Network for Research and Development in Africa
International Journal of Pure and Applied Science Research
ISSN: 2384-5918. Volume 12, Number 2
Pages 28-36 (September, 2020)
nrdajpapers@gmail.com

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

Alhaji Modu Bukar[1*], Hadiza B. Goni[1], Adama B. Bwala[1], Fatima B. Kolo[1], Aliyu Isa[1], Abdulaziz Ibrahim[1], Ibrahim Muhammad[1] & Yusuf A. Isa[2]

[1]Biology & Microbiology Unit, Department of Science Laboratory Technology, Ramat Polytechnic Maiduguri, PMB 1070 Maiduguri, Borno state, Nigeria

[2]Department of Consultancy Services, Ramat Polytechnic Maiduguri, PMB 1070 Maiduguri, Borno state, Nigeria

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 sanatory 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 heath 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 settings 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).

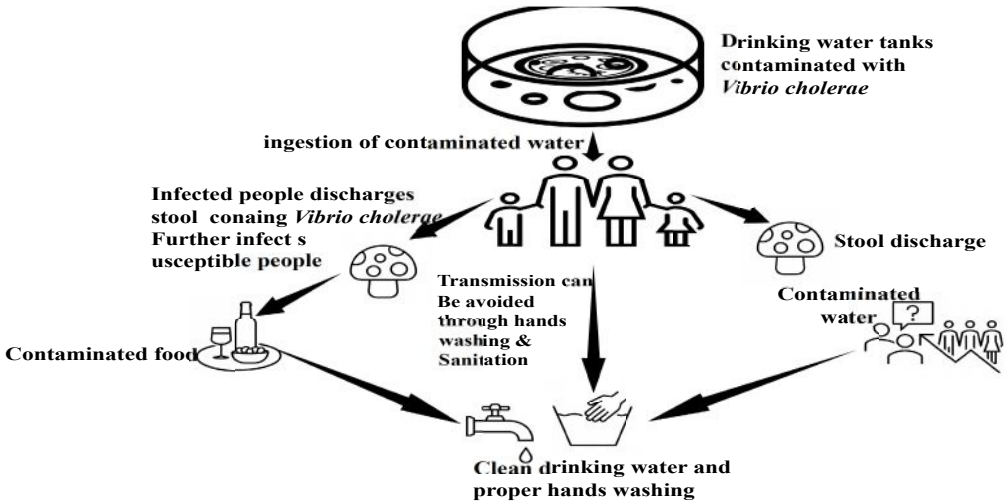

Figure 1: Schematic diagram showing how improper hygiene in different emergency settings can lead to epidemic. Drinking clean water and proper hands washing can play key roles towards preventing cholera in the societies.

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.

