ISOLATION OF CHOLERA VIBRIOS BY POSITIVE-RECOGNITION PLATING PROCEDURES

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Received for publication May 5, 1962

Lankford (J. Microbiol. Soc. Thailand **3**:10, 1959) and Smith et al. (J. Infectious Diseases **109**:31, 1961) emphasized recently the value of noninhibitory rather than inhibitory media for isolation of *Vibrio comma*. During temporary duty at the Pakistan-SEATO Cholera Research Laboratory, I compared the methods of these workers. Lankford recommended examination of plate cultures for presence of typical colonies by low-power stereoscopic oblique-light microscopy, and Smith et al. recognized the colonies by cloudy zones due to gelatinase activity on Taurocholate Gelatin Agar (TGA).

The plating media employed are given in Table 1. Alkaline Peptone Water (APW) for enrichment consisted of Peptone (Difco), 1.0%, plus NaCl, 1.0% (pH 8.5).

Rectal swabs, transported to the laboratory in phosphate-buffered saline (pH 8.0), were streaked directly on isolation media, incubated in APW medium for 8 hr, and replated. Plates were incubated for 18 to 24 hr at 35 C. Nutrient agar (NA) plates were examined by the oblique-light technique for typical chromatic greenish to red-bronze finely granular colonies of *V. comma* (Husain and Burrows, J. Infectious Diseases **99**:90, 1956). TGA plates were examined macroscopically for colonies, each surrounded by a cloudy zone. MacConkey Agar (MA) and SS plates were observed for lactose-negative colonies. Identification was confirmed by serological and biochemical tests (Burrows and Pollitzer, Bull. World Health Organization **18**:275, 1958) on three to five fishings from each plate showing suspect colonies.

A total of 50 rectal swab specimens was examined: 35 from cholera cases admitted to Mitford Hospital, 7 from field cases, and 8 from contacts.

Table 1 shows the high efficiency of isolation of *V. comma* after primary plating on TGA and NA media. No additional isolations resulted from APW enrichment. Nearly identical isolation rates were obtained. TGA yielded positive isolations from three specimens negative on NA, and NA yielded two isolations negative on TGA. None of the contact specimens was positive by either method. Thus, the two methods appear of equal efficiency.

Among the colonial fishings from TGA plate cultures of the 50 specimens, there were only six "nonvibrio" bacteria (all *Pseudomonas* sp.) and from NA only three (two *Pseudomonas* sp. and one *Alcaligenes faecalis*). More than 95% of all fishings from each medium were *V. comma*. Hence, both methods have definite differential value. Nonagglutinable vibrios were isolated from two specimens on TGA but were not recognized on NA.

Isolation of cholera vibrios on MA was much

TABLE 1. *Isolation of Vibrio comma on various media*

Medium	Specimen source	Number of specimens	Number of specimens positive	Per cent positive
Taurocholate Gelatin Agar (TGA)*	Cases	42	29	69.0
	Contacts	8	0	0.0
Nutrient Agar (NA)†	Cases	42	28	66.7
	Contacts	8	0	0.0
MacConkey Agar (MA)‡	Cases	42	10	23.8
	Contacts	8	0	0.0
SS Agar (SS)‡	Cases	42	0	0.0
	Contacts	8	0	0.0
All media	Cases	42	31	73.8
	Contacts	8	0	0.0

* Peptone (Difco), 1.0%; Yeast Extract, 0.1%; Sodium Taurocholate, 0.5%; NaCl, 1.0%; Gelatin (Difco), 3.0%; agar, 1.5% (pH 8.0). Modified after Smith et al., employing Peptone instead of Trypticase (BBL).

† Beef Extract (Difco), 0.3%; Peptone (Difco) 0.5%; NaCl, 0.5%; agar, 1.5% (pH 8.0).

‡ Dehydrated formulations (Difco).

less efficient; SS was completely inhibitory. No salmonella, shigella, or Arizona group cultures were detected.

The incidence of isolation of *V. comma* from patients in the present study was higher than that experienced by Smith et al. using TGA and other media during the 1959 Bangkok epidemic (21 positive of 80 "cholera" admissions). The findings of each of these studies demonstrate the value of simple noninhibitory media in the bacteriological diagnosis of cholera. Although the TGA and NA oblique-light techniques were of equal efficiency in the present study, the former possesses advantages for workers inexperienced with *V. comma* and for those laboratories lacking adequate microscopic equipment. Further, colonies on TGA in obliquely transmitted light are similar in appearance to those on NA. Hence, with one medium, colonies may be recognized by either a cloudy zone, by iridescence, or by both properties which together tend to reduce false positives. These advantages have been confirmed by examination of artificially infected stool specimens in the Division of Biologics Standards laboratory.

INSTABILITY OF CAPSULATION IN A STRAIN OF *KLEBSIELLA PNEUMONIAE*

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Received for publication May 7, 1962

This note describes a strain of *Klebsiella pneumoniae* which shows extreme variability of the mucoid form.

Strain 1.2 of *K. pneumoniae* was obtained from J. P. Duguid. This strain, derived through several serial subcultures from NCTC strain 5054, is of capsular serotype 1 (A). A derivative of the same NCTC strain was used by Ciuca et al. (Arch. roumaines pathol. exptl. microbiol. **18**:347, 1959), who reported it to be lysogenic for an inducible bacteriophage.

Highly sectored mucoid colonies of strain 1.2, showing opaque and translucent radial striations, were first observed on plates of doubly enriched minimal medium (Davis's minimal medium A + 10% nutrient broth) on which cells had been spread after penicillin screening. These highly sectored colonies had the appearance shown in Fig. 1.

Sectored colonies were picked and restreaked on seven media and incubated at 15, 25, 30, and 38 C.

Colonies were examined by obliquely transmitted light (Braun, *Bacterial Genetics*, p. 106, 1953). Sectoring developed independently of medium or incubation temperature. Restreaking of the sectoring colonies yielded not only further

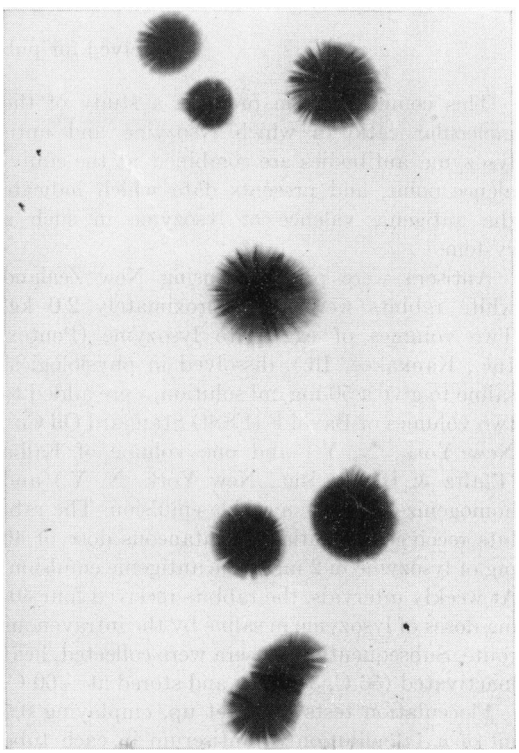

FIG. 1. *Colonies of the sectoring mucoid type on minimal medium A + 10% nutrient broth (doubly enriched minimal medium).*

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