Determination of Cholera Outbreak among Internally Displaced Persons (IDPs) In Complex Emergency Settings within Maiduguri, Borno State-Nigeria

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Abstract: Cholera is an acute diarrhoeal infection that occurs as the result of the ingestion of Vibrio cholerae bacterium aetiologic agent in contaminated food and water. Cholera occurs as the results of the ingestion of Vibrio cholerae bacterium remain a major global threat to the public. Inadequate sanitation, water supply, and poor hygiene are therefore significant to predetermine conditions for the well-being of internally displaced persons residing at various camps across Maiduguri observed after series insurgent attacks. The present study is aimed at determining the incidence of cholera infections amongst the IDPs setting in Maiduguri, which suggests possible outcomes of management of the disease. A total of 120 stool samples were collected from suspected cholera (health complexes with acute watery diarrhea 3 or more loose stools over a 24 period) that residing at four different IDP camps within Maiduguri from 2nd April to 31st August 2018. Samples were swabbed with a sterile swab and inoculated into a sterile a vial containing 20 mL alkaline peptone water (APW), pH 8, and transported to the Microbiology Laboratory University of Maiduguri Teaching Hospital for the analysis. The samples were subculture into freshly 2 mL APW and allowed to grow for 5 h and then inoculated streaked onto thiosulfate citrate bile salts sucrose (TCBS) and blood agar subsequently incubated at 37°C for 24 respectively. Furthermore, the presumptive colonies were confirmed by gram reaction and biochemical tests including; catalase, citrate, cholera red, oxidase, and urease reaction. Of 51 isolates were showed positive based on colony morphologies on TCBS and blood agar. The highest one appeared at the Muna IDP camp with 21(41.18%), followed by Dalori 11(21.57 %), Bakassi camp with 10(19.61%), and the lowest Teacher’s village (TV) with 9(17.65%). The outcome of this work justifies the need for interventions by improving the food, water, and environmental hygiene in the various IDP camps to prevent the occurrence V. cholerae infections.

Keywords: Vibrio cholerae, cholera, hygiene, diarrhea, alkaline peptone water
INTRODUCTION
Cholera is global public health challenges that occur as the result of insufficient sanitation, inadequate water supply, and improper hygiene in different emergency settings across various internally displaced persons (IDPs). In addition, victims of insurgency (such as Borno), and other social conflict are susceptible to numerous infectious diseases, especially diarrheal diseases transmitted by faeco-oral route as the results of poor sanitary conditions (Rosewell et al., 2013) and lack proper food and water hygiene (Ngwa et al., 2020). Cholera with estimated cases of 2.9 million worldwide, the World Health Organization reported 95,000 cases of cholera fatalities every year (Lam, McCarthy, & Brennan, 2015). The existing literature has unmasked possible risk factors associated with cholera infection (Byleveld, Deere, & Davison, 2008), such as drinking vendor water (Eweka & Olusegun, 2016), age (Twedt et al., 1981), placing contaminated hands in drinking water tanks (Wang et al., 2015), discharging faeces from the body to drinking water sources (Uppal, Mehra, Panda, & Kumar, 2017), eating contaminated foods, and poor sanitary conditions across the IDPs camps (Toole & Waldman, 1997). Thus, these results indicate that the major source of cholera transmissions are by ingestion of contaminated food and water (Al-hadrawi, Al-harmoosh, & Al-fatlawy, 2019; Eweka & Olusegun, 2016).

The recent insecurity situation is a disaster caused by man has resulted to disruptions in the health architecture and related social activities such as water and environmental sanitation. Several studies have indicated a link between cholera and other predisposing factors including poverty (Talavera & Pérez, 2009), poor water sanitation, poor hygiene, poor toilet facilities, poor nutritional status and limited access to healthcare (Dureab, Shibib, Al-Yousufi, & Jahn, 2018). Nevertheless, environmental health is always one of the top priorities, although continue and sustainable monitoring of water and foods are quite challenging at the internally displaced persons (IDP) camps and other emergency settings across the world. IDP and refugee camps are usually designed as a temporary emergency setting where people can only live for a short-term period, but at least 80% of emergency settings sets during crises last for more than 10 years and 40% last for 20 years. Thus, it is paramount important to make the IDP and refugee camps very lively for the displaced people (Uppal et al., 2017; Behnke et al., 2020).

The human-made disaster in Borno and the north eastern region of the country has led to the disruption of the health system and associated social services, such as water and sanitation. This in turn has increased the rate of transmission of infectious diseases such as during the current cholera outbreak. Many studies showed a close link between cholera and other disaster-related factors such as unplanned overcrowding, internally displaced people, poor water and sanitation, poor nutritional status, poor personal hygiene, limited access to health care and low coverage of vaccination (De Sousa, Dos Fernandes Vieira, De Menezes, Dos Reis, & Hofer, 2004; Twedt et al., 1981).

