RESEARCH STUDY

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Rapid and Reliable Method for Identification of V. Cholerae O1 and V. Cholerae O139 Serotypes in Diarrheal Cases in Baghdad

ARTICLE INFORMATION

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ABSTRACT

Background: Cholera is gastroenteritis caused by enterotoxin producing Vibrio cholera. Cholera is predominantly a waterborne disease especially in countries with inadequate sanitation. Several rapid methods have been developed and used to detect V. cholerae serotypes directly from stools.

Objectives: to evaluate a rapid and accurate method for the diagnosis of cholera caused by V. cholerae O1 and O139 serogroups d to find the incidence of sporadic cases of cholera in Baghdad.

Methods: Sixty four stool samples were collected from four hospitals in Baghdad. The age of patients ranging from two months to 12 years, 26 were females and 38 males. Immunochromatographic visual test for qualitative detection of O1 and /or O139 serogroups was used as well as routine culture procedure for isolation of V. cholerae.

Results: Immunochromatographic visual test shows that, out of 64 stool samples only 16 was positive (23.4%). Fifteen of them belong to O1 serotype and one belong O139 serotype. Stool sample culture on alkaline peptone water and then on Thiosulfate Citrate Bile salts Sucrose (TCBS) agar enhance the growth of 11(68.75%) V. cholerae isolates out of 16 that were positive by using immunochromatographic visual test. Sensitivity of culture and immunochromatographic test was 68.75% and 100% respectively.

Conclusions: V. cholerae O1 is more predominant than V. cholerae O139 among V. cholerae strains isolated from sporadic cases of cholera in Baghdad. Immunochromatographic test is rapid, accurate and more sensitive than culture method in recover V. cholerae strains.

Introduction:

Cholera is gastroenteritis caused by enterotoxin producing Vibrio cholera [(1)]. Transmission of cholera vibrios to humans occurs through eating or drinking contaminated food water. Aquatic environments can serve as good reservoirs of the bacteria [(2)]. Cholera is predominantly a waterborne disease especially in countries with inadequate sanitation. Water maybe contaminated with fecal matter [(3)]. A lack of clean drinking water caused some outbreak in different part of the word. Recently an outbreak of cholera was occurred in Iraq in 2007 because of lacking appropriate sanitation [(4)]. In Iran, an epidemic cholera spread throughout 1959 [(5)]. Inadequate sanitation facilities was likely responsible for this spread of endemic cholera. There was rapid spread of disease in Iraq and Iran in 1965-1966 [(6)].

Although V. cholerae O1 and O139 will grow on a variety of commonly used agar media, isolation from fecal specimens is more easily accomplished with specialized media. Alkaline peptone water is recommended as an enrichment broth, and Thiosulfate Citrate Bile salts Sucrose (TCBS) agar is the selective agar medium of choice for isolating V. cholerae O1 and O139 [(7)]. Several rapid methods have been developed and used to detect V. cholerae O1 directly from stools [(8)]. We try here to evaluate a rapid and accurate method for diagnosis of cholera caused by V. cholerae O1 and O139 serogroups, to find the incidence of sporadic cases of cholera in Baghdad.

Methods:

Sixty-four stool samples were collected from four hospitals, Al Kadhymia Teaching Hospital, Al Kadhymia Hospital for Children, Children Protection Hospital in Al-Mansur and Baghdad Teaching Hospital. Twenty six of the patients were females and 38 were males, and their age ranging from two months to 12 years.

Immunochromatographic one step visual test for Vibrio cholera (Crystal VC-India), for qualitative detection of O1 and/or O139 serogroups was used. This test based on the principle of immunochromatography, in which the nitrocellulose membrane is coated with monoclonal antibodies to V. cholerae O1 and O139 LPS as two separated bands. When the stool sample flows through the nitrocellulose membrane, the colloidal gold, coupled with anti-V. cholerae O1/ O139 Lipopolysaccarides antigen (LPS) monoclonal antibodies, binds with the respective antigen in the stool sample. This antigen-antibodies complex moves through nitrocellulose membrane and bind with corresponding immobilized antibodies against V. cholerae O1/O139 and form magenta red color band. Control band is always appears, irrespective of reactive non-reactive samples, so as validate the test procedure.

