VALIDITY OF ONE STEP RAPID VISUAL TEST IN DETECTION OF CHOLERA CARRIERS IN NAJAF, IRAQ

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ABSTRACT

Background: Cholera is an endemic disease in Iraq, the most severe epidemic had occurred in 1966 as a continuity of the seventh pandemic. Then subsequent less serious outbreaks occurred in 1999, 2003, and 2008 with a case fatality ratio of about 2%.

Objective: To verify the sensitivity and specificity of immunochromatographic one step rapid test for V. cholerae among asymptomatic carriers using the culture as a validating diagnostic test.

Design: a cross – sectional study

Materials & Methods: A total of 28000 stool samples were collected from individuals that were attending the primary health care centers in Najaf governorate during the months (August, September, October and November, 2008) which are considered the months of cholera season in Iraq. The individuals were asymptomatic or suffering from mild diarrhea. All the stool samples were examined for Vibrio cholerae by two methods; the rapid visual test as a screening and the culture as a diagnostic test. The ability of the rapid visual test had been compared with results of culture to assess the validity and prediction of this rapid test in our locality.

Results: From 28000 stool samples, 51 samples found to be carriers of V. cholerae O1 of Inaba serotype with carrier detection rate of 182 per 100000 population. The sensitivity and specificity of the one step rapid visual test were 84% and 100% respectively with ten false negative individuals. The test revealed high predictive value for both positive and negative results.

Conclusion: The validity of the immunochromatographic one step rapid visual test (VC Dipstick) is so high that it can be applied to detect the carriers of Vibrio cholerae O1 during the expected cholera season.

INTRODUCTION

Cholera is an endemic disease in Iraq, the most severe epidemic had occurred in 1966 as a continuity of the seventh pandemic. Then subsequent less serious outbreaks occurred in 1999, 2003, and 2008 with a case fatality ratio of about 2%. The last outbreak was reported in 1998-1999 with attack rate less than 0.2%. The only isolated strain of vibrio was Group O1 with majority of cases were of Inaba serotype (Iraqi Ministry of Health). Stool culture is the main diagnostic procedure used in public health laboratory in Najaf city, Iraq to confirm the infection with Vibrio. In the previous years, sea salt water (alkaline pepton) was used as a transport media to transfer the samples to the laboratory. Recently, it is replaced by Carry-Blair media which can reserve the viability of Vibrio for about one week to be cultured.[¹,²] The problem in the control of cholera is the undetected carriers of the disease who are usually a hidden source of infection in the community.[³,⁴] Fall is the expected season of cholera in Iraq with some sporadic cases of cholera O1 that may be reported during other seasons. During the period between August 20th and September 29th, 2008, the health authorities of Iraq reported the first cholera cases of the year. At the 28th of September 2008, a total of 341 laboratory-confirmed cholera cases, including five deaths, had been verified (case-fatality ratio: 1.5%). Nine provinces were affected, with Babil accounting for the majority of cases (58%), followed by Baghdad (18%) and Karbala (9%). Other provinces in which cholera cases have been reported include Anbar, Basrah, Diala, Diwanyia, Misan and Najaf (Iraqi Ministry of Health).

The aim of this study was to verify the sensitivity and specificity of

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immunochromatographic one step rapid test for V. cholera among asymptomatic carriers using the culture as a validating diagnostic test.

MATERIALS AND METHODS

Stool samples were collected from 28000 individuals of primary health care (PHC) centers visitors (daily random sample of 200-300 from 30 PHC centers) in Najaf governorate during the expected months of cholera (August September, October and November 2008) which represent the season of cholera in Iraq. The individuals were asymptomatic or suffering from mild diarrhea. Stool samples were collected by the laboratory technicians who were trained on the cholera programme. All the stool samples were examined for Vibrio cholerae by two methods; the rapid visual test with dipstick as a screening test and the culture as a diagnostic test. The ability of the rapid visual test had been compared with results of culture to assess the validity and prediction of this rapid test in our locality. Collection and transportation of stool specimens for culture in the public health laboratory in Najaf city were done by Carry-Blair transport media. For confirmation of infection, stool samples were collected only from PHC center visitors and patients of previously untreated cases who have not received antibiotics treatment before collecting stool samples or have no or mild diarrhea.

