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LABORATORY DIAGNOSIS OF CHOLERA IN CASES AND CARRIERS

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Laboratory diagnosis of a case of cholera consists of isolating the vibrios agglutinating with anticholera serum in the patient's excreta. The presence of large numbers of vibrios makes the task simple, though at times the organism evades detection. The diagnosis of the first few cases of an outbreak is particularly difficult. Cholera is a disease of poverty, hunger and dirt and formulation of methods for diagnosis, treatment and control should be simple but effective.

Collection of Samples

Stools collected early in the course of the disease, particularly before giving any antimicrobial drug, are the most rewarding. They are best collected in a sterile bottle or test-tube by No. 26 or 28 rubber catheters lubricated with sterile paraffin or glycerine. A rectal swab or glass rod is often used when it is essential to make sure that the swabbing is done properly. The rectal swab should be made of absorbent cotton-wool so that it can absorb about 0.1-0.2 ml of fluid. Contamination from the perineum should be avoided. It is preferable to moisten the swab with peptone water and press it against the side of the tube to remove excess fluid before swabbing convalescents who do not have watery diarrhoea. Collection of stools from the pan into which they are voided is often practised in rural areas. However, the organism may remain to contaminate otherwise negative stools or may be killed by the disinfectants generally used in such pans.

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Methods for Rapid Diagnosis

Though not required for treatment, a quick method of diagnosing cholera would be helpful for planning control measures, epidemiological and clinical investigations and for quarantine purposes. Bandi's procedure, requiring two to seven hours of incubation of cholera stool in peptone water with anticholera serum, is not practised since Ghosal & Paul found routine methods giving 38 per cent. more positive results. By dark-field microscopy about 80 per cent. of the cases can be correctly diagnosed within a few minutes if examined early in the course of the disease; after six to 10 hours of enrichment of faecal matter more cases can be diagnosed. No special skill or equipment is required besides a dark-field microscope, particularly the Cook McArthur model. The fluorescent antibody technique can also diagnose about 90 per cent. of the cases correctly in about two hours but it requires special skill and equipment. Both these methods are complementary to the usual method of isolation. It is possible, however, to have a correct bacteriological diagnosis in four to five hours with the help of a stereoscope. The faecal matter is properly streaked on a non-inhibitory nutrient agar plate with or without 0.1 per cent. Teepol and incubated for four to five hours, when vibrio colonies can be easily spotted by a stereoscope. Then they can be confirmed by slide agglutination with O-group serum. Stereoscope alone is not dependable and the colony characteristics are not fully developed. This procedure has been successful on more than 100 specimens collected within the first two days of the disease.

Transportation of Specimens

The Venkataraman-Ramakrishna's Fluid may be used when at least 1-3 ml of the inoculum is available. If the inoculum is small, e.g., a rectal swab of about 0.1 ml, it should be transported in alkaline-trypticase-taurocholate-tellurite medium (pH 9.2) or in alkaline peptone water (pH 8.0-8.5) which both have enriching as well as preservative properties. Presence of a single vibrio can be detected by enrichment in any of these two fluids. Its simplicity and low cost plus its good performance in the field make alkaline peptone water a more suitable media for general purposes. It should be distributed in screw-capped or rubber-capped bottles rather than in test-tubes. The vibrios may be overgrown by commensals

when kept too long in a tropical climate before plating but this can be avoided by transferring about 0.1-0.2 ml to a fresh tube of the medium for proper incubation in the laboratory. Cholera vibrios kept at room temperature in this media were found to remain viable for more than two to six weeks; during the first 14 days no vibrios were lost from the 35 samples tested. In Teepol-peptone water (alkaline peptone water with 0.1 to 0.2 per cent. Teepol) the growth of cocci and aerobic spore bearers is prevented and vibrios remain viable for a longer period but grow slowly. For specimens coming from great distances, this may be the more suitable medium. If none of these media are available, strips of thick unsterile blotting paper soaked with stool may be used. This may be placed in a plastic envelope and sealed properly to avoid drying en route to the laboratory. Vibrios have been found to survive on such strips for about five weeks, or as long as moisture is preserved.

Enrichment before Plating

Direct streaking on a solid medium of stools collected on the first or second day of illness has yielded better results than enrichment followed by plating though for obvious reasons the latter procedure is the better method for specimens collected during convalescence. Direct plating has the additional advantage of not allowing the associated non-agglutinable vibrios to overgrow the agglutinable ones as is possible in tubes of enrichment medium. However, both direct streaking and inoculation for enrichment should be made routinely whenever possible. Of the various media available for enrichment, simple alkaline peptone water (pH 8.0-8.5) is preferred.