Alkaline peptone water was prepared as an enrichment broth, as follows 10g of peptone (Difco-USA) and 10g of sodium chloride (BDH-UK) were dissolved in one liter of distilled water. The pH was adjusted to 8.5 then autoclaved. Thiosulfate Citrate Bile salts Sucrose (TCBS) agar (BDH-UK) is the selective agar medium of choice for isolating V. cholerae. Ten milliliters of alkaline peptone water were inoculated with about 1 ml of stool sample that was positive when tested by Immunochromatographic one step visual test and then incubated 6 hrs at 35-37°C. After 6 hrs of incubation, about 0.1 ml was inoculated on the surface of TCBS Agar [9].

Sensitivity of culture was measured using the following equation [10]:

Sensitivity = [(number of isolates positive by culture / (total number of positive isolates)] × 100.

Sensitivity of immunochromatographic test was measured using the following equation:

Sensitivity = [(number of positive isolates by immunochromatographic test / (total number of isolates positive)] × 100.

Results:

Immunochromatographic one step visual test shows that, out of 64 stool samples only 16 was positive (23.4%). Fifteen of these samples were positive for O1 serogroup and just one was positive for O139 serogroup (table 1). The age of the patients with positive stool results range from 3 months to 12 years. Ten of them were males and 6 females. Patients with cholera are lived in different parts of Baghdad city including Dora, Al-Sadir, Ur, Al-Kadhymia, Bnook, Jamila, Al-Amin, Al-Qahira, Sabh Al-boor, and Al-Wazyrhia neighborhoods.

Table1: Number of Vibrio cholerae O1 and O139 positive and negative results with immunochromatographic test.

Result	Vibrio cholerae O1	Vibrio cholerae O139
Positive	15	1
Negative	49	63
Total	64	64

Stool sample culture on alkaline peptone water and then on TCBS agar enhance the growth of 11(68.75%) V. cholerae isolates out of 16 that were positive by using immunochromatographic one-step visual test, and yield sucrose fermentative yellow colonies of V. cholerae. Sensitivity of culture and immunochromatographic test was 68.75% and 100% respectively (table 2).

Table 2: Number and percentage of positive V. cholerae with immunochromatographic test and with routine culture.

Result	Positive with immunochromatographic test	Positive with routine culture
Number (%)	16 (100%)	11 (68.75%)

Discussion:

Cholera can spreads as an epidemic, endemic or pandemic disease [11]. Thousands of people were infected with Vibrio cholera during the outbreak in Iraq in 2007-2009 [12]. In this study, almost all of cholera serogroups was V. cholerae O1 except one; Shahcheraghi et.al [9] found that all of serogroups obtained belong to V. cholerae O1. Saleh et.al [12] obtained the same result in the outbreak in Iraq in 2007-2009.

Successful intervention and containment depends largely on early detection of cholera outbreak [13]. Culture still remains the gold standard for diagnosis of cholera but requires a functional microbiology laboratory and is time consuming. Vibrio spp. grows very rapidly in alkaline peptone water, and at 6 to 8 hours will be present in greater numbers than non-Vibrio organisms [7]. Nevertheless, as shown in this study, 6 out of 16 failed to yield yellow colonies of V. cholerae in spite of that these isolates were positive when tested with immuno-chromatographic test, and there is obvious difference in sensitivity of culture and immunochromatographic test (68.75% and 100% respectively).

Prompt and accurate diagnosis of cholera is of much importance in order to implement proper epidemiological measures [14-15]. Prompt laboratory diagnosis of cholera is often advantageous for monitoring the spread of the disease and rapidly instituting control measures [16]. Several rapid methods have been developed and used to detect V. cholerae O1 directly from stools of acutely ill patients or from enrichment broth. In certain situations, these rapid methods may be practical [10].

Crystal VC is lateral flow immunochromatographic test for qualitative determination of lipopolysaccarides antigen (LPS) of both V. cholerae O1 and O139 LPS. Many rapid diagnostic tests for cholera targets only V. cholerae O1, whereas immunochromatographic test is used for the rapid diagnosis of both V. cholerae O1 and O139 in endemic areas [8].

We conclude that V. cholerae O1 is more predominant than V. cholerae O139 among V. cholerae strains isolated from sporadic cases of cholera in Baghdad. Immunochromatographic test is rapid, accurate and more sensitive than culture method in recover V. cholerae strains.

Conclusions:

We conclude that V. cholerae O1 is more predominant than V. cholerae O139 among V. cholerae strains isolated from sporadic cases of cholera in Baghdad. Immunochromatographic test is rapid, accurate and more sensitive than culture method in recover V. cholerae strains.

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