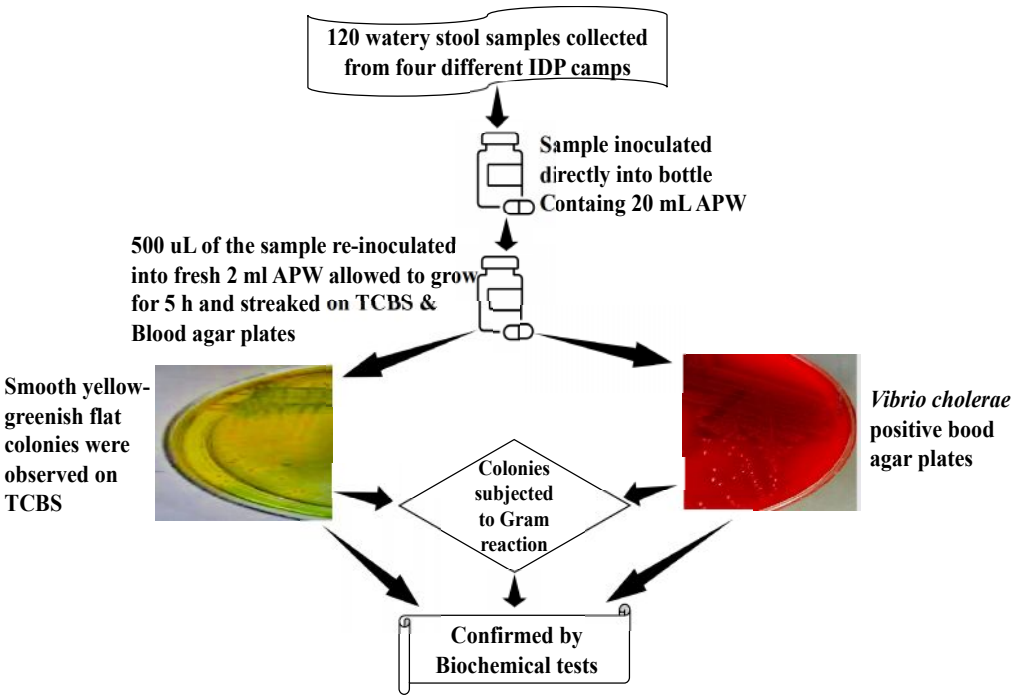

Figure 2: Schematic flowchart summarizes the sequence of events involves from sample collections, conventional culture, gram reaction and firnal confirmation of *Vibrio cholerae* from the samples analysed.

Rice like watery stool sample

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

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

Total number of stools samples processed (N = 120)	Isolation of *V. cholerae* by culture (%)		*V. cholerae confirmed* by Biochemical tests (%)
	TCBS Agar	Blood Agar	
Number of positive	51(42.5)	51(42.5)	51 (42.5)
Number of negative	69 (57.5)	69 (57.5)	69 (57.5)

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

IDP Camps	No. specimens tested	Tested positive	% frequency
Bakasi	30	10	19.61
Dalori	30	11	21.57
TV	30	9	17.65
Muna	30	21	41.18
Total	120	51	100.0

TV =Teacher's village

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

Isolation/identification	Morphological appearance	Results
TCBS Agar	yellow-greenish flat colonies	+
Blood Agar	clear zones β-hemolysis	+
Gram reaction		+
Catalase reaction		+
Citrate reaction		+
Cholera red		+
Oxidase reaction		+
Urease reaction		-

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; Abdulabbas 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.

ACKNOWLEDGMENTS

The work was supported by the TETFund under the Institution Based Research 2015/2016. We would like to thank the management of Ramat Polytechnic, Maiduguri for the technical supports. We also thank the study participants and the following research assistants who conducted the fieldwork for this study: Mrs. Adama B. Bwala, Mrs. Fatima B. Kolo, Tahiru Alfa, Mr. Aliyu Isa Gunda and Yusuf Alhaji Isa.

REFERENCE

Abdulabbas, H. T., Bunyan, I. A., & Abdul-Lateef, L. A. (2019). Isolation and genotyping of vibrio cholerae isolates from patients with cholera disease in Babylon province. *Annals of Tropical Medicine and Public Health*, *22*(8). https://doi.org/10.36295/ASRO.2019.22086

Al-hadrawi, H. A. N., Al-harmoosh, R. A., & Al-fatlawy, H. N. K. (2019). Detection of Some Virulence Factors of Clinical V . cholerae isolates in Najaf / Iraq, *11*(2), 375–379.

Behnke, N. L., Cronk, R., Shackelford, B. B., Cooper, B., Tu, R., Heller, L., & Bartram, J. (2020). Environmental health conditions in protracted displacement: A systematic scoping review. *Science of the Total Environment*, *726*, 138234. https://doi.org/10.1016/j.scitotenv.2020.138234

Byleveld, P. M., Deere, D., & Davison, A. (2008). Water safety plans: Planning for adverse events and communicating with consumers. *Journal of Water and Health*, *6*(SUPPL. 1), 1–9. https://doi.org/10.2166/wh.2007.019

