Development of a more sensitive and specific chromogenic agar for the differentiation and detection of *Vibrio* species: a focus study on *V. parahaemolyticus*

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Since 2000, foodborne infections in the US due to *Vibrio* species have shown an upward trend. In fact, the relative incidence rate has been doubled compared to the mean data from 1996 to 1998, while the rate of other major foodborne pathogens – *Campylobacter* spp, shiga toxin- producing *E. coli* O157, *Listeria* spp, and *Salmonella spp* – remained unchanged or lower.

Within the *Vibrio* genus, *V. parahaemolyticus* is responsible for the majority of these illnesses. Therefore, accurate differentiation among Vibrio species and detection of *V. parahaemolyticus* is critically important to ensure the safety of our food supply. Although molecular techniques are increasingly common, the culture method is considered the standard approach. In addition, the molecular approach is still cost-prohibitive in many clinical laboratories. Hence, we developed a chromogenic agar to provide better detection method for *V. parahaemolyticus*.

To test the sensitivity of the newly developed agar, twenty one strains representing diverse serotypes and source of origins were used. These strains were isolated from clinical, environmental and food sources, and included the O3:K6 pandemic clone. They were previously identified by FDA and CDC, and were further verified in our laboratory by using *tlh*-PCR.

In at least four separate trials, these strains were heavily plated on the chromogenic agar and thiosulfate-citrate-bile salts-sucrose (TCBS) agar, which is the recommended medium for culturing *V. parahaemolyticus*, followed by incubation at 35-37^{o}C for 24-48 h. Three strains (14.3%) did not grow optimally on TCBS, nonetheless exhibited green colonies if there was growth. Two strains (9.5%) did not show the characteristic cyan colonies on chromogenic agar.

The chromogenic agar also displays enhanced differential ability compared to TCBS. While *V. parahaemolyticus* were of cyan colonies, *V. cholerae* (*n=4*) and *V. algnolyticus* (*n=2*) yielded magenta and yellow colonies, respectively. In addition to these species, 25 other *Vibrio* species and non-*Vibrio* species, including closely related Gram-negative bacteria, other Gram positive bacteria, and fungi were also tested to determine the specificity.

Of the total 31 non-*V. parahaemolyticus* strains, 30 (96.8%) either did not grow on the chromogenic agar or exhibited other colony morphologies. The false positive strain, *V. proteolyticus*, yielded a dark hue of the expected cyan color. In addition to the excellent sensitivity and specificity, the mean recovery of the chromogenic agar was found to be 96.4% (*n=17*) relative to tryptic soy agar supplemented with 2% NaCl.

Thus, this chromogenic agar can be effectively used in routine testing for the presence of *V. parahaemolyticus*.