigens on the slid to see the agglutination reaction.

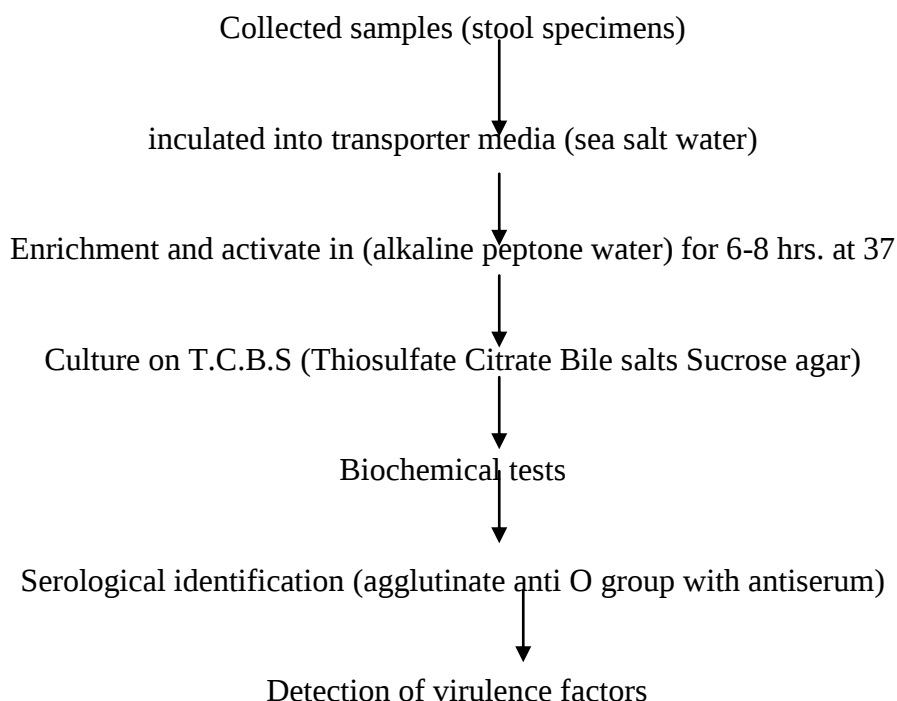


Figure 1 Diagnostic steps of laboratory tests.

Siderophores Production Test:

M9 Media was prepared and then supplemented with 2% agar. After sterilization in autoclave and cooling to 50C°, 0.25 mg/L glucose (sterilized by filtration) and 200 µmol/L of dipyrityl

were added. Then the organisms were inoculated into this media and it was incubated for 24 hours at 37C°. The results were seen when the growth of organism was present or not [19].

Haemagglutination Test (HA):

It was performed to show the ability of bacterial group to produce colonization factors antigen. RBC suspension was prepared from the human blood (group A). Human blood washed with phosphate buffer saline (repeated 3 times), 3% suspension from RBC (V/V) was then prepared and at the same time, blood agar was prepared, then it was inoculated with bacteria and incubated at 37C° for 24 hours, after that, place 1 drop of human red blood cells with loopful onto a clean slide, to this drop added 1 drop of bacterial culture by using a flamed loop and bacterial culture were mixed with human red blood cells on clean slide. The blood agglutination with bacteria was detected in room temperature during (1-5) minutes. Agglutinated red blood cells in suspension (positive reaction) had a clumped appearance

distinct from non-agglutinated red blood cells (negative reaction) [20].

Result and Discussion

A total of 136 patients diagnosed with cholera were admitted to Hilla teaching hospital (Emergency department). They were 44 (32.35%) suspected infections which caused by *V. cholerae* while the rest infections were caused by *Aeromonas*.

Among the 44 infections, 23 (52%) males and 21 (48%) females with a male: female ratio of 52.3: 47.7 respectively as shown in figure (2).

These results are similar to Iranian study which found that suspected patients with cholera were (58%) males and (42%) females [16] and to [8] study who found there is no difference between infected male and female.

