AN EVALUATION OF ALKALINE PEPTONE WATER FOR ENRICHMENT OF *VIBRIO CHOLERAE* IN FECES

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INTRODUCTION

Alkaline peptone water (APW) is extensively used as an enrichment for *Vibrio cholerae* from feces. It has a simple composition and can also be used to maintain viability of the bacterium during specimen transport at 22-27°C (Barua, 1974). However, several investigators (Ahuja, *et al.*, 1951; Feeley, 1962; Sack and Barua, 1964; Gangarosa *et al.*, 1968) questioned the effectiveness of only using APW. They reported that direct streaking of fecal specimens on a plating medium gave a better isolation rate than enrichment in broth followed by plating. They also found that direct plating had an additional advantage over enrichment in that the associated faster growing commensal organisms had less opportunity to overgrow *V. cholerae*. Barua (1974) suggested that both direct plating and enrichment should be done at the same time when investigating acute cases of possible cholera.

The purpose of this study was to evaluate the effectiveness of APW enrichment and compare it with direct plating in the recovery of *V. cholerae* from fecal specimens.

This study was supported bv the Indonesian Ministry of Health and the Naval Medical Research and Development Command, Navv Department for Work Unit MR-041.09-002-5037. The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department of the Naval Service at large or that of the Indonesian Ministry of Health.

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MATERIALS AND METHODS

Clinical specimens: Rectal swab specimens (1262) were collected during April to December, 1981 from patients admitted to the Infectious Diseases Hospital (IDH), Jakarta with a diagnosis of gastroenteritis. The specimens were transported within 4 hours after collection to the laboratory in Amies transport medium.

Media: Alkaline peptone water (APW) enrichment broth was prepared from peptone, 10 g; sodium chloride, 10 g; and distilled water, 1 litre. The pH was adjusted to 8.4, 8 ml added to screw-cap tubes and sterilized at 121°C for 15 minutes. Thiosulfate citrate bile salts sucrose (TCBS) agar (Eiken Chemical Co., Ltd., Japan) was used as the plated medium.

Bacteriology: Upon arrival in the laboratory, the rectal swab specimens were directly streaked onto TCBS agar. The specimens were then put in APW enrichment broth, incubated for 6 to 8 hours at 37°C and TCBS inoculated with an aliquot from the APW culture. All TCBS cultures were incubated at 37°C for 18-20 hours. Flat, yellow colonies on TCBS resembling *V. cholerae* were picked for biochemical and serological identification. Additionally, other non-yellow colonies resembling *V. parahemolyticus* were also selected and identified.

RESULTS

V. cholerae biotype El Tor was isolated from 611 of 1262 fecal specimens; 535 by

both the TCBS-direct plate method and APW enrichment followed by plating; 15 by direct plating alone and 61 only after APW enrichment.

There were also 21 specimens containing isolable *V. parahemolyticus;* 14 by both direct plating and APW enrichment, 2 only by direct plating and 5 only by APW enrichment. Eleven specimens contained non-agglutinating vibrios (NAG); 7 by both direct plating and APW enrichment, 2 only by direct plating and 2 only by APW enrichment.

DISCUSSION

There was a significant difference ($p \leq .001$) between the direct plating method only and the APW enrichment followed by plating in the recovery of *V. cholerae* biotype El Tor from the stool specimens. APW enrichment gave four times more *V. cholerae* isolates than direct plating. This was somewhat different from the findings of others who claimed the superiority of the direct plate method (Ahuja *et al.*, 1951; Feeley, 1962; Gangarosa *et al.*, 1968).

Although the associated faster growing organisms may have the possibility of overgrowing *V. cholerae* in the APW enrichment fluid, we did not find that this occurred. The APW enrichment also seemed to be somewhat better for the isolation of *V. parahemolyticus* than the direct plate method. Unfortunately, the number isolated was too small to draw a conclusion. Likewise, the difference between the 2 methods was not statistically significant for either the isolation of *V. parahemolyticus* or NAG vibrios.

The results from this study supported Barua's (1974) contention that both direct plating and APW enrichment should be done to recover the maximum number of *V. cholerae* isolates from feces of cholera patients.

SUMMARY

A comparison was made to determine the sensitivity of direct inoculation of thiosulfate citrate bile salts agar (TCBS) and alkaline peptone water (APW) enrichment prior to direct inoculation of TCBS to culture *Vibrio cholerae* from feces of patients with gastroenteritis. *V. cholerae* was isolated from 611 feces specimens. Of those, 535 were isolated in TCBS and APW-TCBS, 15 in only TCBS and 61 in only APW-TCBS. *V. parahemolyticus* (21) and non-agglutinating vibrios (11) were also isolated but more often in direct inoculated TCBS than APW-TCBS cultures. Maximum isolation sensitivity of *V. cholerae* and *V. parahemolyticus* from feces is obtained by both direct inoculation of TCBS and enrichment in APW prior to TCBS inoculation.

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