BACTERIOLOGICAL DIAGNOSIS OF CHOLERA UNDER FIELD CONDITIONS

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Introduction:

In underdeveloped countries where laboratory facilities are very meagre bacteriological diagnosis of cholera in outlying areas always presents a difficult problem. Recent studies of media at the Pakistan-SEATO Cholera Research Laboratory show that it is possible to make an accurate bacteriological diagnosis of cholera even in the remotest areas of the province. Two media found most useful are (1) a solid alkaline, bile salt, tellurite, gelatin agar plate which acts as a highly selective medium for Vibrio cholerae (Monsur, 1961), and (2) a liquid alkanine, bile salt, peptone, tellurite medium which acts both as an enrichment and as a preservative fluid. With these two media the bacteriological diagnosis of cholera in rural areas can be approached from two different angles, viz:

A) on the spot diagnosis by a mobile team in the field, supplied with media sent out periodically from the base laboratory; and

B) central laboratory diagnosis of specimens posted in a preservative fluid medium; reliable results can be had even after a week or more.

Materials and Methods:

A) Field diagnosis by bacteriological technicians with minimum equipment. The technician is supplied from a base laboratory with prepared solid medium in flasks and with sterile petri dishes. Plates are poured in the field by melting the medium over boiling water. Before pouring plates the requisite quantity of potassium tellurite solution is added to the melted medium. The medium is highly inhibitory for other organisms, especially the anthracoids, and accidental contamination should not be troublesome. If plates are poured in small lots and used in a day or two, refrigeration is not necessary. The plates may be inoculated from rectal swabs directly or after enrichment in liquid medium. V. cholerae grows abundantly on the plate at room temperature and icubation is not essential. Because of the marked inhibitory effect of the medium on other organisms and the large numbers in which vibrios are generally present in the stool in early stages of cholera the growth is generally pure and abundant. It is usually possible to confirm the diagnosis serologically in the field by slide agglutination.

Previous workers have taken advantage of the fact that V. cholerae is aproteolytic organism for its isolation on gelatin-agar plates. On gelatin-agar each colony of V. cholerae develops a characteristic halo (Smith and Goodner, 1958). Useful though this gelatin-agar is for the isolation of V. cholerae, it is non-selective and is not inhibitory of competing organisms. During a study for a preservative fluid for V. cholerae it was found that sodium taurocholate and potassium tellurite greatly enhanced the chances of isolation of V. cholerae from material heavily contaminated with other organisms. The medium prepared by combining these ingredients with those of gelatin-agar plates is highly selective for V. cholerae. The organism grows abundantly on this plate and gives typical colony morphology, permitting easy identification (Monsur, 1961). The composition of the medium is as follows:

(more)

Trypticase	... ...	10.0	gms
Sodium chloride	... ...	10.0	gms
Sodium taurocholate	... ...	5.0	gms
Sodium carbonate	... ...	1.0	gms
Gelatin (Difco)	... ...	30.0	gms
Agar agar	... ...	15.0	gms
Distilled water	... ...	1	litre

The medium is sterilized by autoclaving and potassium tellurite is added to a final concentration of 0.5 to 1 x 10^{-5} (from one part in 200000 to one part in 100,000) before pouring plates.

B) Studies at the Cholera Research Laboratory show that at least two fluid media not only enrich but also preserve V. cholerae. One of these is alkaline-peptone water containing 10.0 gms trypticase, 10.0 gms sodium chloride and 1.0 gm sodium carbonate per litre, with a pH of about 9.2. The other is the same as the selective medium described above with omission of the two solidifying ageats, gelatin and agar. This medium is now used as a preservative for specimens coming from a distance.

The medium is distrubuted in 2 oz screw-capped bottles in 25 ml. quantities and potassium tellurite is added to the medium at the time of inoculation to a final concentration of 1 x 10^{-5}. Separate addition of potassium tellurite in the field can be avoided by pretreating the rectal swabs used for collecting specimens with the proper amount of potassium tellurite beforehand. These swabs, if kept well-dried, have given satisfactory results when used up to 3 months after preparation.

Observations:

A) The solid medium has a high pH of about 8.5; this, together with the sodium taurocholate and potassium tellurite, makes the medium highly inhibitory for most organisms. On the other hand V. cholerae grows very well producing characteristic colonies which attain a large size (3-4 mm.) in 48 hours with typical black centres and well-defined halos around each colony. As a rule, the colonies can easily be identified at 24 hours by their characteristic grayish appearance and surrounding halo. Quantitative studies with artificially infected stool suspensions have shown that the medium is capable of detecting a single viable V. cholerae in the inoculum, even though it be heavily contaminated with other faecal organisms.

B) The two liquid media have a pH of about 9.2. Experiments were carried out in which stool suspensions were added to a series of screw-capped bottles containing 25 ml quantities of these media. The bottles were then inoculated with serial dilutions of cultures of V. cholerae and a loopful from each of these inoculated bottles was plated out on different media at various intervals. The results of these experiments, when compared with the viable count of the original peptone water culture of V. cholerae used for inoculation, indicate that both media are capable of detecting even a single viable organism per ml of stool suspension. The purity of the final growth and the persistance of V. cholerae in the presence of other organisms seem to be better in the medium containing bile salts and potassium tellurite than in plain alkaline peptone water.

These two fluid media were tested with swabs from cholera cases. Rectal swabs from cases were put in screw-capped bottles containing the medium to be testested and the caps were screwed down tightly. In most cases two pairs and in some three pairs of bottles were used. One of each pair contained plain alkaline peptone water and the other had bile salt and potassium tellurite added. One loopful from each of the first pair of bottles was plated out some hours after incubation and, if this was negative, again the following morning. The remaining pair were kept at room temperature without being opened and were plated out on the 7th day. When a 3rd pair of bottles was available these were tested after 15 days. In a small number of cases where it was not possible to follow this routine rigidly, only the relevant information has been included in the analysis of data. During the period from May to December 1961, (a) 73 positive cultures were put in alkaline peptone water and kept at room temperature; 62 were positive and 11 negative on the 7th day; (b) 74 positive isolations, were put in alkaline bile salt-tellurite peptone water and kept at room temperature; 71 were positive and 3 were negative on the 7th day. There was then a loss of 11 in 73 in plain alkaline peptone water and 3 in 74 in alkaline bile salt tellurite peptone water. This result fits the experimental observations described above. The growth obtained from alkaline bile salt tellurite peptone was definitely purer than that obtained from plain alkaline peptone water. Alkaline bile salt tellurite peptone succedded in growing the organism after overnight incubation in every case in which the other medium was successful. From the point of view of rapidity of isolation, alkaline peptone water seems to give a slightly earlier positive result on a larger number of cases than does alkaline bile salt tellurite peptone, although in every case, the latter medium gave a similar positive or perhaps a better growth within 24 hours. Enrichment and preservation of the culture for a prolonged period (about a week or so) is best gotten with alkaline bile salt tellurite, whereas plain alkaline peptone water is preferred for a rapid diagnostic growth.

Conclusion:

Two additional tools are available, to be used singly or together for the field diagnosis of cholera. A mobile team can now make a spot diagnosis with plates and simultaneously send control specimens to base laboratories for confirmation. When field teams are not available, specimens from suspect out-breaks may be sent routinely by local lay representatives to base laboratories by post or otherwise. Under this system a base laboratory can serve an extensive area with reliable diagnoses on material sent in by non-technical representatives.

These methods offer new possibilities for the early diagnosis of out-breaks of cholera permitting health authorities to take effective steps early in an out-break before extensive spread occurs.

References:

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