intravenously to give the patient bicarbonate and potassium.

If the signs of dehydration get worse or remain unchanged, it is may be necessary to speed up administration of the solution being used.

The ORS can be administered via nasogastric tube to a patient who is *not* in shock but who cannot drink (due to fatigue, sleepiness, or other reasons) at a rate of 20 ml/kg per hour if there are personnel available who have been trained to do this. In children who are in shock, this procedure should be used only when it is not possible to give the solution intravenously.

Antibiotics

Antibiotics are very important in the treatment of cholera because they reduce the duration of diarrhea and the shedding of vibrios within 2 or 3 days.

Oral tetracycline is the antibiotic of choice: 500 mg should be given 4 times/day for 3 days. Doxycycline (300 mg), a form of long-acting tetracycline that is given only once, is preferred when it is available. Other alternatives, when the strains are resistant, are furazolidone and trimetroprim-sulfamethoxazole.

No other antidiarrheal, antiemetic, antispasmodic, cardiotonic, or corticosteroid products should be used.

Maintenance Therapy

After the initial fluid and electrolyte imbalance has been corrected and the signs of dehydration have disappeared, it is important to replace the abnormal losses due to diarrhea or vomiting and, in addition, to meet the normal daily requirement for liquids until the diarrhea ends. During maintenance therapy the ORS should be used after every evacuation (1/2 to 1 cup depending on the patient's age). Maintenance therapy also includes continued feeding throughout the course of the disease.

References

American Public Health Association. *Control of Communicable Diseases in Man.* Abram S. Benenson (editor), Washington, D.C., 15th edition, 1990.

(*Source*: Diarrheal Disease Control Program, PAHO.)

Laboratory Diagnosis of Cholera

A request for laboratory diagnosis is most important upon an initial suspicion of cholera based on the recognition of the typical clinical features and the appropriate epidemiologic setting. Because most bacterial diarrheas are self-limited, stool cultures are generally limited to cases with severe symptoms requiring hospitalization, persistent or recurrent and dysentery-like clinical presentation.

The clinical or public health laboratory is usually organized to process the specimens following an algorithm designed to identify a list of enteric pathogens prevalent in the Region. Most laboratories may not inoculate media suitable for the isolation of vibrios unless specifically requested to do so. *Vibrio cholerae* is not the only organism to cause watery diarrhea or *rice water* stools, although it produces the most severe disease. The approach adopted by a particular laboratory for the isolation of vibrios will depend on the frequency anticipated, and the cost-effectiveness of incorporating agar thiosulfate-citrate-bile salt-sucrose (TCBS) medium on a routine basis. Vibrios may be isolated in other plating media, but a particular search may need to identify *V. cholerae* or to screen for Gram negative bacilli, oxidase-positive colonies.

Stool specimens should be collected early in the disease and preferably within the first 24 hours of illness, and before the patient has received any antimicrobial agents. Rectal swabs are probably highly efficient in the acute phase of illness, but less satisfactory for convalescent patients or transiently infected asymptomatic persons. Specimens should be inoculated onto isolation plates with minimun delay. The viability of vibrio species is well maintained in an alkaline pH of *rice water* stool but is unpredictable in formed stools. Vibrios are very susceptible to desiccation; hence, specimens must not be allowed to dry. When there will be a delay in plating a culture, the rectal swabs or fecal material should be placed in the semisolid transport medium of Cary and Blair, which maintains the viability of vibrio cultures for up to 4 weeks. Buffered glycerol-saline, often used in enteric bacteriology, is an unsatisfactory transport medium even for short periods. In the absence of available suitable transport media, strips of blotting paper may be soaked in liquid stool and inserted into airtight plastic bags to prevent drying, and the organism will remain viable for up to 5 weeks. Specimens in transport medium may be shipped to the laboratory without refrigeration.

Microscopic examination of a diarrheal stool may be helpful only in certain circumstances. A methylene blue stain for leukocytes may be helpful in differentiating invasive and enterotoxigenic causes of diarrhea. However, a direct examination of stool material is not recommended for general purposes.