De Sousa, O. V., Dos Fernandes Vieira, R. H. S., De Menezes, F. G. R., Dos Reis, C. M. F., & Hofer, E. (2004). Detection of Vibrio parahaemolyticus and Vibrio cholerae in oyster, Crassostrea rhizophorae, collected from a natural nursery in the Cocó river estuary, Fortaleza, Ceará, Brazil. *Revista Do Instituto de Medicina Tropical de Sao Paulo*, *46*(2), 59–62. https://doi.org/10.1590/s0036-46652004000200001

Dureab, F., Shibib, K., Al-Yousufi, R., & Jahn, A. (2018). Yemen: Cholera outbreak and the ongoing armed conflict. *Journal of Infection in Developing Countries*, *12*(5), 397–403. https://doi.org/10.3855/jidc.10129

Ebob, T. J. (2020). A Review on Diagnostic Methods for the Identification of Vibrio cholerae. *Journal of Advances in Medicine and Medical Research*, (July), 136–164. https://doi.org/10.9734/jammr/2020/v32i830474

Eweka, O., & Olusegun, T. O. (2016). Management of Internally Displaced Persons in Africa: Comparing Nigeria and Cameroon. *African Research Review*, *10*(1), 193. https://doi.org/10.4314/afrrev.v10i1.15

George, C. M., Hasan, K., Monira, S., Rahman, Z., Saif-Ur-Rahman, K. M., Rashid, M. U.,&Alam, M. (2018). A prospective cohort study comparing household contact and water Vibrio cholerae isolates in households of cholera patients in rural Bangladesh. *PLoS Neglected Tropical Diseases*, *12*(7), 1–13. https://doi.org/10.1371/journal.pntd.0006641

Hendriksen, R. S., Price, L. B., Schupp, J. M., Gillece, J. D., Kaas, R. S., Engelthaler, D. M., &Aarestrupa, F. M. (2011). Population genetics of vibrio cholerae from Nepal in 2010: Evidence on the origin of the haitian outbreak. *MBio*, *2*(4). https://doi.org/10.1128/mBio.00157-11

Israil, A., Nacescu, N., Cedru, C. L., Ciufecu, C., & Damian, M. (1998). Changes in Vibrio cholerae O1 strains isolated in Romania during 1977-95. *Epidemiology and Infection*, *121*(2), 253–258. https://doi.org/10.1017/S0950268896001100

Jesudason, M. V., Thangavelu, C. P., & Lalitha, M. K. (1984). Rapid screening of fecal samples for Vibrio cholerae by a coagglutination technique. *Journal of Clinical Microbiology*, *19*(5), 712–713. https://doi.org/10.1128/jcm.19.5.712-713.1984

Keddy, K. H., Sooka, A., Parsons, M. B., Njanpop-Lafourcade, B. M., Fitchet, K., & Smith, A. M. (2013). Diagnosis of vibrio cholerae o1 infection in Africa. *Journal of Infectious Diseases*, *208*(SUPPL. 1), 23–31. https://doi.org/10.1093/infdis/jit196

Lam, E., McCarthy, A., & Brennan, M. (2015). Vaccine-preventable diseases in humanitarian emergencies among refugee and internally-displaced populations. *Human Vaccines and Immunotherapeutics*, *11*(11), 2627–2636. https://doi.org/10.1080/21645515.2015.1096457

Lesmana, M., Richie, E., Subekti, D., Simanjuntak, C., & Walz, S. E. (1997). Comparison of direct-plating and enrichment methods for isolation of Vibrio cholerae from diarrhea patients. *Journal of Clinical Microbiology*, *35*(7), 1856–1858. https://doi.org/10.1128/jcm.35.7.1856-1858.1997

Manuscript, A., Chen, A., Manuscript, A., Imai, M., Herfst, S., Sorrell, E. M., … Manuscript, A. (2013). *Transmission of influenza A/H5N1 viruses in mammals*. *Virus Research* (Vol. 178). https://doi.org/10.1002/9780471729259.mc06a05s26.Detection

Nagamani, R. (2017). Case Report Non O1 Vibrio cholera : An emerging pathogen in blood ? - A review and report of cases from a regional laboratory at the Eastern Province in Saudi Arabia, *5*(3), 109–112.

