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ISOLATION AND SERODIGNOSTIC OF *VIBRIO CHOLERAE* FROM PATIENTS SUFFERED FROM WATERY DIARRHEA IN SUWAYRAH, WASIT GOVERNORATE, IRAQ

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ABSTRACT

Background: Cholera has been recognized as a killer disease since earliest time. The disease is caused by infection of the small intestine by *Vibrio cholerae* O1 and O1391 which is characterized by severe dehydrating diarrheal condition and is one disease in modern times that is epidemic, endemic and pandemic in nature.
Objective: This study was carried out to detect and isolate *V. cholerae* from patients suffered from watery diarrhea, which may cause severe complications such as dehydration, shock followed by death.
Materials and methods: stool specimens were collected from 308 patients with watery diarrhea. These samples were tested with many criteria such as TCBS agar, gram stain, biochemical tests and VITEK-2 system to improve the isolation and diagnosis of *V. cholerae*. Serotyping test was done to detect the predominant serotype that responsible for the disease.
Results: The results showed that 24 cases (7.8%) of 308 cases were *V. cholerae* positive. These positive cases were distributed on different age periods. All the isolates were belong to the Inaba serotype.
Conclusions: the efficacy of the conventional methods was equal to the VITEK-2 system in *V. cholera* detection. Serotyping test used to detect the *V. cholerae* that cause the outbreak.

Keywords : *V. cholerae*; Inaba serotype; Ogawa serotypes; TCBS and VITEK-2 system.

Introduction

Invasion of *Vibrio cholera* to the gut will raise the mucus formation, which lead to vomiting and rice-water diarrhea that cause dehydration followed by death if not treated. Contaminated drinking water and food with infected people's stool are usually the rout of *V. cholerae* transmission (Centers for Disease Control, 2005; Tamrakar *et al.,* 2009). Communities with poor sanitation levels or proper water sources face the danger of cholera outbreaks. Every year, 3-5 million persons infect with *V. cholera* and about 100000-120000 of them die. Additional to its short incubation period that ranged from two hours to five days, cholera has the capability to kill the untreated individual within few hours (WHO, 2012).

Vibrio cholerae is a worldwide-distributed bacterium that responsible for cholera disease. It is a gram-negative, comma-shaped rod, facultative anaerobe and motile via a single polar flagellum (Willey *et al.,* 2008). Although its ability to growth in temperature ranged from 10 to 43°C in pH 5.0 to 9.6, *V. cholerae* grow rapidly at 37°C in pH of 7.6, while the inactivation of these bacteria will occur in less than 4.5 pH at 25°C (ESR Ltd, 2001).

Filippo Pacini discovered this bacterium in 1854, while first isolation of it in pure culture occurred by Robert Koch in 1883 (Lippi and Gotuzzo, 2014). Among more than 200 serotypes of *V. cholerae*, there are two serotypes considered as the predominant causes of cholera disease called O1 and O139, they hold the genes encoding cholera toxin (CT) and the toxin co-regulated pilus (TCP) (Chatterjee *et al.,* 2007; Gaffga *et al.,* 2007). Both of these biotypes could be further classified into 3 serotypes (Ogawa, Inaba and rarely Hikojima) (WHO, 2010).

Motility, toxin co-regulated pillus, and cholera toxin are virulence factors of *V. cholerae* that related to its pathogenic nature. Lose one or more of these factors will decline the infection aptitude of it (CNN Library, 2018). Another risk factor of cholera bacteria is antibiotic resistance, it is not susceptible to tetracycline, trimethoprim-sulfamethoxazole, and erythromycin (Sack *et al.*, 2006). Therefore, new generation of antibiotics have been discovered which are effective against cholera bacteria in *in vitro* studies (Faruque and Nair, 2008).

Materials and Methods

1. Samples collection

A total of 308 stool samples were obtained from patients who attended *Suwayrah* General Hospital in *Wasit* Governorate/Iraq during the period from August 2017 to January 2018.

Patients included children, teenagers, adults and elderly from both genders suffering from watery diarrhea. The stool samples were collected in sterile plastic containers. Then, a small quantity of samples was injected in to 5 ml of alkaline peptone water (APW) prepared previously in sterile tubes and incubated over 6-8 hours at 37 ºC for culture (Oliver and Kaper, 1997).

