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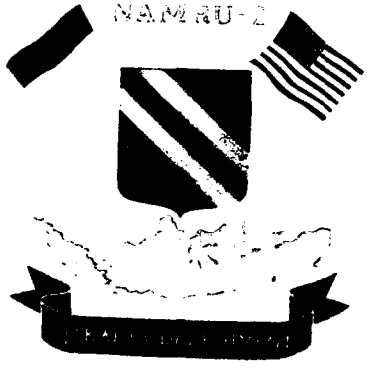

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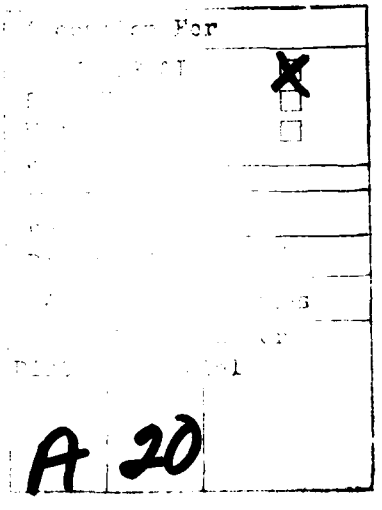

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SLIDE COAGGLUTINATION FOR *SALMONELLA TYPHI* ANTIGENS IN BROTHS INOCULATED WITH FECES FROM TYPHOID FEVER PATIENTS

ROBERT C. ROCKHILL, LARRY W. RUMANS* and MURAD LESMANA

U.S. Naval Medical Research Unit No. 2, Jakarta, Detachment, APO San Francisco, California 96356. *Internal Medicine Associates, Topeka, Kansas 66606.

INTRODUCTION

The method of bacterial coagglutination was developed by Kronvall for serotyping *Streptococcus pneumoniae* (1973). Since that time, it has been used to successfully identify *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *N. meningitidis* and Lancefield groups A, B, D and G streptococci (Danielsson and Kronvall, 1974; Edwards and Larson, 1974; Thirumoorthi and Dajani, 1979). The method can also be used to detect homologous soluble bacterial antigens in body fluids (Suksanong and Dajani, 1977). This coagglutination method has been applied in the detection of *S. typhi* D, Vi and d antigens from the urine of patients with suspected typhoid fever and have been found to be reliable, allowing early presumptive diagnosis of typhoid fever (Lesmana *et al.*, 1980).

The present study was designed to determine if antigens of *S. typhi* could be detected in an enrichment broth before either bacteriologic or serologic confirmation of typhoid fever was available.

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MATERIALS AND METHODS

Patients: Individuals suspected of having typhoid fever were interviewed and examined upon admission to the Special Hospital for Infectious Diseases in Jakarta, Indonesia. Minimal criteria required for clinical diagnosis of typhoid fever were history of fever for at least seven days, an admission temperature $\geq 38.5°$ C, and at least two of the following: abdominal pain, constipation, mental confusion or delirium, hepatomegaly or splenomegaly. Specimens were obtained from 88 consecutive patients meeting the clinical criteria for typhoid fever. Fifty healthy individuals served as a control group.

Specimen collection: Ten ml of blood were withdrawn from each patient and 3 ml added to 10 ml of 10% oxgall (Oxoid) in water medium. The remainder was saved for Widal serology. Four rectal swab specimens were obtained, 2 placed in Amies transport medium and one each directly into 3 ml of MSB (Hobbs and Allison, 1945) and DSB (Raj, 1966) (Oxoid) while at the patient's bedside. Blood specimens for culture were collected daily until the patient became afebrile. Acute and convalescent sera were drawn at least two weeks apart for the determination of *Salmonella* O antibody levels by Widal agglutination. All specimens were transported to the laboratory within 1-2 hours after collection and the blood, MSB and DSB cultures incubated at 37°C. Rectal swabs were collected from 50 healthy employees of NAMRU-2 and

the Infectious Disease Hospital and cultured the same way as the patients.