Solid Media for Plating and Isolation

The choice of a plating medium depends upon the experience of the individual worker and the facilities available. Some well-tried media are non- or slightly inhibitory (nutrient agar, pH 7.6; Teepol agar with 0.1 per cent. Teepol, pH 7.6; bile salt agar, pH 8.2, and gelatin agar, pH 8.0) while others are highly selective (cholera medium, oxoid, pH 8.4; TCBS medium, Eiken, pH 8.6 and gelatin trypticase taurocholate tellurite agar, pH 8.5). It is better, if possible, to use two types of media for each specimen to avoid unpredictable lapses, one-half of each being used for direct streaking and the other half after enrichment. The selective medium

is not necessary for cases in the first or second day of illness when it may be more economical to use one or two plates of freshly prepared, non-inhibitory media (not older than two or three days).

The colonies can be spotted with the naked eye after overnight incubation, and can be seen even more easily on transparent media with the help of a stereoscope using transmitted oblique light. By stereoscopic examination, one can differentiate colonies of vibrios from those of many species including *Aeromona* but not always from colonies of some strains of *Pseudomona*, *Comamonas* and non-agglutinable vibrios.

Identification and Characterization

Suspected colonies should first be tested with standard anticholera O-group I serum, and if positive, by slide agglutination with Ogawa and Inaba type-specific antisera. If the suspicious colony fails to react with the group-serum, at least five to ten colonies and finally a sweep from the confluent area should be similarly tested before considering the case negative for agglutinable vibrios, as they may coexist with the non-agglutinable ones. It may be better to pick up five to ten colonies on KIA or nutrient agar slants for further study.

Remnants of an agglutinable colony should be picked up with a straight wire and put onto a KIA or TSI slant, which, after overnight incubation, should show typical biochemical characteristics. The growth should be serologically confirmed and then subjected to tests for fermentation of mannose, sucrose and arabinose, production of acetylmethyl carbinol by Baritt's technique, haemagglutination of chicken or sheep cells, sensitivity to 50 unit polymycin B discs and cholera-phage group IV at routine test dilution. When facilities are available, some representative strains of each outbreak should be tested for sensitivity to chloramphenicol and tetracycline, using 1.6, 3.2, 6.3 $\mu\text{g/ml}$, and streptomycin, using 5 $\mu\text{g/ml}$ concentration by an agar plate diffusion technique. A few strains resistant to these antibiotics were found in the Philippines in 1965. Representative strains should also be sent to the International Cholera Phage Reference Centre of WHO in Calcutta.

Serological tests, running paired sera (acute phase serum collected within 48 hours of onset, and convalescent serum collected between the seventh and tenth day) have been found very reliable for retrospective diagnosis of cholera. The

agglutination test using live antigen and the vibriocidal antibody tests have been tried widely when the results were closely parallel to bacteriological findings. The serological test may act as a check on the bacteriological procedures of a laboratory.

Laboratory Diagnosis of Carriers of Cholera

Contact carriers and post-cholera carriers have been found to excrete about 10^2 - 10^5 vibrios per gram of stool along with large numbers of commensals, while cholera patients excrete about 10^7 - 10^9 vibrios per ml with a few commensals. This makes the diagnosis of a carrier more difficult than that of a case.

The contacts should be approached as soon as possible after the detection of the index case. There should also be daily follow-up, as more positive contacts are diagnosed in this way.

It is generally advisable to inoculate about 3 g of stool into 50-100 ml of alkaline peptone water soon after evacuation. However, moistened rectal swabs or glass rods or stool swabs may be used for convenience. Vibrios have been found to be more or less uniformly distributed on the surface as well as within the faecal mass; a properly collected rectal swab or stool swab may, therefore, also be a reliable method. The specimen should be collected in alkaline peptone water with or without Teepol or in alkaline-bile salt-tellurite peptone water in the field. At the laboratory, it should be incubated for five to six hours and then plated on a selective and non-selective medium. About 0.1-0.2 ml should be transferred to a second peptone water tube for proper incubation. Plating should be done from this tube in case of failure to isolate the vibrio from the first tube. This procedure of second enrichment in a group of carriers yielded about 11 per cent. more positive isolates. The detection of colonies of vibrios on a non-inhibitory medium may at times be very difficult without the stereoscope, and selective media are definitely helpful. The rest of the procedure for identification and characterization is the same as with patients.

Contact carriers also develop antibody in high titre but serological examination of contacts may not be practicable for diagnosis.

For detection of post-cholera carriers, serological tests appear to be of special value. About 50 bacteriologically proven cases of cholera were serologically examined 12 weeks or more after attack. The agglutinin titre was found to vary from less than 20 to 160 (only four had a titre of 160) in all except one who has an agglutinin time of 640-1280 almost four years after the disease and is harbouring vibrios in her gall bladder to excrete them intermittently. Serological follow-up of the former cases, in the absence of vaccination, may be the easiest way to trace such post-cholera carriers. Persistence of the antibody in a high titre 12 weeks or more after illness followed by discovery of vibrios in the stool and, if possible, in the bile will confirm the diagnosis of such a carrier state.