DETECTION OF SYNERGISTIC HEMOLYSIS BETWEEN *VIBRIO CHOLERAE* NON-O1 AND STAPHYLOCOCCAL BETA-LYSIN WITH MODIFIED CAMP TEST

Murad Lesmana, Decy Subekti, Periska Tjaniadi, Gary Pazzaglia, Sri Sugianingsih, and Triyani *

ABSTRAK

Suatu hemolisis sinergistik pada lempeng agar darah domba diperlihatkan oleh Vibrio cholerae *non-O1 dengan beta-lisin dari stafilokok. Sebanyak 167 galur (strains) non-O1 yang diuji dengan metode modifikasi CAMP menunjukkan suatu gambaran hemolisis berupa bulan sabit yang tipis; sedangkan* V. cholerae *biotipe El Tor dan biotipe klasik yang digunakan sebagai galur kontrol memperlihatkan reaksi CAMP berupa gambaran seperti sosis untuk biotipe El Tor dan negatif untuk biotipe klasik.*

Introduction

The test first described by Christie, Atkins, and Munch-Petersen in 1944 [1] for the presumptive identification of *Streptococcus agalactiae* (group B streptococci), and later termed by Murphy et al [2] as CAMP reaction after the initials of the surnames of its discoverers, based on synergistic hemolysis between *Staphylococcus aureus* beta-lysin and a group B streptococcal factor (CAMP-factor). Positive CAMP-like reactions were also reported in other organisms, including *Aeromonas* spp [3], *Listeria monocytogenes* [4], and *Clostridium perfringens* [5].

In 1985, Lesmana and Rockhill [6] reported the occurence of CAMP-like phenomenon between Staphylococcal beta-lysin and *Vibrio cholerae* O1 biotype El Tor. The test was performed using the standard streak method [7] in which a streak of beta-lysin producing *S.aureus* strain was made across the middle of a sheep blood agar plate and perpendicular to this, inoculum of strains of *V.cholerae* were streaked; the two streaks were carefully placed 1-2 mm apart. The positive reaction appeared as a crescent shaped zone of complete hemolysis at the juncture of the growth of the eltor vibrio and *S. aureus*. The test was negative in classical *V. cholerae*.

Although the CAMP test could be used to

* U.S. Naval Medical Research Unit No.2, Jakarta; and Center for Communicable Disease Research, National Research and Development, Indonesian Ministry of Health.

distinguish biotypes of *V. cholerae* (eltor and classical), and has been used by Kohler (8) to examine further species of the genus *Vibrio* for their ability to induce a CAMP reaction, the method has not been tested for examining *V. cholerae* non-O1.

It was the aim of this study to further evaluate the use of the CAMP test on *V. cholerae* non-O1 and to investigate whether the characteristic of CAMP-like reactions of *V. cholerae* non-O1 differs from those reported in *V. cholerae* O1 (6).

Materials and Methods

Bacterial strains. One hundred sixty-seven *V.cholerae* non-O1 strains used in this study were obtained from clinical specimens of diarrheal patients in Jakarta during 1988-1992. Isolation and identification of the organisms were performed according to the standard culture methods (8), involving conventional biochemical and serological tests. All media and reagents were purchased from Difco Laboratories, Detroit, MI.

Collection of stock cultures were maintained at -70°C in tryptic soy broth with 20% added glycerol. For testing, isolates were thawed and were subcultured onto thiosulfate citrate bile salts sucrose (TCBS) agar, incubated aerobically at 35°C for 18-20 h. *V. cholerae* non-O1 grew on TCBS with typical colonial morphology: yellow colonies, alkaline over acid reaction in Kligler iron agar (KIA), motile, ornithine-positive and did not agglutinating in polyvalent O1 antiserum, were subjected for the CAMP test.

Media for CAMP test. Blood agar medium was prepared from tryptic soy agar(Difco) added with 5% washed (x3 in normal saline) defibrinated sheep red blood cells. The medium was poured into 100 x 15 mm disposable petri dishes to a depth of approximately 3 mm.

