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MOST RECOVERABLE METHODS FOR QUANTITATIVE DETECTION OF *Vibrio parahaemolyticus* IN SHRIMPS (*Penaeus monodon*)

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Three strains of *V. parahaemolyticus* were separately inoculated into black tiger shrimps and recoveries were evaluated by using nine standard procedures base from International Standard Organization (ISO), Australian/New Zealand Standard (AS/NZS), American Public Health Association (APHA) and Food and Agriculture Organization (FAO) incorporating most probable number (MPN) method and direct plating. The enrichment step in alkaline salt peptone water (ASPW) and 20 h direct plating on thiosulfate citrate bile salts sucrose (TCBS) agar gave the highest recovery (75.7%, 56.5% and 54.9% for strain I, strain II and strain III respectively) of *V. parahaemolyticus* in shrimps and followed by primary and secondary enrichment steps in alkaline peptone water (APW) using MPN method (71.6%, 53.5% and 46.4% respectively for three strains). *V. parahaemolyticus* was not recovered in salt polymyxin broth (SPB) using either direct plating or MPN methods. Three tube MPN method using ASPW as enrichment gave recoveries of 64.2%, 43.4% and 44.1% respectively for three strains while recoveries were 62.1%, 47.4% and 44.8% for APW (APHA). The enrichment in glucose salt teepol broth (GSTB) using MPN method resulted in 57.1%, 46.1% and 44.1% recoveries whereas it was 24%, 19.9% and 21.9% in saline glucose sodium dodecyl sulphate peptone water (GST). The recoveries in GST using direct plating were 50.4%, 47.2% and 40% respectively for three strains. ASPW is recommended as the most superior enrichments for enumeration of *V. parahaemolyticus* in tiger shrimps using direct plating method.

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INTRODUCTION

Vibrio parahaemolyticus is a major food borne pathogen that causes rejection of consignments of black tiger shrimps (*Penaeus monodon*) at the export market. There are various methods available for the detection of *V. parahaemolyticus* in food. Quantitative procedures either direct plating or most probable number (MPN) are occasionally used for the enumeration of *V. parahaemolyticus* (Klontz et al. 1993). It has been noted that different methods to detect *V. parahaemolyticus* have different sensitivities (EC 2001). There are different methods using different enrichments such as alkaline peptone water (APW), alkaline salt peptone water (ASPW), glucose salt teepol broth (GSTB), saline glucose sodium dodecyl sulphate peptone water (GST) etc. for selective isolation of *V. parahaemolyticus*. The objective of this study was to find the most recoverable method for quantitative detection of unstressed *V. parahaemolyticus* in shrimps.

MATERIALS AND METHODS

Comparison of methods

Brackish water black tiger shrimps (*Penaeus monodon*) were used in this study. Nine different standard methods for testing of *V. parahaemolyticus* were evaluated. Three strains of *V. parahaemolyticus* were used for the study. Strain I was a clinical O3:K6 strain which was *tdh* positive and *trh* negative while strain II was non pathogenic. ATCC 17802 was used as strain III. 25g or 50g muscle portion of shrimp was weighed into a sterile stomacher bag and blended in a stomacher with 225ml or 450 ml diluent described in each method at low speed for 60 seconds. The blended sample of shrimps constituted the 10^{-1} dilution and it was inoculated with *V. parahaemolyticus* at approximately 10^4 to 10^5 cfu/ml. This was mixed well in a stomacher at low speed for 60 seconds and serially diluted using 9 ml sterile diluent. Appropriate dilutions were plated in duplicate agar plates or MPN tubes were inoculated according to each method. Samples were analyzed separately following standard procedures (ISO 1990; Andrews 1992 (FAO); Aus/NZ Standard 1997; Charles & Angelo 2001 (APHA) for quantitative detection of *V. parahaemolyticus.*

RESULTS AND DISCUSSION

Data comparing methods of testing *V. parahaemolyticus* by quantitative recoveries are presented in Figure 1a, 1b and 1c. The performances of 20 h direct plating method in ASPW was superior to that of other methods and gave the highest recoveries of 75.7%, 56.5% and 54.9% for strain I, strain II and strain III respectively (ISO method). The recovery was significantly higher for strain I than for strain II and strain III. The next highest recoveries of 71.6%, 53.5% and 46.4% were obtained from primary and secondary enrichments in APW using MPN method for (AS/NZS) strain I strain II and III respectively.

V. parahaemolyticus was not recovered in SPB using either direct plating or MPN method. Three tube MPN method using ASPW as enrichment gave recoveries of 64.2%, 43.4% and 44.1% respectively for the three *V. parahaemolyticus* strains (ISO method), while recoveries were 62.1%, 47.4% and 44.8% for APW (APHA). The enrichment in GSTB using MPN method (FAO method) resulted in 57.1%, 46.1% and 44.1% recoveries whereas it was 24%, 19.9% and 21.9% in GST (ISO method). The recoveries in GST using direct plating (ISO method) were 50.4%, 47.2% and 40%.