Bacteriological isolation: MacConkey (MAC), desoxycholate citrate lactose sucrose (DCLS) and *Salmonella-Shigella* (SS) agar plates were inoculated with 1 swab from the Amies medium. The swab was then put into 5 ml MSB. The second swab cultures were incubated 18-24 hours at 37° C and aliquots from the MSB and DSB used to inoculate MAC, DCLS and SS media which were then incubated overnight.

Salmonella-like colonies from the plated media were subcultured to Kligler iron agar (KIA), lysine iron agar, motility indole ornithine medium and urea agar slants. Growth was taken from the KIA, in the 4 tube screen that gave a presumptive *Salmonella* biochemical profile and used for serogrouping to confirm *S. typhi* (Ewing and Martin, 1974).

Blood cultures were subcultured daily to MAC, DCLS and SS and any suspicious Salmonella-like colonies identified with the 4 tube screen and serogrouping. All agar media came from a commercial source (Difco).

Stabilized Staphylococcus preparation: The Cowan 1 strain *Staphylococcus* was grown confluently on Mueller-Hinton agar medium for 18 hours at 37°C without added carbon dioxide. Cells were harvested by emulsifying in 5 ml phosphate buffered saline (PBS: 0.03M phosphate, 0.12 M NaCl, pH 7.2) with a glass rod. Washing and heat treatment were performed according to the method of Edwards and Larson (1974). Briefly, the harvest was washed 3 times with PBS suspended in 0.5% formaldehyde in PBS for 3 hours, again washed 3 times with PBS and made up to a final 10% suspension in PBS. The suspension was then heated at 80°C for 1 hour with constant stirring, washed 3 times in PBS and stored at a 10% stabilized suspension in PBS at 5°C until coupled to the antiserum.

Staphylococcus-antibody reagent: The method of Kronvall was used to coat the cells (1973). One ml of the 10% *Staphylococcus* preparation was mixed with 0.1 ml each of monovalent *Salmonella* D, Vi and d antisera (BBL). The individual mixtures were left at room temperature for 3 hours and gently agitated at ½ hour intervals. Prior to use, 1 ml of the sensitized suspension was diluted with 9 ml PBS. This suspension was used as the final coagglutination reagent and called D-COAG, Vi-COAG and d-COAG.

Coagglutination procedure: Aliquots were withdrawn from the 3 and 5 ml MSB and DSB enrichment cultures 4 and 18 hours after inoculation with the rectal swabs. One drop (50 µl) each was added to 4 ringed areas on a glass slide. Equal volumes of D-, Vi-, and d-COAG were then added to each of the first 3 drops and stablized *Staphylococcus* only to the fourth drop to serve as a negative control. The drops were mixed with an applicator stick, the slide rotated by hand for a maximum of 5 minutes and the time noted when visible agglutination appeared. The magnitude of the agglutination reaction was also noted and judged as 1 + (weak), 2 + (moderate), 3 + (good) or 4 + (strong).

RESULTS

Salmonella typhi was isolated from the first blood and/or Amies rectal swab cultures taken on admission from 60 out of 88 patients. The bacterium was also isolated from 60 MSB and 50 DSB media inoculated at the bedside from the same 60 patients.

The D-, Vi-, and d-COAG reactions were optimally positive, giving a 2-3 + reaction in 0.5-1 minute when 5 ml of MSB or DSB broth were used that had been incubated for 18 hours. However, only a 1 + reaction occurred in 2-3 minutes at the early testing period after 4 hour incubation. When the 3 ml volume of

the 2 media was tested, optimum agglutination occurred following the 4 hour incubation period and extending the incubation period to 18 hours did not noticeably increase the intensity of agglutination. All agglutination reactions with the 3 ml volumes were distinct giving 2-3 + reactions in 0.5-1 minute. There were no instances when the 3 COAG reagents failed to react with the same positive culture. The negative controls were never equivocal but in 10 instances testing with the DSB resulted in a very light granularity following 18 hour incubation. However, there was a definite difference between the minimal granularity observed and the true appearance of coagglutination. No such granularity was apparent at the 4 hour testing period.