2. Samples processing

A loopful was taken from the top layer of the APW, and streaked on Thiosulphate Citrate Bile salt Sucrose agar or TCBS (Difco-BD, Sparks, MD, USA). Then, incubated overnight at 37ºC.

3. Identification of the isolates

The suspected colony further identified according togram stain, biochemical testinclude Catalase, Oxidase, Methyl red, Indole, Urease, simmon's citrate, Glucose and lactose Fermentation on KIA, Voges-Proskauer (Difco, USA), and Growth in 1% NaCl) and Vitek-2 system (bioMe´rieux, Marcy l'Etoile, France) (Huq *et al.*, 2012).

4. Serotyping of *V. cholerae*

For further confirmation to *V. cholerae* isolates, serological serotyping was done (Koskela *et al.*, 2009).Slide agglutination test using commercially available polyvalent, anti-Ogawa, and anti-Inaba antisera from Plasmatec Laboratory Products Ltd (Plasmatec/ UK).

Statistical Analysis

The Statistical analysis of the presented study was performed by Statistical Package for the Social Sciences (SPSS) version 20.

Results and Discussion

The distribution of the tested groups according to the age in the presented study summarized as follow: ≤10 years 127 (41.2 %), 11-20 years 86 (27.9%), 21-30 years 32 (10.4%), 31-40 years 28 (9.1%), 41-50 years 15(4.9%) 51-60 years 8(2.6%), 61-70 years 5(1.6%) and >70 years 7 (2.3%).

From a total of 308 stool samples analyzed, 24 cases (7.8%) showed positive results. The distribution of the positive samples according to the age was as shown in table 1.

Table 1: Distribution of positive and negative cases among tested patients.

Age groups	≤10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years	>70 years	Total
No. of positive cases	3(0.9%)	4(1.3%)	5(1.6%)	0(0%)	9(2.9%)	1(0.3%)	0(0%)	2(0.7%)	24(7.8%)
No. of negativecases	124(40.3%)	82(26.6%)	27(8.8%)	28(9.1%)	6(1.9%)	7(2.3%)	5(1.6%)	5(1.6%)	284(92.2%)
Total	127(41.2%)	86(27.9%)	32(10.4%)	28(9.1%)	15(4.8%)	8(2.6%)	5(1.6%)	7(2.3%)	308(100%)

The above results showed variation in age groups who appeared positive to *V. cholerae* infections because fecal-oral rout is the main strategy for transmission. Contaminated food and water with feces of human or animal infected with *V. cholera*e considered as the source of infection (CDC, 2014). Therefore, all human with various ages face the risk of cholera acquirement.

In the presented study, there was a significant difference ($P \leq 0.05$) in the incidence of cholera in age group (41-50 years) than other age groups. This result differs from those of Malik and Hasan (2018), who fixed most susceptible age period to infection is 5-20 years (Malik and Baiee, 2018) and Al-Abbassi *et al*. (2005), who established the highest occurrence of cholera at age group <15 years (Al-Abbassi *et al.*, 2005). Most of the infected persons in age 41-50 years are living in rural slums, in which there are inadequate good hygienic managements, insufficient sources of drinking water and improper sanitation that encourage incidence of cholera infections (Nelson *et al.*, 2015).

The conventional laboratory diagnosis such as culture and biochemical methods were considered as one of the main strategies in detection of *V. cholera* (Chakraborty *et al.*, 2008).

On TCBS agar, the *V. cholerae* colonies appeared yellow, smooth and slightly flattened colonies with opaque centers and translucent margins (Figure 1).

The colonial appearance of *V. cholera* on TCBS agar was agreed with Kaysner and De Paola, who established that *V. cholerae* showed Large elevated yellow colonies on this media (Kaysner and DePaola, 2013).

Fig. 1: Growth of *V. cholerae* isolates on TCBS.

The microscopic examination of grow colonies revealed gram-negative, non-spore forming, slightly curved rods arranged as single or double of bacteria. This features distinguish it from other gram- negative bacilli, this agreed with Brooks *et al* (Brooks *et al.*, 2007).

On the other hand, the results of biochemical tests were as shown in Table 2.

