

Contemporary understanding of *Vibrio cholerae* and cholera outbreaks.

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Abstract

Background: Cholera is an infectious disease caused by *Vibrio cholera* and transmitted by the fecal-oral route. The major source of *V. cholerae* outbreaks infection in human is infected water and food which is the most common vehicle and cholera toxin has been incriminated in the virulence.

Objectives: This review is to give previous and recent knowledge *V. cholerae* and cholera outbreaks.

Material and Method: Searching engines, electronic databases and web sites (Google, Bing, PubMed, ASM etc.) were searched randomly for epidemiology, symptoms, transmission, laboratory diagnosis, treatment, control and prevention of cholera.

Results: This review relates both the existing and current knowledge of *V. cholerae* and cholera outbreaks. Cholera is a rare disease in developed countries; but, still exists in countries in Sub Saharan Africa, Indian subcontinent, Latin and Central American countries (like Haiti). Sign and symptoms starts with an acute onset of painless rice watery diarrhea which can rapidly become voluminous. Several methods of laboratory investigation such as microscopy, culture, biochemical test, molecular testing and cholera toxins can assist in knowing epidemiology, identification of cholera strains, monitoring treatment among others. An antibiotic regimen is also recommended. In addition, adequate hydration is essential. Health education and education in environmental control are critical for the prevention of cholera. We found many papers that discussed about cholera, but we screened out mainly those that discussed about epidemiology, symptoms and transmission, laboratory diagnosis, treatment, control and prevention of cholera.

Conclusions: Adequate case search, laboratory diagnosis with sufficient safe water supply, sanitation, hygiene, community engagement, leadership commitment and oral cholera vaccine are the most important mechanism for the control and prevention of cholera disease.

Keywords: *Vibrio cholerae*, Cholera, Outbreak, Cholera toxin.

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Introduction

Cholera is a sudden diarrheal infection that is highly virulent which lead to death within short time if leave without any intervention [1]. Vibrios are the most widely found organisms on water globally. They are motile, bend aerobic bacilli and having

a pointed end slender cellular appendages. *Vibrio cholerae* serogroups O1 and O139 infect humans, while other vibrios may cause bacteremia or intestinal inflammation [2]. Also Strains of *V. cholerae* are identified with different categories based on the carbohydrates found on the body O antigen. Reports has shown more than 200 categories of *V. cholerae* identified so far, 2 of

the serogroups, O1 and O139, are emerged to cause outbreaks [3]. In the past time history, cholera began in 1817, during the period, India recorded outbreak in which it subsequently moved across the Asian continent and was then regarded as the first outbreak of cholera infection in southeast Asia. More so in 19th century six cholera pandemics took place, ending in 1923 and affected most of the people in the region. Also in 1961, the seventh epidemic began in Indonesia, then moved to the India subcontinent and the Middle East, then spread across Africa in the 1970s and more recently invaded South America in the early 1990s by Reidl and Klose [4]. In Nigeria total cases of approximately 199,000 and total of 4,600 approximate deaths between 2004 and 2014 was recorded. Within the number of years over 50 notified cholera cases were found in Northern part of the country [5].

Infection is by eating or drinking of food contaminated with stool of infected person [6]. The diagnosis is by frequent profuse rice water diarrhea. For fast laboratory testing, a wet preparation of watery stool should be examined microscopically; the motility feature of vibrios is observed and can be discontinued with monovalent antibody. Other methods include culturing the mop of the rectum or the rice water stool specimen on Thiosulfate citrate bile salt agar (TCBS) agar, differential and non-differential media; [3] the colonies were tested for clumping with monovalent anti sera on slide. Biochemical tests were performed using KIA (Klingler Ion Agar) and TSI (Triple Sugar ion), oxidase positive, alkaline peptone water (APW) a selective medium, immunofluorescent tests and polymerase chain reaction (PCR) which is faster and more specific test. Protocols and procedures are developed to be use by specialized laboratories [7]. Dynamic strategies are required to regulate the disease and to minimize the mortality such as the use of case base surveillance, laboratory diagnosis, availability of treated water, environmental sanitation, hygiene, social mobilization, drugs and vaccines can be used to reduce the burden of infection [8].

