Laboratory investigation and infection control of cholera in Kingston, Ont.

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Vibrio cholerae, **biotype El Tor, was isolated in a hospital laboratory in Kingston, Ont. in 1974. Confirmation and complete identification by the Ontario regional and provincial public health laboratories was obtained within 3 days. Institution of well established infection-control and public health measures prevented spread of the infection within the hospital and the community.**

Vibrio cholerae, **biotype El Tor, a été isolé dans le laboratoire d'un hôpital de Kingston, Ont. en 1974. La confirmation et l'identification complète de la souche ont été obtenues en moins de 3 jours des laboratoires provinciaux et régionaux de la santé publique de l'Ontario. La mise en place de moyens reconnus de contrôle contre l'infection et de mesures de santé publique a permis d'empêcher la propagation de l'infection à l'hôpital et à la communauté environnante.**

Most physicians and microbiologists in Canada have no experience with cholera or isolation of the etiologic agent, *Vibrio cholerae*. However, with today's rapid transportation systems and mobile population this situation could change rapidly. Sporadic cases in nonendemic areas may occur in persons with a history of travel to endemic areas. The first isolation of *V. cholerae* in North America since 1911 was made in Texas in August 1973.[1] The source of that organism is still unknown.

We report in detail the isolation and identification of *V. cholerae* in Kingston, Ont. in the hope this will prepare Canadian health personnel to meet this challenge.

Laboratory investigation

A patient presented at the emergency department of Hotel Dieu Hospital in Kingston with profuse diarrhea; his clinical course is detailed in the accompanying paper by Bourdages and Beck (page 393). Stool specimens were examined immediately. A Gram-stained preparation of the liquid feces revealed a predominance of short gram-negative organisms, many of which were slightly curved or comma-shaped. A wet preparation demonstrated numerous highly motile bacilli. On the basis of the clinical picture, the history of travel from South Africa to Angola and Portugal, and the results of microscopic examination of the stool, a diagnosis of cholera was suspected.

Since cholera-specific medium was not on hand in the hospital laboratory, a fecal sample was forwarded to the Kingston Regional Public Health Laboratory, where this medium was available. In the hospital laboratory the stool specimen was processed according to the usual routine for suspected enteric pathogens.[2] After 18 hours' incubation at 37°C, several of the lactose-negative colonies from the MacConkey agar plate were found to be urea-negative. The growth on the urea slopes was found to be positive for oxidase and *o*-nitrophenyl-β-D-galactopyranoside. Colonies demonstrating these reactions, suggestive of *V. cholerae*, were examined further. At the same time the Public Health Laboratory reported the presence of large yellow colonies typical of *V. cholerae* on thiosulfate citrate bile salts sucrose medium (TCBS), an alkaline medium designed for the isolation of *V. cholerae*. Preliminary serologic examinations at that laboratory, using *V. cholerae* polyvalent antiserum, gave strongly positive results. Thus, 24 hours after receipt of the specimen the presence of *Vibrio* organisms was certain.

Cultures and other fecal specimens were forwarded immediately to the Central Public Health Laboratory in Toronto, where the identification of *V. cholerae* was confirmed. The complete characterization of the isolate is given in Table I. On the basis of the positive Voges-Proskauer reaction, resistance to polymyxin B and the phage susceptibility pattern, the isolate was classified as *V. cholerae*, biotype El Tor. Serologic techniques identified the isolate as serotype Inaba. The organism was further classified as Mukerjee El Tor phage type IV by Dr. A.L. Furniss of the Public Health Laboratory Service, Preston Hall Hospital, Maidstone, England (personal communication). This isolate was thus fully identified as *V. cholerae*, biotype El Tor, serotype Inaba, phage type IV.

Infection control

A prominent feature associated with cholera is profuse diarrhea and unexpected vomiting. Since the infectious agent is present in feces and vomitus the question arises, What procedures does the hospital implement to prevent spread of the infection to other patients, personnel and the community?