Steps in collection of stool samples

The stool samples were examined by two methods: The first method was the direct Rapid Dipsticks of Cholera (Crystal VC, India) for liquid sample and diluted viscous stool, and the second part of the sample was collected in Carry-Blair transport media to be cultured in the public health reference laboratory in Najaf city. Approximately, a swab of about 200 µl of faeces was transferred from each sample cup to a clean test tube using the plastic dropper included with the test kit. Semisolid samples were diluted with saline if necessary. The Vibrio chromatography dipstick (manufactured in India) was placed in the test tube such that approximately the last centimeter of the strip was immersed in the feces as described in the company instruction form. After 10 minutes (or upon appearance of the positive control band), the dipstick was removed and test results were read positive according to the manufacturer criteria. Tests were judged as positive, negative, or indeterminate (ambiguous results) for V. cholerae O1 and for V. cholerae O139. The personnel collected a swab from a freshly passed stool specimen (Fresh stool should be less than 1 hour old) or from a swab of the rectal contents. According to laboratory procedure, a swab is dipped into the Carry-Blair medium first, in order to moisten it, then the moistened swab is immersed into the tube of transport medium, pushing the swab to the bottom of the medium tube. The top part of the stick is broken and discarded and the tube is screwed tightly. The media tubes were labeled with the date of collection, name of patient, and reference number if required. All the samples were daily sent to the laboratory for culture. Cholera specimens in transport medium were sent unrefrigerated to be used in the laboratory for 7-14 days, depending on environmental conditions and cultured by standard laboratory techniques of cholera culture and serotyping. The validity of the dipstick cholera test was evaluated by estimating the sensitivity (ability to identify the true positive) and specificity (ability to identify the true negative) of the screening test considering the stool culture as a diagnostic validating test.

RESULTS

From 28000 stool samples, 51 samples found to be carrier of V. cholerae O1 of Inaba serotype with carrier detection rate of 182 per 100000 population. The sensitivity and specificity of the one step rapid visual test were 84.3% and 100%
respectively with ten false negative individuals. The predictive value for positive test was 100% versus 99.97% for negative test. (Tables 1, 2).

Table 1. One step rapid visual test screening in relation to stool culture in detection of cholera carriers.

<table>
<thead>
<tr>
<th>TEST</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Rapid visual test (crystal VC Dipstick)</td>
<td>43</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 2. The validity estimation of rapid dipstick of vibrio crystal to detect cholera carriers in relation to stool culture results.

<table>
<thead>
<tr>
<th>Rapid dipstick of vibrio cholerae (Crystal VC Test )</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>84.3</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
</tr>
<tr>
<td>Predictive value for positive test</td>
<td>100</td>
</tr>
<tr>
<td>Predictive value for Negative test</td>
<td>99.97</td>
</tr>
</tbody>
</table>

DISCUSSION
Cases of cholera are still reported annually in Iraq, and cholera is considered as endemic disease, with interrupting limited outbreaks since 1966.[2] The preliminary diagnosis of cholera cases depends on standard case definition confirmed by culture technique. Cholera in Iraq is of El-Tor biotype group O1 in all governorates. There is some evidence that Vibrio O139 was isolated from Euphrates river in middle and south of Iraq. Between outbreaks, the vibrios persist in the community through asymptomatic carriers and in those with mild diarrhea, and play a role in transmission of the disease during outbreaks. Therefore, early detection of the carriers and those with mild symptoms of cholera can help in their early and prompt treatment to prevent the transmission of the disease.[15] The results of this study revealed that colorimetric immunochromatographic test is rapid and commercially available instead of culture which is expensive, need more materials and longer time to confirm the diagnosis.[16] The identification of carriers is so difficult that they remain an important source of infection and for outbreaks in the community.[17] So the rapid test of cholera by crystal VC dipstick which is commercially available and can be easily used during outbreaks to control the infection, by treating the detected carriers to stop the transmission of the disease.[18] The high sensitivity (84.3%) and specificity (100%) of the test in this study make it valid to be applied in our area and help to identify both cases and carriers. The validity of the test in this study was consistent with that reported in other studies.[14,18] The high predictive value for both positive and negative tests (100% and 99.97% respectively) make the test more dependable to predict outbreaks and as basis for proper treatment of cases and carriers. It is important to identify the true positive (sensitivity) because of the severity of cholera and the potential for rapid disease spread. However, low false positive test results will save unnecessary mobilization of resources for disease control in this governorate during outbreaks, that making the high specificity of such a test is of importance also, and similarly desirable.[13]

Some countries like Guinea-Bissau revealed a lower specificity (approximately 71-76%) which is much lower than the current study versus higher results in other localities (91%) which makes this laboratory rapid dipstick test is more reliable for use.[19]

CONCLUSION
The validity of the chromatographic one step rapid visual test is high. It is highly sensitive and highly specific screening test with excellent
predictive value to be used for detection of carriers of *Vibrio cholerae* O1 in addition to its validity in detection of acute cases.

**Acknowledgement**

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**REFERENCES**