Methods

We utilized mixed-methods of approach, that combined qualitative review from electronic databases and searching engines (Google, ASM, PubMed among others). We randomly searched regarding cholera outbreaks, epidemiology, sign and symptoms, laboratory diagnosis, treatment, control and prevention. We found many papers that talk about cholera, but we selected mainly those that discussed cholera outbreaks, epidemiology, sign and symptoms, laboratory diagnosis, treatment, control and prevention. We then performed a systematic review of articles published on peer-reviewed scientific journals, books, web sites information's [9].

Result

Epidemiology

The world is yet under the seventh cholera outbreak since 1960, when the El Tor lineage evolved. Cholera has continuously appeared in places where it has been absent for a century and recently occurred in Haiti in October, 2010 [10]. Cholera is still a community health problem affecting populations at risk, that their drinking water is from contaminated source and it's

below required sanitary conditions. An approximately between 3 and 5 million cases of new infections are reported annually and about 120,000 are linked to mortality worldwide [8]. Africa was noted to have a highest report in cholera outbreaks than other continents. For example, during 1995 and 2005, 417 out of a total world report of 632 outbreaks happened in Africa [11], but recent report [2] by WHO [12] shows in 2016, total of 132, 121 cases were reported by 38 countries with 2420 [2] deaths, leading to case fatality rate (CFR) of 1.8%. Even though it shows a 23% reduction in the cases reported when related with 2015 (172 out of 454 cases). The decrease is not appreciable, because there is an increase in CFR (0.8% in 2015). All continents across the globe has reported cases of *Vibrio cholerae* infection which include; Europe, Americas (4 each), Africa (17), Asia (12) and Ocean (1). Of all the cases reported, Africa account for 54% followed by Hispaniola with 32% and 13% from Asia furthermore 80% of whole world burden come from 5 countries such as Yemen, Haiti, Democratic Republic of Congo, Tanzania and Somalia [2]. Imported cases are from 9 countries [12].

Transmission

Cholera infection is through ingestion of food containing *Vibrio cholerae* in company of fecal material via oral route. The bacteria dislike acid and they tend to die due to presence of gastric juice [2].

Signs and symptoms

Almost 50% of cholera disease with classic *V. cholerae* is without obvious sign; also 75% of disease is in company of El Tor biotype. The generation time is between 1 to 3 days in case of individuals who came down with the disease, but vary on the number of the infective dose ingested. The infection begins with nausea, vomiting and copious stools, which looks like "rice water," containing slimy secretion, epithelial cells, and numerous quantity of the bacteria. There is sudden excess excretion of body fluid which contains electrolytes, which leads to severe dehydration and death if leave untreated. About 50% of the deaths are without treatment. The investigation of cholera disease shows no problem during outbreaks, but however, if cases are occurring irregularly it is difficult to distinguish from other diarrheal infection except with other differential diagnosis [2].

Cholera toxin

The living poisonous *V. cholerae* will attach to the wall of digestive tract, in which potent toxin (Cholera Toxin CT, also called "cholera toxin") is released where it links and localize on the wall of digestive tract and secretes substances that induces the release of cyclic adenosine 51-monophosphate (cAMP). This rise of cellular substances leads to the accumulation of body fluids in the digestive tract [7].

Laboratory diagnosis of *V. cholerae*

There are different techniques and methods of diagnosis of cholera but detection of *V. cholerae* from stool sample by culture remains the old standard method. Blair medium and alkaline peptone water is best for transportation of sample and TCBS has been good selective medium for the culture [13,14].

Microscopy: The use of light microscope, immunofluorescence,

dark-field and phase-contrast microscopy have aid in the laboratory investigation of cholera. In this test, direct wet mount of watery stools are tested for the existence of cholera with characteristics of darting movement. The stools are examined together with the addition of polyvalent antisera and subsequently with monovalent antisera [15].

Culture: Isolation of *V. cholerae* is essential for the diagnosis and management of client. The recommended selective and differential media for culturing *V. cholerae* is Thiosulfate citrate bile salts sucrose agar (TCBS) which is used globally. It is available in industrial form ready to use, simple to prepare, no autoclaving is required and the limitation of the media is when prepared has limited life span and cannot be used for serology directly. The large inoculum is done with the aid of wire loop on to the medium to obtain a discrete colony which is incubated at 37°C aerobically for 24 hours [15,16] (Figure 1).