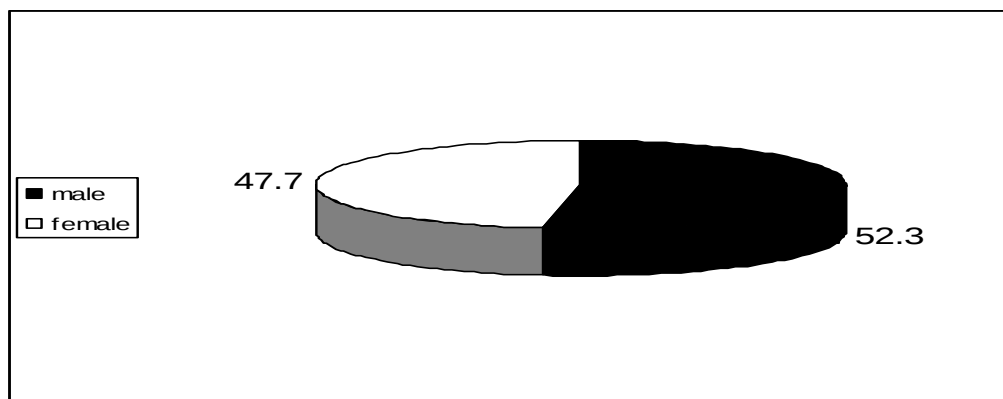


Figure 2 shows sex distribution of the patients with cholera.

All ages were affected, but the majority infections were between > 1and 20 years as shown in table (1). These findings are similar to those reported from other cholera-endemic areas, where the highest incidence of

clinical cholera is usually observed among toddlers, pre-school children and women of childbearing age in Iraqi study [8] that is likely to be related to the lower level of immune competence in this age [7]

Table 1 shows the age distribution of the cholera patients.

Age groups(year)	No.(%)
> 1-10	14(31)
10-20	13(30)
20-30	7(16)
30-40	4(9)
40-50	3(7)
50-60	3(7)
Total	44(100)

Virulence factors:

Colonization factors antigen was detected in this study. The result of Colonization factors antigen revealed that all *V. cholerae* isolates have colonization factor antigens (Table 2).

Many bacteria that cause diseases must be able to survive inside and outside the host. Attachment to and colonization of biotic surfaces is a common mechanism by which various microorganisms enhance their ability to survive in diverse environments. The pathophysiology of cholera is a result of the effects of cholera toxin on intestinal epithelial cells. For sufficient quantities of cholera toxin to reach the intestinal epithelium and to produce

clinical symptoms, colonization of the small bowel must occur [17].

On the other hand, the other virulence factors, Siderophores were detected in this study. The result of Siderophores showed that all *V. cholerae* isolates was producing Siderophores (Table 2). It was known that bacteria which were able to produce Siderophores have no ability to produce hemolysin. So *Vibrio* species, like many other pathogens, would possess high affinity iron transport systems to compete with their vertebrate hosts for the limited supply of iron [18]. Iron can increase disease risk by functioning as a readily a viable essential nutrient for invading microbial to survive and replicate in host.

Table 2 Type of virulence factors detected in *Vibrio cholerae*

No. of Isolates	Colonization factors antigen	Sidrophores
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	+
7	+	+
8	+	+
9	+	+
10	+	+
Total	100%	100%

Serotyping variation:

The present study found that all isolates have **Inaba** serotype. Al-

Abbassi *et al.* reported an epidemic of cholera in Baghdad, Iraq during 1999 were serotypes Ogawa (79.6%) and Inaba (12.1%) [8]. While in Hamadan province in the west of the Islamic Republic of Iran in 2005, found that all their isolates have serotype **Inaba** [16]. This result may be explained by Iraq is at risk of epidemics spreading from neighbouring countries because it lies on the routes of pilgrimage to of holy shrines.

Conclusion

Thus it was concluded that male susceptible for **Vibrio cholerae** more than female. In this study the Inaba serotype of **Vibrio cholerae** was predominant. **Vibrio cholerae** was produce (siderophores and colonization factor antigens). Further studies can be done for the identification other virulence factors responsible for the **Vibrio cholerae** pathogenicity characterization.

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