Ngwa, M. C., Wondimagegnehu, A., Okudo, I., Owili, C., Ugochukwu, U., Clement, P., & Sack, D. A. (2020). The multi-sectorial emergency response to a cholera outbreak in Internally Displaced Persons camps in Borno State, Nigeria, 2017. *BMJ Global Health*, *5*(1), 1–12. https://doi.org/10.1136/bmjgh-2019-002000

Ramamurthy, T., Das, B., Chakraborty, S., Mukhopadhyay, A. K., & Sack, D. A. (2020). Diagnostic techniques for rapid detection of Vibrio cholerae O1/O139. *Vaccine*, *38*, A73–A82. https://doi.org/10.1016/j.vaccine.2019.07.099

Rosewell, A., Clark, G., Mabong, P., Ropa, B., Posanai, E., Man, N. W. Y., & MacIntyre, C. R. (2013). Concurrent outbreaks of cholera and peripheral neuropathy associated with high mortality among

persons internally displaced by a volcanic eruption. *PloS One*, *8*(9). https://doi.org/10.1371/journal.pone.0072566

Shaban, L., Ahmed, S. F., Materu, S., & Klena, J. D. (2009). Outbreak investigation: molecular characterization of Vibrio cholerae isolates from Horn of Africa. *BMC Proceedings*, *3*(Suppl 3), O9. https://doi.org/10.1186/1753-6561-3-s3-o9

Shrestha, S. D., Malla, S., Adhikari, B. R., Shakya, G., Basnyat, S. R., & Sharma, S. (2010). Antibiotic susceptibility patterns of Vibrio cholerae isolates. *Journal of the Nepal Medical Association*, *49*(3), 232–236. https://doi.org/10.31729/jnma.95

Talavera, A., & Pérez, E. M. (2009). Is cholera disease associated with poverty? *Journal of Infection in Developing Countries*, *3*(6), 408–411. https://doi.org/10.3855/jidc.410

Toole, M. J., & Waldman, R. J. (1997). The public health aspects of complex emergencies and refugee situations. *Annual Review of Public Health*, *18*, 283–312. https://doi.org/10.1146/annurev.publhealth.18.1.283

Twedt, R. M., Madden, J. M., Hunt, J. M., Francis, D. W., Peeler, J. T., Duran, A. P., &Wazenski, T. J. (1981). Characterization of Vibrio cholerae isolated from oysters. *Applied and Environmental Microbiology*, *41*(6), 1475–1478. https://doi.org/10.1128/aem.41.6.1475-1478.1981

Uppal, B., Mehra, B., Panda, P., & Kumar, S. (2017). Changing epidemiology and antimicrobial resistance pattern of Vibrio cholerae isolates at a tertiary care health laboratory in North India (2011–2015). *Tropical Journal of Medical Research*, *20*(2), 132. https://doi.org/10.4103/tjmr.tjmr_17_17

Varela, P., Pollevick, G. D., Rivas, M., Chinen, I., Binsztein, N., Frasch, A. C. C., & Ugalde, R. A. (1994). Direct detection of Vibrio cholerae in stool samples. *Journal of Clinical Microbiology*, *32*(5), 1246–1248. https://doi.org/10.1128/jcm.32.5.1246-1248.1994

Wang, L., Chen, Y., Huang, H., Huang, Z., Chen, H., & Shao, Z. (2015). Isolation and identification of Vibrio campbellii as a bacterial pathogen for luminous vibriosis of Litopenaeus vannamei. *Aquaculture Research*, *46*(2), 395–404. https://doi.org/10.1111/are.12191