Biochemical tests: The biochemical tests are other methods for identifying the bacteria based on their phenotype and metabolism. Several tests were developed for detection of

bacteria. String and oxidase test *Vibrio* shows positive other tests on carbohydrates which use (TSI) triple sugar ion are used. Also, other tests which include gas from glucose, sucrose, lysine, arginine, ornithine, VP, 0% NaCl₂ and 1% NaCl₂ can aid in diagnosis of *V. cholerae* and the use of polyvalent and monovalent antisera from fresh overnight growth culture on nutrient agar [15].

Typing methods: There are also different methods of typing *V. cholerae* in the laboratory this include bacteriophage typing, serological, ELISA and agglutination techniques. Phages are recovered from the environment in which host of *V. cholerae* strains generally survive and can be found in sewage, feces, soil and water [17].

1. Bacteriophage: There are many methods of detecting cholera using bacteriophages but the circular spot or streak cultures shows more sensitive of the test strains which when made on marked areas in Petri dishes, with the aid of a platinum loop of 3 mm in diameter and delivering 0.1 ml volume at a time. One loopful

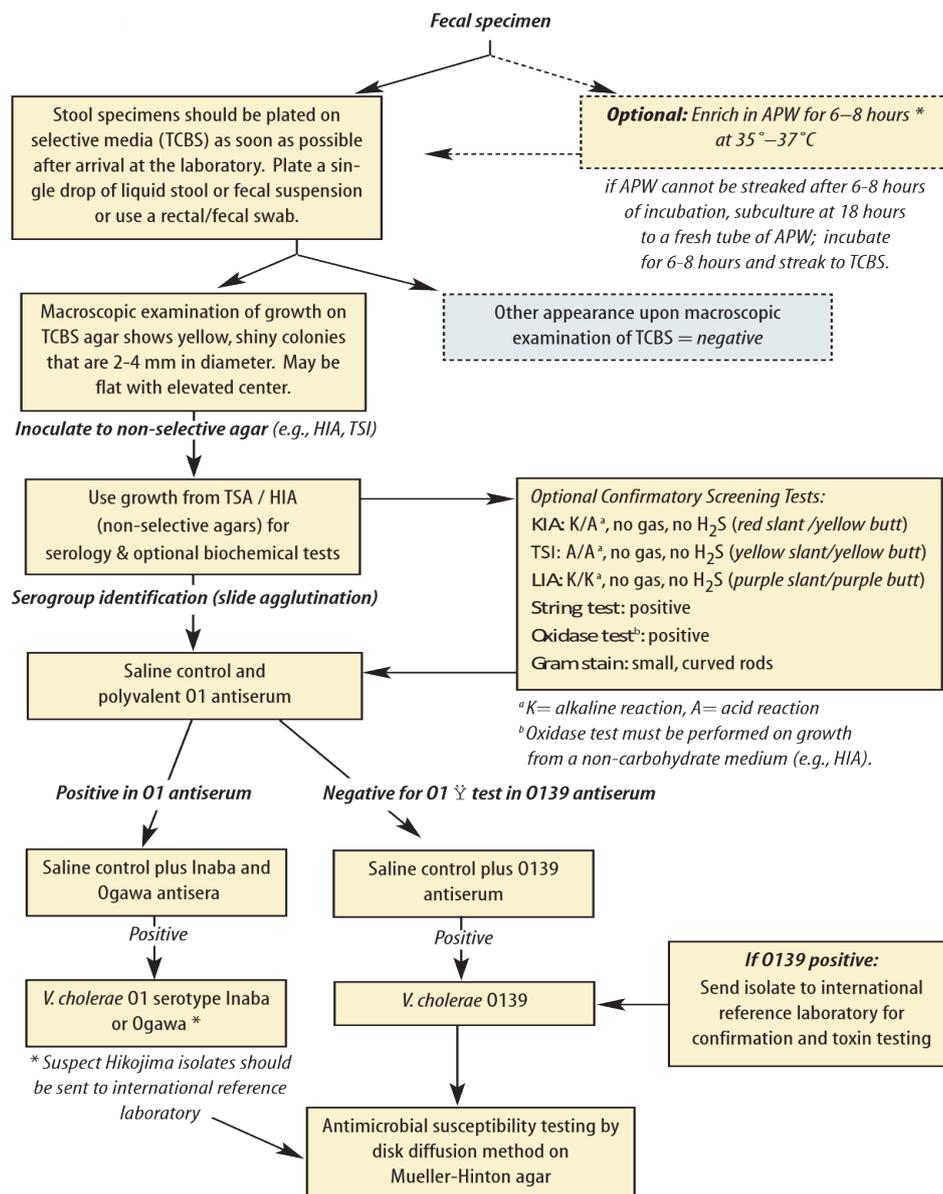


Figure 1. Steps to isolate and identify *Vibrio cholerae* in the laboratory.

of a phage preparation was added on them. The results should read after overnight incubation at 37°C. Cholera bacteriophages showed noticeable variations in affinities for different varieties of vibrios as well as for different strains belonging to the same species [18].