CAMP test. With a marking pen, the bottom of the blood agar plate was divided equally into 16 quadrants. At each vertex, a beta-lysin producing *S. aureus* was stab-inoculated into the blood agar medium, followed with stab-inoculation of a strain of *V. cholerae* non-O1 within each quadrant, approximately 7-9 mm from the point of *S. aureus* inoculation (Figure 1). Plates were incubated in a candle jar at 35°C for 18 to 20 h. For control comparison, strains of *V. cholerae* O1 biotype El Tor and Classical were also included in the test. Positive CAMP-like reaction appeared as a distinct, elongated zones of complete synergistic hemolysis (perpendicular to the axis between colonies) at the periphery of normally circular zone of hemolysis demonstrated by *V. cholerae* strains.

Results

All 167 strains of *V. cholerae* non-O1 tested demonstrated positive CAMP-like reactions (Figure 1) which appeared as narrow crescent-shaped zones of complete synergistic hemolysis (1.00 mm at the widest point). The inner concave portion of the crescents appeared to lie on the edge of the wider circular zone of non-CAMP beta-hemolysis. In contrast, strains of *V. cholerae* biotype El Tor produced a broader zone of complete synergistic hemolysis (>2 mm) extending well beyond its periphery, of sausage-shaped, substantially overlapping the circular smaller zone of non-CAMP beta-hemolysis; whereas the Classical strains of *V. cholerae* were CAMP-negative, demonstrating no beta-hemolysis nor evidence of hemolytic synergism (Figure 1).