Cholera is not highly communicable. Transmission is through the fecal–oral route — that is, the organism excreted in the feces of a carrier, or the feces and vomitus of a victim, gains access to drinking water or food. The ingestion of sufficient numbers of organisms (estimated at 10^8 to 10^{10}) causes clinical symptoms.[3] Cholera is then in the category of infection requiring "enteric isolation". The infection control procedures to be carried out with a cholera victim are the same as for any patient with an enteric infection, such as typhoid fever. These are clearly defined in the US Public Health Service publi-

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Table I—Characteristics of *Vibrio cholerae* isolate*

Test or culture medium	Reaction or result	Test or culture medium	Reaction or result
Oxidase	+	Trehalose	+
Indole	+	Xylose	−
Motility	+	Sorbitol	−
Simmons' citrate	+	Rhamnose	−
Christensen's citrate	+	Salicin	−
Methyl red	−	Inositol	−
Methyl red 22°C	−	Adonitol	−
Voges-Proskauer	+	Cellobiose	+
Voges-Proskauer 22°C	+	Galactose	+
Lysine decarboxylase	+	Melezitose	−
Ornithine decarboxylase	+	Melibiose	−
Arginine dihydrolase	−	Esculin	−
Gelatin (charcoal)	+	Nitrate	+
Christensen's urea	−	Sodium acetate	+
Malonate	−	Oxidation-fermentation (dextrose)	Fermentation
Phenylalanine deaminase	−	Jordan's tartrate	+
H_2S (triple sugar iron)	−	String test	++
Dextrose, acid	+	0/129 (vibriostatic agent)	Sensitive
Dextrose, gas	−	Polymyxin B	Resistant
ONPG†	+	Mukerjee phage IV	Resistant
Sucrose‡	+	Mukerjee phage V	Sensitive
Arabinose‡	−	Tube hemolysis (sheep erythrocytes)	−
Mannose ‡	+		
Mannitol	+	Growth on Salmonella - Shigella	−
Maltose	+	Growth on MacConkey	+

*Incubated for 48 hours at 36°C if not otherwise specified.
†*o*-nitrophenyl-β-D-galactopyranoside.
‡Heiberg group I.

cation "Isolation Techniques for Use in Hospitals".[4] Such procedures were implemented immediately when the diagnosis of an enteric infection had been made.

The patient's stool specimens were examined daily for *V. cholerae*. Within 3 days the feces were negative for cholera vibrios. No secondary cases of cholera appeared in the hospital within 5 days of the patient's admission. Extensive investigations were undertaken in Kingston to identify all contacts of the patient. Multiple stool samples were taken from persons at the home where the patient had stayed prior to coming to hospital. No excretors of the organism or persons with cholera or symptoms of enteric infection were reported among the patient's contacts. No cases of cholera were reported among passengers on the same flights as the patient. Follow-up by provincial and federal epidemiologists was not able to pinpoint the source of this infection, although both Luanda (Angola) and Lisbon, the two areas the patient had visited, are good possibilities because cholera was endemic in both.

Discussion

Identification of an organism as *V. cholerae* requires differentiation from *V. parahaemolyticus*, an enteric pathogen, and from the *Aeromonas* group, which are also oxidase-positive, motile and fermentative. This differentiation is easily performed in the laboratory by means of a small number of biochemical tests. At one time the El Tor vibrio was regarded as a separate species. However, it is now considered only a biotype of *V. cholerae*, differing from the "classic" biotype in a few characteristics, such as hemagglutination of chicken erythrocytes, hemolysis of sheep erythrocytes and resistance to *V. cholerae* phage type IV. Of importance with this isolate is the fact that the El Tor organism is considered hardier: it persists longer in nature, is excreted longer and is more resistant to chemical agents than classic *V. cholerae*. Furthermore, asymptomatic infection in humans occurs more frequently with the El Tor biotype; thus, there is greater danger of the disease being transported to North America and remaining undetected.

Suspicion of cholera in the admitting hospital and good cooperation with the Ontario public health laboratories expedited laboratory confirmation of the diagnosis of cholera and resulted in the complete identification of this unusual organism (in our country) in the shortest possible time.

Institution of the routine enteric isolation procedures, as directed by the infection control program at Hotel Dieu Hospital, relieved the staff of any unnecessary concern for their safety and allowed them to concentrate their efforts on restoring the patient to health.

Most cases of infectious diarrhea in Canadians are due to the more common agents, and therefore we do not recommend that specimens from all patients with diarrhea be examined for *V. cholerae*. However, physicians must consider this disease in the differential diagnosis of diarrheal illness in individuals returning to Canada from countries where cholera is endemic and must communicate this information to the bacteriologist so that the TCBS medium required to facilitate the isolation and identification of *V. cholerae* can be used. Without such clinical suspicion it is doubtful that a laboratory isolation or the correct diagnosis would be made.

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