2. Typing Serological and Agglutination Techniques: Use of polyvalent or monovalent sera makes a fastest and specific procedure of detecting *V. cholerae* O1. Knowing what the genus and species of cholera is important in management of cases and for the selection of vaccine for cholera prevention and control. Biochemical tests including serology is generally from fecal specimens isolates to suspect *V. cholerae*, biochemical tests are needed due to the fact that the sera is not always readily available when intend to use [15].
3. ELISA: The Enzymes Linked Immuno Sorbent Assay is a serological method that is widely used to detect either by using corresponding antibody to detect antigen or vice versa using enzyme labeled color [19] (Figure 2).

Automated systems: This involves the use of commercially prepared reagents, consumables and automated system which may include open or close system. Some of this material involves, API 20E, VITEK and BIOLOG identification system [20].

1. API 20E: The Analytical Profile Index (API 20E) (BioMerieux, Inc., Hazelwood, MO) system has been the most widely used for determination of bacteria that loses the primary gram stain and pick up the secondary stain. The plastic strips contain 20 small wells with dehydrated media components. The suspected colony is mixed in physiological solution following McFarland standard concentration and is introduced into each pouch and incubated at 37°C for about 24 hours. The test inference can be read following manufacturer’s instructions. Initial evaluation of this system gave the indication that the accuracy of API 20E is equivalent to that of traditional

biochemical methods [19].

2. VITEK: The VITEK is a fully automated bacteriology system that performs bacterial identification and antibiotic susceptibility testing analysis (BioMerieux, Inc., Hazelwood, MO) compared to conventional methods that take up to 2 days to perform. This system can provide results within hours, making same-day reporting possible. VITEK is known to be rapidly and relatively accurate for the detection of *Staphylococci*. The set back of this system is that this system could have challenge in correctly distinguishing the closely related bacteria. Research shown that two *Aeromonas veronni* biovar sobria isolates were misidentified as *Vibrio alginolyticus* using the VITEK approach [19].
3. Biolog: The Biolog Inc., California, USA is a company that establishes detection of organism on the fundamental principle of electron transfer or swap which is produced during an organism’s respiration. The swap of ions is visualized as tetrazolium pigment change. The equipment tests the organism being able to utilize panel of 95 alternates carbon origin. A database containing 434 species or groups of mostly gram-negative bacteria has been compiled by the manufacturer for Biolog associated identification. In a preliminary evaluation of the Biolog system, two out of three tested *V. cholerae* strains were correctly identified and eight out of a possible ten *V. cholerae* isolates were correctly identified when automated scoring was done, with nine out of ten identified when scoring was done manually [19].

Rapid diagnostic methods

Rapid diagnosis of *V. cholerae* is essential to effectively control the outbreak of the disease. Several rapid detection techniques have been developed and are used for the detection of *V. cholerae* even though some of the methods require equipments and reagents but the use of crystal VC RDT shows significant result [21].