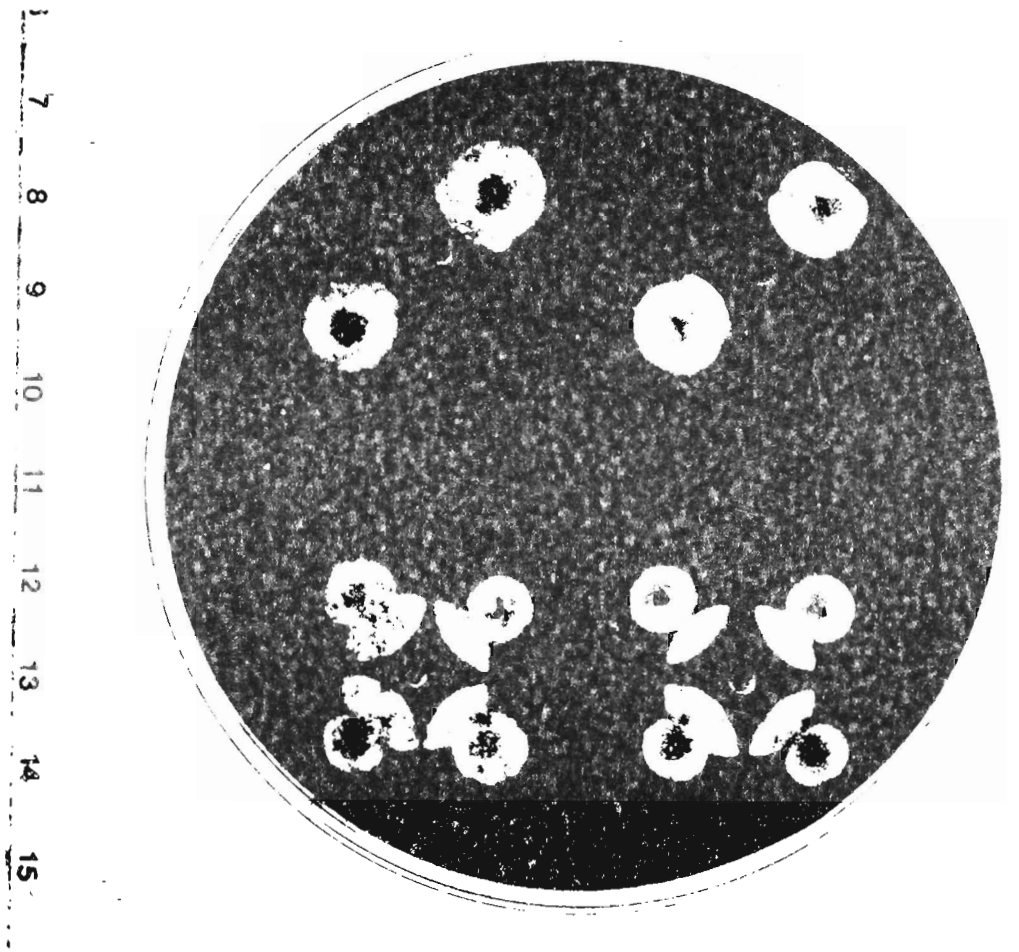

Figure 1. **CAMP reactions for *V. cholerae* strains. Left upper & lower quadrant: *V. cholerae* non-O1 (No.1, 3, first column; No.2, 4, second column); *V. cholerae* classical (No.2, 4, first column; No.1, 3, second column); right upper and lower quadrant: *V. cholerae* eltor; at the center of each quadrant is *S. aureus* (beta-hemolysin producing).**

Discussion

Unlike *V. cholerae* biotype El Tor which demonstrated a typical large crescent-shaped complete synergistic hemolysis as also reported previously [6], strains of *V. cholerae* non-O1 showed a small intermediate response of CAMP-like reactions. These characteristics distinguished non-O1 strains from the eltor biotype.

The modified CAMP test using stab inoculation instead of streak as described in the standard conventional method [7], allowed 16 strains to be tested compared to 10 strains in the latter system. In addition, stab-inoculation method produced a more distinct picture of positive and negative CAMP reaction. Gubash[5] reported that the synergistic hemolysis in the conventional CAMP test was affected by the inoculum size of the test organism; if the inoculum was too light, so that growth was less than confluent, the reaction tended to be very weak or small-sized. This was not found in the modified CAMP test using stab-inoculation technique. The stab method almost always produced uniform size of growth on blood agar plate.

As also reported by Gubash [5], we found that for the best results, test plates should be immediately read or interpreted following 18-20 h incubation. If the plates were kept at room temperature or at 2-8°C or incubated beyond the usual incubation time (18-20 h), reading or interpretation of the test results was difficult due to "hot-cold" lysis of the sheep erythrocytes. Differences in the performance of the media may occur with different batches of sheep blood agar. Variations in the production of the crescent-shaped lysis by the same strain at different times and different batches of blood agar might be due to variations in the production of the responsible factor with time, or to other as yet unexplained influences [5]. Lesmana and Rockhill [6] recommended that test plates should be incubated in the candle jar. They reported that only half (52%) of the CAMP-positive *V. cholerae*, which the test plates were incubated aerobically produced strong CAMP reaction, 15% were moderately positive and the remaining 33% were CAMP-negative. Not only was there a significant difference of the results between aerobic and CO_2 incubation, there was also a difference in the size of the zone of synergistic hemolysis. We did not perform aerobic vs CO_2 incubation comparison as it has been established that aerobic incubation led to 33% false negative CAMP reaction [6].

Recently, there were reports on the newly emerged *V. cholerae* serogroup O139 ("Bengal" strain) which has been associated with large epidemics of cholera-like diarrhea in india [10] and Bangladesh [11], and has the potential to become a new pandemic cholera strain. In contrast to local *V. cholerae* non-O1 strains which produced narrow crescent-shape of synergistic hemolysis, CAMP test which was performed on 5 strains of *V. cholerae* O139 reportedly to show that all were strongly positive, yielding reactions identical to those produced by *V. cholerae* biotype El Tor strains

(large sausage-shaped zone of synergistic hemolysis) [12].

Further study with the CAMP test on additional O139 strains may demonstrate the test to be an inexpensive and reliable adjunct for distinguishing this new *V. cholerae* non-O1 serotype which does not agglutinate in O1 antiserum, but yield a strong, large sausage-shaped synergistic hemolysis typical for eltor strains, from other *V. cholerae* strains (serogroup O1 and other non-O1).

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