Positive coagglutination also occurred with inoculated MSB and DSB from 16 patients with negative bacteriological findings. Twelve of these patients later developed four-fold or greater titers of *Salmonella* O antibody. The remaining 12 patients from whom the bacterium was not isolated and the coagglutination test was negative also failed to demonstrate seroconversion. None of the negative control group gave positive MSB or DSB coagglutination results.

DISCUSSION

Typhoid fever is endemic in Indonesia and other developing nations of the world (Anderson *et al.*, 1976). The lack of appropriate early treatment may be associated with high mortality due to severe toxemia, gastrointestinal hemorrhage and/or perforation. Prompt treatment significantly shortens the febrile course and reduces mortality (Woodward and Smadel, 1964; Butler *et al.*, 1977). Since the introduction of effective chemotherapeutics in the late 1940's, e.g., chloramphenicol, mortality from typhoid fever has been significantly reduced. However, in developing countries with substandard nutrition and poor medical facilities, the mortality in unselected hospitalized patients may exceed 15% in spite of the absence of chloramphenicol resistance.

Currently, two methods form the basis for the confirmation of typhoid fever in an individual with a compatible clinical syndrome. Direct bacteriologic confirmation resulting from the isolation of *S. typhi* from clinical sources represents the preferred means. This method is frequently not available in areas of high endemicity, however, due to economic and logistic reasons. The Widal test is dependent upon the demonstration of a four-fold or greater rise in agglutinins to both O and H antigens of *S. typhi* between acute and convalescent specimens. However, the convalescent specimen is rarely obtained. Single specimens obtained during the acute phase of illness are frequently collected and reliance on the results of a single Widal test is common even though the reliability of this method may be disputed. Certainly, the Widal test, by itself, can never provide more than a presumptive diagnosis of typhoid (Woodward and Smadel, 1964).

The standard approach for hospitalized patients with suspected typhoid in Indonesia and elsewhere in Southeast Asia is to obtain blood and stool cultures and administer specific therapy without waiting for culture results. Patients seen in satellite clinics infrequently have cultures performed and are often treated with inappropriate antibiotics given for an inadequate duration resulting in increased morbidity and ultimate hospitalization. Since the drug of choice for typhoid fever (chloramphenicol) carries a risk of potentially serious toxicity, even though it is rarely observed, the need for a rapid, sensitive, and specific confirmatory test for typhoid would be of great assistance to the clinician faced with an uncertain diagnosis. Similarly the exclusion of typhoid fever from diagnostic consideration is equally important since the

clinical presentation of other etiologic agents may resemble the appearance of typhoid fever.

The three coagglutination reagents used in this study successfully identified one hundred percent of the MSB and DSB fecal cultures incubated only 4 hours after inoculation from which *S. typhi* was isolated. In addition, 16 patients with a compatible typhoid fever clinical syndrome also gave positive COAG results when the bacterium was not cultured. Twelve of these later demonstrated seroconversion to *Salmonella* O antigen. Widal agglutination performed during the acute phase of illness were non-diagnostic from 11 of these 12 patients. Therefore, these patients with typhoid fever were successfully identified by the method of bacterial coagglutination long before they would have been confirmed by other means.

The coagglutination test, as demonstrated in this study, has potential to detect major *S. typhi* characterizing antigens, D, Vi and d in MSB and DSB enrichment media inoculated with feces from patients with typhoid fever.

SUMMARY

Salmonella typhi antigens D, Vi and d were readily detected, by slide coagglutination, in mannitol selenite (MSB) and dulcitol selenite (DSB), *Salmonella* enrichment broths 4 hours after inoculation with feces from 60 patients with bacteriologically confirmed typhoid fever. Positive coagglutination also occurred using MSB and DSB inoculated with fecal specimens obtained from 16 patients from whom *S. typhi* was not cultured. Twelve of these later seroconverted to *Salmonella* O antigen. None of the MSB or DSB inoculated with feces from 50 healthy control subjects, gave a positive coagglutination test. The coagglutination method appears to have potential as a rapid test for the detection of antigens of *S. typhi* in MSB and DSB broths inoculated with feces from patients with suspected typhoid fever.

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