Currently, cholera infection is responsible for numerous outbreaks in developing countries, some of which have reported serious mortality cases (Eweka & Olusegun, 2016). Even though the extensive characterization of virulent strains has been conducted in some parts of the world, there is little literature available about the characterizations of _V. cholerae_ causing an epidemic in Africa (Lesmana, Richie, Subekti, Simanjuntak, & Walz, 1997). There’s a
need to address this shortfall of information to address feature outbreaks among IDPs and non-IDPs across the globe (Keddy et al., 2013).

*Vibrio cholerae* is the etiologic agent of cholera infection is a motile gram-negative curved shaped bacillus, oxidase-positive, catalase-positive but urease negative non-capsulated facultative bacterium that can grow best in salty environments including aquatic, freshwater, and marine environments (Manuscript et al., 2013; Shrestha et al., 2010; Byleveld et al., 2008). Nevertheless, outbreaks of cholera have been linked with the causative agent *Vibrio cholerae* (Dureab et al., 2018). The disease is responsible for serious cases epidemic in emergency situations where toilets facilities and clean water are the major challenges in socio-economic situations around developing countries (Abdulabbas, Bunyan, & Abdul-Lateef, 2019). Thus, cholera still remains the most prevalent disease among people, particularly in developing countries as the results of poverty and poor sanitary facilities (Eweka & Olusegun, 2016).

![Diagram of cholera transmission](image)

*Figure 1: Schematic diagram showing how improper hygiene in different emergency settings can lead to epidemic. Drinking clean water and proper hands washing can play key roles towards preventing cholera in the societies.*

The prompt and timely diagnosis of cholera can be archive through isolation and identification of the etiologic agent *V. cholerae* from stool culture and confirmed by biochemical tests (Ebob, 2020; Ramamurthy, Das, Chakraborty, Mukhopadhyay, & Sack, 2020). Thus, the conventional culture methods of isolation and identification of the bacterium may take three or more days to achieve (Abdulabbas et al., 2019). Therefore, detection of cholera cases at the initial stage and subsequent confirmation for the timely interventions, it is crucial (Ramamurthy et al., 2020; George et al., 2018).

**MATERIALS AND METHODS**

**Study area**
The research work was conducted in Microbiology Laboratory, University of Maiduguri Teaching Hospital (UMTH). Maiduguri is the capital of Borno State. It is located in the Sahel Savanna region of north-eastern Nigeria at latitude 11°05' North and longitude 13°05' east and at about 350 m above sea level. Maiduguri has mean annual rainfall and temperature of about 630 mm
and 32°C, respectively, but temperature can go as high as 45 to 48°C in the month of March to May.

**Samples collection and transportation**

A total of 120 stool samples were collected from suspected cholera (health complexes with acute watery diarrhea 3 or more loose stools over a 24 period) that residing at four different IDP camps within Maiduguri from 2nd April to 31st August 2018. All the feces samples were swabbed with sterile swab and inoculated into a sterile a vial containing 20 mL alkaline peptone water (APW), pH 8, and transported to the Microbiology Laboratory University of Maiduguri Teaching Hospital for standard microbiological analysis.

**Isolation and identification of V. cholerae**

All the culture media were prepared aseptically following the manufacturer’s instructions. On arrival at the laboratory, 500 µL of each of the samples were subculture into freshly prepared 2 mL APW and allowed to grow for 5 h and then streaked onto enriched media thiosulfate citrate bile salts sucrose (TCBS) and blood agar (BA) subsequently incubated at 37°C for 18 h (Varela et al., 1994). Then the plates were examined macroscopically and the colonial

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**Figure 2:** Schematic flowchart summarizes the sequence of events involves from sample collections, conventional culture, gram reaction and final confirmation of *Vibrio cholerae* from the samples analysed.

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Rice like watery stool sample
morphologies of the bacteria on the two-culture media were recorded based on the characteristics of the bacterial colonies (Keddy et al., 2013). The discrete suspected bacteria colonies were further subjected to gram reaction to study the microscopic properties of the bacteria were examined and recorded based on the shapes and colors. The suspected positive colonies were further confirmed by biochemical tests including catalase, oxidase test, Simmon citrate test, indole, and cholera red test. The results obtained from the culture and biochemical tests were summarized in table 1 and 2.