ASPW enrichment broth with direct plating on TCBS appears to supply the most suitable environment for recovery of the organism. Nordstrom and DePaola (2003) found significantly higher detection of *tdh* positive *V. parahaemolyticus* in oyster samples using spread plating method (50.8%) compared to the streak-plating method (7.9%). The qualitative procedure (enrichment of 25-g portions of oyster homogenate, followed by isolation on TCBS) yielded a lower rate of pathogenic *V. parahaemolyticus* detection (9.6%) than the quantitative (0.1 g of oyster homogenate) spread plating procedure (12.8%) (DePaola et al. 2003). The concentration of PB used in SPB was 12.34 µg/ml or 100 IU/ml taking 1 mg as equivalent to 8100 IU, as with PB according to manufacturer's instructions. Ottaviani et al. (2003) also found that growth has inhibited in SPB for many

microorganisms. Karunasagar et al. (1986) mentioned that even in the absence of competing flora, SPB with 2.5 µg of Polymyxin per ml did not yield good recovery, suggesting the toxicity of Polymyxin to *V. parahaemolyticus*.

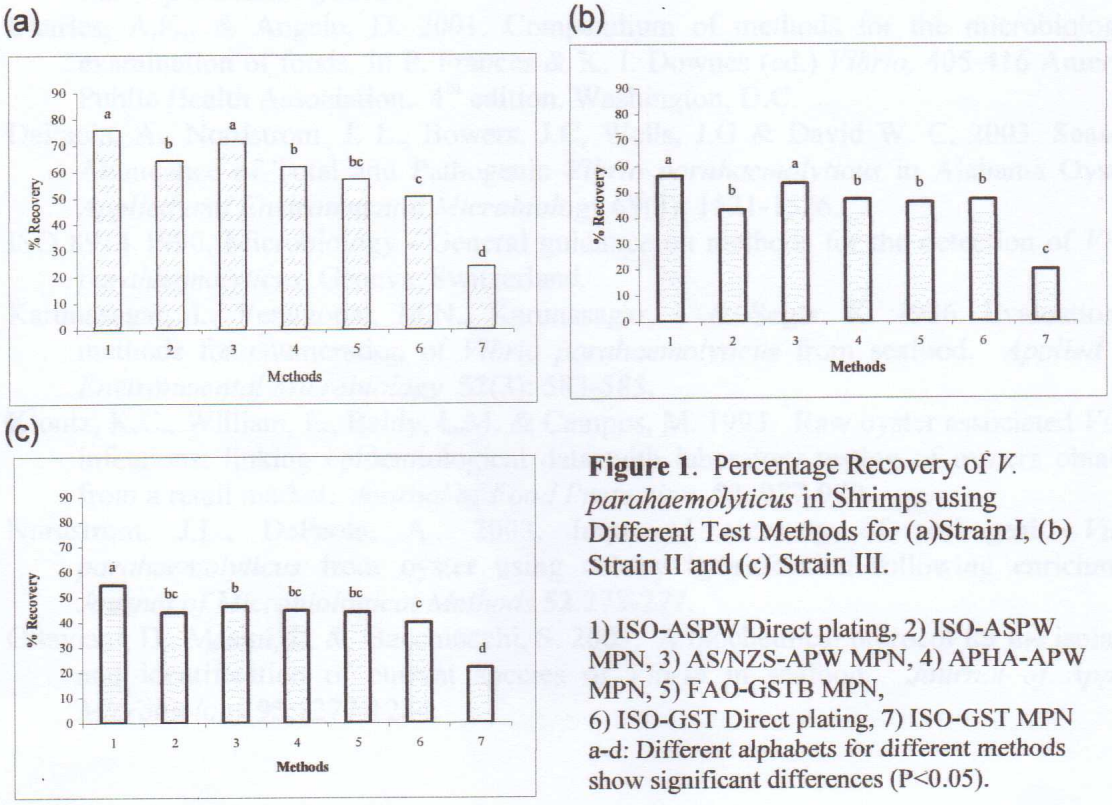

Figure 1. Percentage Recovery of *V. parahaemolyticus* in Shrimps using Different Test Methods for (a)Strain I, (b) Strain II and (c) Strain III.

1) ISO-ASPW Direct plating, 2) ISO-ASPW MPN, 3) AS/NZS-APW MPN, 4) APHA-APW MPN, 5) FAO-GSTB MPN, 6) ISO-GST Direct plating, 7) ISO-GST MPN
a-d: Different alphabets for different methods show significant differences ($P<0.05$).

CONCLUSIONS

Based on these results, ASPW enrichment broth with direct plating on TCBS agar was more efficient than other methods for recovery of unstressed *V. parahaemolyticus* from shrimps.

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