Short Communication

Early detection of cholera cases and contacts by polymerase chain reaction

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Abstract

Amplification of cholera toxin *ctx* gene in *Vibrio cholerae* by polymerase chain reaction (PCR) allowed early detection of cholera cases and contacts when compared to conventional culture methods. All specimens that were positive by PCR (12 out of 74) were also culture positive. Diagnosis of cholera together with detection of toxin producing *Vibrio cholerae* strains can be achieved on the same day of specimen collection.

Key words: *ctx* gene; cholera; PCR diagnosis.

Laboratory diagnosis of cholera currently relies on the isolation of the causative organism by conventional culture. During the investigation of cholera outbreaks, large numbers of clinical and environmental specimens need to be screened. Stool culture of contacts with symptoms of diarrhoea and of all household contacts, even if asymptomatic, should be undertaken. This will normally increase the workload of the microbiology laboratory and may involve several laboratory technicians, laboratory space and hundreds of culture plates.

Amplification of genes expressing cholera toxin subunits A (*ctx*A) and B (*ctx*B) by polymerase chain reaction (PCR) technique have been shown to be successful in distinguishing toxigenic from non-toxigenic *Vibrio cholerae* strains in pure cultures (Fields *et al.*, 1992). In a recent cholera outbreak, we applied this technique to detect cholera cases and contacts and compared it with conventional culture methods.

As in any investigation of a cholera outbreak, rectal swabs from suspected cases and contacts were taken by public health inspectors and transported to the laboratory in alkaline peptone water (APW). For PCR the specimens were processed immediately on arrival. 500 µl of the APW were taken just below the surface of water, transferred to a sterile microcentrifuge tube and centrifuged 12000 rpm for 5 minutes. The pellet was resuspended in 500 µl sterile distilled water, boiled for 10 minutes to release the DNA and recentrifuged to remove cell debris. Two primers (GTG GGA ATG CTC CAA GAT CAT CG, positions 1129 to 1151 and ATT GCG GCA ATC GCA TGA GGC GT, positions 1625 TO 1647) that amplify a 519-bp region spanning *ctx*A and *ctx*B cistrons were used. The PCR was carried out in 50 µl reaction mixture consisting of 5 µl of 10x PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl; 15 mM $MgCl_2$), 50 µM each of the four deoxynucleoside triphosphates, 0.25 µM of each primer, 1.25 U of *Taq* polymerase and 5µl of the supernatant containing the DNA. PCR was carried out in a GeneAmp 2400 PCR System thermal cycler (Perkin Elmer Cetus, USA) with the following conditions: denaturation at 94°C for 5 min; 30 cycles of 1 min at 94°C, 1 min at 61°C and 2 min at 72°C and a final extension step at 72°C for 5 min. 8 µl PCR products were electrophoretically separated in 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide and visualised under UV transilluminator (Fig. 1). For culture methods, a minimum of 6 hours of incubation in the APW was required before the swab is plated onto a solid culture media.

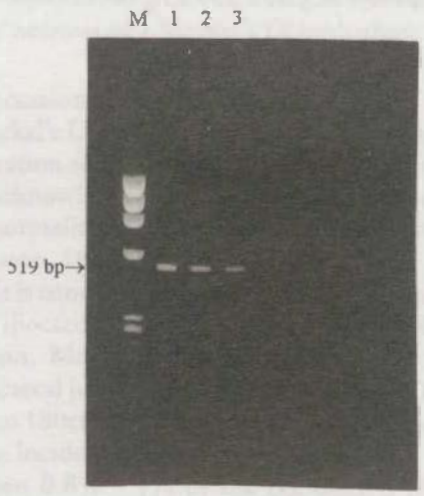

Fig. 1. Detection of *ctx* gene in cholera case and contact. M = ϕX174/*Hae*III marker; 1 = DNA from *Vibrio cholera* 01:2 = stool sample from a cholera; 3 = stool sample from a cholera contact

Out of 74 rectal swabs collected during the investigation of cholera outbreak, 12 were culture positive. All the culture positive cases were also positive by PCR technique. PCR detection of cholera toxin can be carried out on the same day of collection. Extraction of DNA by boiling eliminated the time consuming step of phenol-chloroform extraction and isopropanol precipitation of DNA thus enabling diagnosis of cholera to be made on the same day. With culture methods, optimal growth of *Vibrio cholerae* is required before subculturing on Monsur's or Thiosulfate Citrate Bile Salt agar (TCBS) can be carried out. A minimum of 6 hours is usually needed for optimal growth but often subculturing is carried out the next day if the APW arrived late in the afternoon. Familiarity of the laboratory technologist in identifying the colonies growing on the agar is another factor to be considered. Toxigenic and non-toxigenic *Vibrio cholerae*, *Aeromonas* spp., *Plesiomonas* spp., and *Staphylococcus aureus* may appear similar on Monsur's agar. Serotyping for presumptive diagnosis of *Vibrio cholerae* can be done only if there is confluent growth or otherwise suspected colonies need to be subcultured again on blood agar. All these would add to the delay in diagnosis of cholera. Conventional culture methods would need at least 2 days for presumptive diagnosis by serotyping and 4 days for definitive identification of the organism. All these steps are labour intensive and will delay the diagnosis of cholera. Early identification of cholera cases and contacts would allow early identification of the source of infection and tracing of healthy carriers so that treatment and effective preventive and control measures can be taken. The identification of cholera toxin is an important step in the diagnosis of cholera because only toxin producing strains have been associated with severe, watery diarrhoea and epidemics. *Aeromonas hydrophila* and *Campylobacter* species that were reported to produce proteins similar to cholera toxin (James *et al.*, 1982; Olsvik *et al.*, 1984) were not positive by PCR (data not shown). This technique can be adopted easily in a laboratory equipped with a thermal cycler. Nowadays this equipment is not something new to any laboratory. Molecular techniques should be applied in the diagnosis of infectious diseases because rapid diagnosis can be achieved. There is less space usage as compared to conventional cultures where a number of culture plates and reagents need to be inoculated and lesser number of laboratory technicians involved.

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