The laboratory worker is most familiar with the isolation of the many strains of *Enterobacteriaceae* common in the Region. Many of the techniques used in the microbiology laboratory for the isolation of enteric pathogens work well with the genus vibrio. The organism generally grows well on the common media such as blood agar and MacConkey agar, but its isolation is enhanced by the use of media and growth conditions that favor it selectively. A factor to consider is the halophilic nature of some species, and many laboratory media have suboptimum amounts of Na+ (less than 0.5 NaCl), therefore, some vibrio cultures will not grow well on the highly selective media used to isolate enteric pathogens, probably due to the lack of sufficient NaCl, and it may be necessary to supplement the media with 1 to 3% NaCl. The isolation of vibrios is favored by an alkaline (pH 9.0) liquid medium and TCBS-agar.

TCBS should not be autoclaved, and its final pH should be 8.4. An enrichment broth, such as alkaline peptone water, should also be inoculated and subcultured in 6 to 12 hours to a second set of TCBS plates. Yellow colonies on TCBS (due to sucrose fermentation) should be selected for further study with biochemical and serologic tests. Colonies of *V. cholerae* on TCBS are usually sticky, suitable for the string test, however, making it cumbersome for the slide agglutination test. Typical colonies of *V. cholerae* should be tested with 0 group 1 serum and if positive, a provisional report of *V. cholerae* 01 may be issued.

Agglutination of a saline suspension of the organism by polyvalent antisera against *V. cholerae* should occur within a minute if it is a positive test. In the case of doubtful agglutination with serum which is reacting well with the controls, the oxidase and string test should be repeated to confirm that the colony is that of a vibrio. A part of the colony should be transferred to KIA and, after overnight incubation if there is a K/A reaction with no gas or H2S, the identity should be confirmed by the slide agglutination with 0 group 1 antisera, Ogawa and Inabe sera. If the reactions are still negative the strain should be referred to the WHO Collaborating Laboratory.

Vibrio cultures usually grow on MacConkey agar, but some times with a reduced plating efficiency, and will appear as colorless (lactose-negative) colonies. Vibrios will also grow well on blood agar, where they will be beta-hemolytic (*V. cholerae* non 01 and *V. cholerae* 01 of the El Tor biotype). Oxidase testing is recommended on both hemolytic and nonhemolytic colonies on sheep blood agar plates. Oxidase testing can be done on colonies grown on sheep blood agar and on lactose/negative colonies culture on selective media. Lactose/positive colonies from selective media can give false/negative oxidase reactions.

Once isolated, the organism is readily identified by biochemical reactions, and identification may be confirmed by agglutination with specific antisera.

If triple sugar iron agar (TSIA) and lysine iron agar (LIA) are inoculated for screening purposes, their reactions will be acid slant/acid butt with no gas (A/A-) or H2S and alkaline slant/alkaline butt (K/K), respectively.

Agglutination of a saline suspension of the organism by polyvalent antiserum against *V. cholerae* should occur within a minute if positive. Any doubtful results should be referred to the WHO Collaborating Laboratory of the Region.

(*Source*: Laboratory Services, Health Services Development Program, PAHO.)

Environmental Health, Prevention and Control of Cholera

Unlike the coliform bacteria which are the primary indicators of pollution which decays faster in salt water and brackish water, *Vibrio cholerae* survives better in the marine environment than in a freshwater environment. This means that in addition to the health threat from drinking water supplies and irrigated food crops there is a threat from seafood which is commonly eaten raw or insufficiently cooked. The fact that *V. cholerae* has an affinity for chitin implies a health risk from shellfish harvested in marine waters which are contaminated with sewage from a population with endemic cholera. Shellfish and seafood have been implicated several times in outbreaks of cholera outside of the western hemisphere.

The *V. cholerae* microorganism is relatively large, ranging in length from about 1.5 to 3 microns and about 0.5 microns in diameter. It is thus readily removed in water treatment plants using flocculation, sedimentation and rapid sand filtration as well