Table 2: Biochemical tests results of *V. cholerae* isolates.

Test	Result
Oxidase	+
Methyl red	-
Indole	+
Urease	-
Simmon's citrate	+
Glucose and lactose Fermentation on KIA	*A/K, (no gas/no H_2S)
Voges-Proskauer test	+
Growth in 1% NaCl	+

*A: Acid, K: Alkaline, KIA: Kligler Iron Agar

The results of *V. cholerae* biochemical tests were oxidase positive due to the ability to produce cytochrome oxidase, stable acid end products from glucose fermentation, Voges-Proskauer positive due to production of acetoin, formed from pyruvic acid during glucose fermentation, simmon citrate positive due to citrate utilization as sole carbon source and can grow in NaCl 1% because it is tolerant of moderate salt concentration (Eaton *et al.*, 2005).

Results of VITEK-2 were similar to those of the conventional methods in the presented study, all the 24 isolates were showed positive results when tested with VITEK-2 system. Additional to the consideration of conventional cultural methods as the gold standard for diagnosis of *V. cholera* remains (Alam *et al.*, 2010), we used VITEK-2 system that had an accuracy level near to 90%. Another benefit of this system is its speed in reliably identifying gram-negative rods within 2-3 hours (O'Hara *et al.*, 1997).

In the presented study, all the isolates were positive to anti-Inaba antisera. Slide agglutination was formed after adding of the kit's reagents (Figure 2).

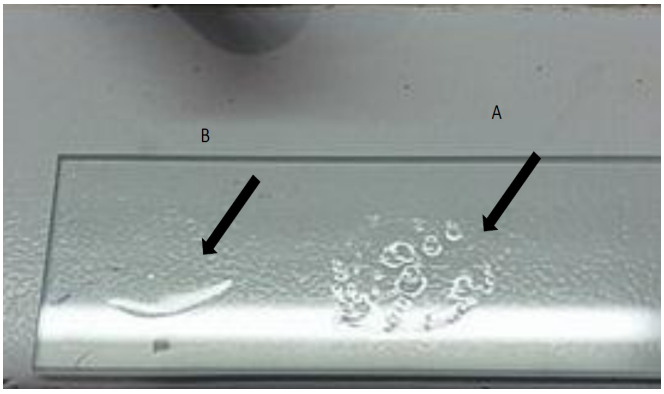

Fig. 2 : Results of serotyping test to *V. cholerae* isolates: (**A)** positive result to anti-Inaba antisera. (**B)** negative result to anti-Ogawa antisera.

From all serotypes of *V. cholera*, only Inaba (AC) and Ogawa (AB) considered as the main causative agents of cholera at the last three decades (USAID, 2014). In the presented study, all the isolated *V. cholerae* were belong to the serotype Inaba, this result agreed with that reported in Baghdad governorate at 2015 (Jameel *et al.*, 2016) and in Babylon governorate in 2014 that affected different age groups and genders (Alaouadi, 2014). Al-Abbasi and Aema, established 2651 positive cases to cholera in fifteen governorates in Iraq, the serotype Inaba was the predominant followed by Ogawa (Al-Abbasi and Aema, 2015).

On other hand, our results were disagreed with a study done in Baghdad governorate at 1999 (Al-Abbassi *et al.*, 2005) and in Haiti from the year 2010 to 2011, which showed predominance of Ogawa serotype as a cholera causative agent (Talkington *et al.*, 2011). This may be due to the ability of Inaba serotype to survive in humidity and high temperature conditions, which made it the major cause of cholera in warm environments. Overcrowded populations may help in increase the transmission level of Inaba serotype from human to human transmission via food and water due to the high efficacy of El Tor strains to transport from host to another in comparison with classical cholera strains (Dougan *et al.*, 2002).

Conclusion

Importance of the good medical cares and prevention strategies in eradication the persistence risk of cholera infection in Iraq provinces. Conventional diagnostic methods are good criteria for *V. cholera* diagnosis but when synergize with another technique such as VITEK-2 system, and serotyping test it will give an excellent results. In the presented study, both conventional and VITEK-2 methods gave the same results due to their high diagnostic efficacy for cholera. Serotyping test benefits in detection of cholera predominant serotypes in order to prevent the outbreak.

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