RESULTS AND DISCUSSIONS

Table 1. Comparison and distribution of total V. cholerae isolates obtained from culture and biochemical tests

<table>
<thead>
<tr>
<th>Total number of stools samples processed (N = 120)</th>
<th>Isolation of V. cholerae by culture (%)</th>
<th>V. cholerae confirmed by Biochemical tests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCBS Agar</td>
<td>Blood Agar</td>
</tr>
<tr>
<td>Number of positive</td>
<td>51 (42.5)</td>
<td>51 (42.5)</td>
</tr>
<tr>
<td>Number of negative</td>
<td>69 (57.5)</td>
<td>69 (57.5)</td>
</tr>
</tbody>
</table>

Table 2. Frequency and distributions of the 51 positive specimens according to IDPs camp that reported the total number of stool specimens tested throughout the study periods

<table>
<thead>
<tr>
<th>IDP Camps</th>
<th>No. specimens tested</th>
<th>Tested positive</th>
<th>% frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakasi</td>
<td>30</td>
<td>10</td>
<td>19.61</td>
</tr>
<tr>
<td>Dalori</td>
<td>30</td>
<td>11</td>
<td>21.57</td>
</tr>
<tr>
<td>TV</td>
<td>30</td>
<td>9</td>
<td>17.65</td>
</tr>
<tr>
<td>Muna</td>
<td>30</td>
<td>21</td>
<td>41.18</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>51</td>
<td>100.0</td>
</tr>
</tbody>
</table>

TV = Teacher’s village

Table 3. Culture, gram reaction and biochemical tests confirmation of V. cholerae obtained from the stool specimens analyzed

<table>
<thead>
<tr>
<th>Isolation/identification</th>
<th>Morphological appearance</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCBS Agar</td>
<td>yellow-greenish flat colonies</td>
<td>+</td>
</tr>
<tr>
<td>Blood Agar</td>
<td>clear zones β-hemolysis</td>
<td>+</td>
</tr>
<tr>
<td>Gram reaction</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Catalase reaction</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Citrate reaction</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Cholera red</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Oxidase reaction</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Urease reaction</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
Discussion
The main aim of this research was to determine the incidence of cholera disease among people leaving at various internally displaced camps in Maiduguri. The existing literature indicated that the movement of mass population increase the risk of infectious disease. Thus, displaced populations may serve as the vehicle of transmissions of pathogens from less endemic to endemic zones (Lam et al., 2015). Cholera is a diarrheal infectious epidemic disease that has been affecting human civilization over a century (Shaban, Ahmed, Materu, & Klena, 2009). The disease has already claimed many lives as the result of the sudden onset that can lead to a serious outbreak, especially amongst fewer privileged people. The specimens were subjected to the conventional methods of isolation and identification of V. cholerae and confirmed through a series of biochemical tests after isolation of the bacterium on two different sets of culture media TCBS and blood agar plates. Of the 120 specimens analyzed, 51 (42.5%) yield V. cholerae, and the remaining 69 (57.5%) are negative to culture and biochemical tests (tables 1 and 2).

There are no previously reported confirmed cases of the cholera epidemic in IDP camps in Maiduguri. However, the incidence of cholera has been reported at the different parts of the word with serious records of mortality, particularly in refugee camps (Nagamani, 2017; Israil, Nacescu, Cedru, Ciufecu, & Damian, 1998; Jesudason, Thangavelu, & Lalitha, 1984; Abdulabbaas et al., 2019). However, the serotype of the bacteria differs based on the locations where the studies have been conducted (Dureab et al., 2018). Comparison of culture and biochemical methods for isolation of Vibrio cholerae for the 120 stool samples analyzed shown in table 1 has indicated almost all the bacteria isolates found positive by couture method were also confirmed positive by gram reaction several biochemical tests shown in table 3. This results agrees with the findings of Lesmana et al. (1997) also found similar results in their study comparison of direct-plating and enrichment methods for isolation of Vibrio cholerae from diarrhea patients. Thus, our findings are consistent with that of Dureab et al. (2018) and Al-hadrawi et al. (2019) who reported in their different studies on “Cholera outbreak and the ongoing armed conflict Fekri amongst people leaving of Yemen” and “Detection of Some Virulence Factors of Clinical V. cholerae isolates in Najaf/Iraq”. However, this finding disagrees with their research findings in terms of the number of positive isolates obtained by plating method differ greatly with the biochemical tests, they found 21% positive by culture and 36% biochemical tests, whereas we found 51% positive by both methods. Nevertheless, it is interesting to know that our findings were close to the findings of other studies reported by Hendriksen et al. (2011) that all suspected watery diarrheic stools may turn to be positive for V. cholerae. It is quite interesting to see that Muna IDP camps reported having the highest cases of cholera with 21(41.18%). Many factors could be associated with a significant increase in the number of V. cholerae isolates in Muna IDP camp including lack of clean tap running water, inadequate toilet facilities, and other abiotic factors (Varela et al., 1994; Jesudason et al., 1984; Ngwa et al., 2020; Rosewell et al., 2013).

CONCLUSION AND RECOMMENDATIONS
Based on the outcome of the present research work, therefore, the following conclusions are put forward: 1- The frequency of V. cholerae from the clinical cases in the four IDP camps and
identification by culture and biochemical tests. 2- all the clinical isolates show to be positive by both methods culture and biochemical tests. The outcome of this work justifies the need for interventions by improving the continues surveillance food, water, and environmental hygiene especially in Muna IDP camp and other various IDP camps across the state to prevent the occurrence V. cholerae infections.

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persons internally displaced by a volcanic eruption. *PloS One*, 8(9).
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