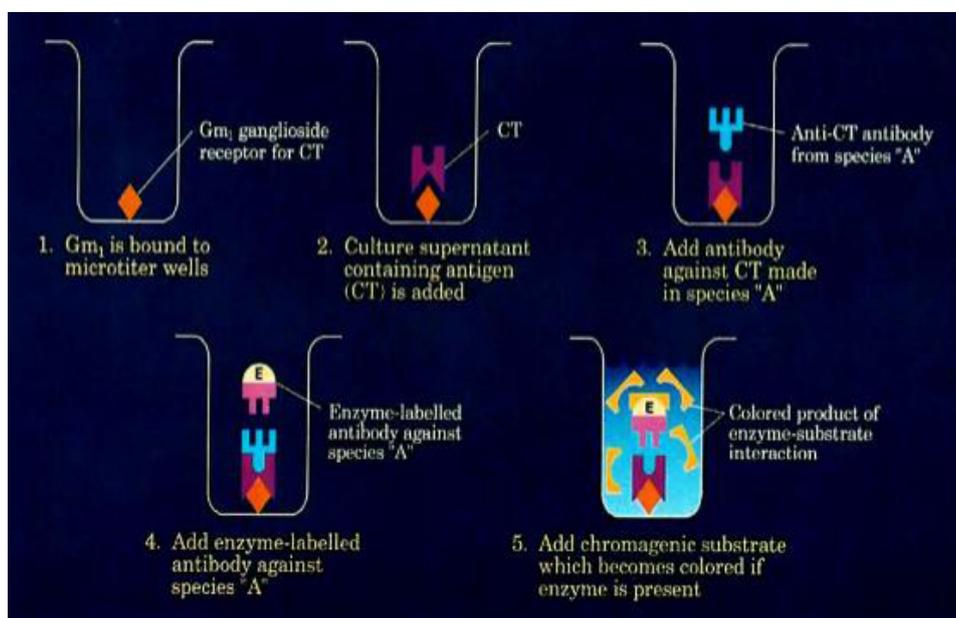


Figure 2. Steps involve in identification of Cholera using GM1-ELISA.

Treatment

The replacement of fluid and ions loss during vomiting and diarrhea is critical in treatment and management of cases which can be administered by oral or intravenous. Several protocols were developed by the World Health Organization for effective rehydration. Many antibiotics are utilized against the organism, but they serve as alternate means of treatment. Oral administration of antibiotics tends to control the intensity of the infection and lessen the time of which vibrio is release. Specific antibiotics are used in children and pregnant women [2]. As long as the patient can tolerate, it is highly recommended for the replacement water and electrolyte loss by Oral Rehydration Solution (ORS) or with Intravenous fluids. Ringer's Lactate is the preferred intravenous solution because it contains an electrolyte composition appropriate for treating cholera patients. Also, for the highly dehydrated client, antibacterial drugs can minimize the amount and time of diarrhea and shorten the duration of infectivity [22].

Prevention and Control

Control strategies for cholera depend upon determining the origin and route of transmission of the disease. Sanitation is effective but inadequate in most outbreaks areas where long time effective vaccine has not yet available and the accessible type has limitation of short term activity and side effect similar to infection. Antimicrobial drugs are good for prevention of cholera among minor number of people [7].

Stakeholders and non-governmental organization call for a declaration to end cholera in a meeting 4th October 2017 Annecy, in France and requested commitment from all stakeholders to assist cholera endemic area and align all energies, efforts, and capacities to put stop transmission. We affirm the vision of a nation that is free of cholera, through fulfillment of eradication process in ending cholera pandemic: a road map to 2030, they devoted to aimed 90 percent decrease in cholera deaths by 2030 [23]. In another development of the WHO position paper on cholera vaccines in August 2017, many countries have integrated cholera vaccine as part of their control campaign. About 25 million doses of OCV were administered in 19 countries since the inception of 2013 stockpile program (WHO, 2017) at 8th May 2018. WHO and partners support Bauchi state with 600,000 doses of oral cholera vaccine (OCV) [24].

The World Health Organization and implementing partners sit in 2016 and revised the kits and materials required for cholera epidemics. In the attempt to end the outbreaks by 2030, the kits include: Study kit, laboratory diagnosis and case management with some basic amenities for local, states and federal with intend each kit can respond to 100 clients and the intervention should be as quickly as possible during the initial month of outbreaks [6].

Discussion

Cholera infection remains a highly potential threat to public and is most likely to occur and reoccur in such area with low pointers of community growth and to states where basic needs for community and human development is not adequate. There is an extreme need for continuously monitoring and quality control testing of water sources to identify *V. cholerae* and

other organism including toxic chemical which may be present in the water supply [25]. With the recent roadmap developed by GTFCC to end cholera by 2030, relevant stakeholder need to be alert and collaborate with multisectorial to prevent, identify and treat cholera transmission through reservoirs, especially within the population at risk during the start of the rainy season.

Conclusion

The emerging and re-emerging cholera outbreaks and CT virulence is a global concern; therefore, all stakeholders are encouraged to be proactive to follow the roadmap strategies of GTFCC to end cholera by 2030.

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