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Opinion Article

Isolation and Identification of Toxin Producing Organism-*Vibrio Cholerae*

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DESCRIPTION

Cholera is a toxin-producing strain of the gram-negative bacterium *Vibrio cholerae* that causes an acute secretory diarrheal illness. Severe cholera is distinguished by significant fluid and electrolyte losses in the stool and the rapid development of hypovolemic shock, which occurs within 24 hours of the onset of vomiting and diarrhea. The use of appropriate rehydration therapy reduces the mortality rate of severe cholera from more than 10% to less than 0.5 percent. The isolation and identification of *Vibrio cholerae* serogroups O1 or O139 from stool specimens remains the gold standard for cholera laboratory diagnosis. Cary Blair Media is ideal for transport, while thiosulfate citrate bile salts sucrose agar is ideal for isolation and identification.

Commercially available rapid test kits are useful in epidemic situations, but they do not produce an isolate for antimicrobial susceptibility testing and subtyping and should not be used for routine diagnosis.

DIAGNOSIS

In many countries where cholera is common but diagnostic laboratory testing is difficult to obtain, the following clinical definition for suspected cholera cases: Detecting and confirming cholera is difficult in remote areas where culture methods are not readily available. There are rapid diagnostic tests available that provide results in 15 minutes after a dipstick is placed into a diluted stool sample however, due to the performance of rapid diagnostic tests, particularly in terms of specificity, positive tests may require confirmation by culture or PCR. Faecal samples that have been spotted on filter paper and dried can be used for PCR confirmation. Such filter paper samples are not biohazardous and do not require refrigeration, and DNA remains stable even after long periods of storage. Such samples can also be used for advanced molecular characterization. In most cases, a *V. cholerae* infection is contracted by consuming contaminated food or water. *V. cholerae* in water is an important reservoir of the organism in endemic areas. Because *V. cholerae* can live on chitinous plankton, filtering water through coarse cloth can reduce cholera incidence in endemic areas. While environmental *V. cholerae* exposure is important, it is also thought that direct person-to-person transmission plays a role in transmission. Individuals suffering from severe cholera can excrete up to 1010–1012 organisms per liter of stool. Organisms recently shed from infected individuals appear to be transiently more infectious than organisms isolated from the aquatic environment, implying that person-to-person transmission of human-shed organisms is possible.

TREATMENT

Following the instructions, 200 liters of watery stool were used for DNA extraction using the QIAamp Fast DNA Stool Mini Kit. A polymerase chain reaction assay was performed with stool DNA, targeting the CT gene (ctxAB) as well as the rfb region, which encodes the O1 serogroup's somatic antigen. The simplex PCR assay was carried out using an Eppendorf Mastercycler instrument. Swabs were wetted with various dilutions of *V. cholerae* O1 culture and stored at room temperature in Cary-Blair transport medium (Difco). APW-enriched cultures were used to test the viability and performance of cholera rapid diagnostic tests at regular intervals for up to 18 days.

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