Rapid diagnosis of typhoid fever by co-agglutination in an Indian hospital

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Summary. Detection of *Salmonella typhi* infection by a co-agglutination assay for specific O, H and Vi antigens and by blood culture were compared for 110 patients with suspected typhoid fever. Blood cultures were positive for *S. typhi* in 25.5 % of patients. Co-agglutination tests with patients' serum and with blood culture supernates gave positive results in 70.9 % and 67.3 % of cases respectively. *S. typhi* antigens Hd and O9 were detected in patients' serum by co-agglutination in 96.4 % of blood culture-positive, and 62.2 % of blood culture-negative patients. Co-agglutination results were uniformly negative with serum samples from a control group of 50 healthy individuals, 20 patients with febrile non-typhoid infectious disease and 20 patients with non-infectious febrile disease. Of the 25 patients with suspected typhoid fever who had not received prior antibiotic treatment, 88 % yielded positive blood cultures and 96 % gave positive results in serum co-agglutination tests. By contrast, of the 85 patients who had received prior antibiotics, only 7 % yielded positive blood cultures, but 63.5 % gave positive results in serum co-agglutination tests. Co-agglutination tests with serum offer a simple, rapid, sensitive, specific and economical method for the early diagnosis of typhoid fever.

Introduction

Typhoid fever is rare in developed countries,¹ but remains one of the most prevalent acute infectious diseases in the developing world.² The disease presents a clinical dilemma because of its varied manifestations and serious complications. Early rapid detection and identification of the aetiological agent, *Salmonella typhi*, is essential in diagnosis and for treatment to reduce morbidity and mortality. Laboratory confirmation of the clinical diagnosis depends mainly upon blood culture, a time-consuming and costly technique. Serological diagnosis is of limited value³ and formal demonstration of a rising antibody titre invariably delays confirmation well beyond any reasonable time for initiation of therapy.

In this study, *S. typhi*-specific antigens were sought in co-agglutination tests with serum samples and blood-culture supernates and the results were compared with those of conventional blood culture.

Materials and methods

The study group comprised 110 patients diagnosed clinically as suffering from typhoid fever. Diagnosis was based on the presence of unremitting fever for

> 5 days, systemic toxicity, fatigue, abdominal symptoms, transient rash and anorexia with a palpable spleen, especially in the second week of illness. A history was taken, including details of antibacterial therapy and duration of symptoms.

The control groups comprised: 50 healthy volunteers with no history of enteric fever or TAB vaccination in the previous year; 20 patients with other non-typhoid infectious disease; and 20 patients with febrile non-infectious disease (table I).

Bacteriological and serological methods

Routine blood culture with citrated glucose broth⁴ was done for all 110 patients with suspected typhoid fever. Blood (5 ml) was collected aseptically by venepuncture and inoculated into 50 ml of nutrient broth containing glucose 0.5 % and sodium citrate 0.2 %. The blood cultures were incubated at 37°C for 14 days and inspected daily for growth.

Co-agglutination⁵ tests were performed on serum samples, and on blood culture supernates after incubation for 24 h. One loopful of serum or blood culture supernate was placed on one half of a clean glass slide and another drop on the other half. A loopful of the appropriate co-agglutination reagent was placed on the first half, and another of normal rabbit serum (control) co-agglutination reagent was added to the other half. The pairs of drops were mixed

Table I. Control groups

Control groups	Number of cases	Diagnoses (number of cases)
1. Healthy volunteers	50	Normal healthy individuals
2. Non-typhoid infective fever*	20	<i>Staphylococcus aureus</i> (7) <i>Escherichia coli</i> (9) <i>Klebsiella</i> spp. (2) Non- <i>S. typhi</i> salmonellae (2)
3. Non infectious febrile illness†	20	Rheumatic fever (10) Ankylosing spondylitis (3) SLE (2) Rheumatoid arthritis (5)

* Pathogens identified by standard procedure from blood culture.

† Diagnosed on the basis of clinical and laboratory findings.

Table II. Co-agglutination and blood culture positivity in relation to duration of fever on admission and prior antibacterial therapy

Antibacterial therapy before blood culture	Duration of illness (weeks)	Number of patients	Total number (%) of patients	Number (%) that were blood-culture		Number (%) that were serum co-agglutination	
				positive	negative	positive	negative
No antibacterial therapy	1	24	25 (22.7)	21	3	23	1
	2	1		1	0	1	0
	3	0		0	0	0	0
	4	0		0	0	0	0
Antibacterial therapy	1	10	85 (77.3)	4	6	9	2
	2	60		2	58	44	16
	3	12		0	12	2	10
	4	3		0	3	—	3
Total			110	28	82	78	32

and the slide was rotated by hand for *c.* 1–2 min. Any visible clumping was noted. Agglutination with the O9 and d reagents and no reaction with the normal rabbit serum reagent was interpreted as a positive test. Negative results were indicated by absence of reaction with the test and control reagents.

Preparation of co-agglutination reagent

A confluent growth of *Staphylococcus aureus* (Cowan 1) on Mueller-Hinton agar, incubated for 24 h at 37°C, was emulsified in phosphate-buffered saline (PBS), pH 7.3, and washed five times with PBS containing formaldehyde 0.5%. After incubation for 3 h at room temperature, the suspension was washed three times with PBS and heated at 80°C for 1 h with constant stirring. The heated suspension was washed three times with PBS, diluted 1 in 10 in PBS and stored overnight at 4°C. One ml of the suspension was mixed with 0.1 ml of antiserum. The antisera were: O9, Hd and Vi salmonella antisera (National Salmonella and Escherichia Centre, Kasauli) and normal rabbit serum. After incubation for 3 h at room temperature, with gentle shaking at 30-min intervals, these working reagents were stored at 4°C.

Results

Blood cultures gave positive results for only 28 (25.5%) of the 110 patients; 25 (89.3%) of these 28 were tested in the first week of illness (table II). Of the 25 patients with no history of antibacterial therapy, 22 (88%) gave blood cultures positive for *S. typhi* and 24 (96%) gave positive serum co-agglutination results. Of the 85 patients who had received antibacterial agents, 54 (63.5%) gave positive co-agglutination results and only 6 (7.1%) were blood-culture positive.

Serum co-agglutination tests gave positive results in 78 (70.9%) of the patients. Of these, 85.3% were tested in the first week of illness, 77% in the second week, 16.7% in the third week and none in the fourth week. For the 28 blood culture-positive patients, serum co-agglutination tests gave positive results in 27 (96.4%) (table III). Of the 82 clinically diagnosed but blood-culture-negative patients, 51 (62.2%) gave positive results in serum co-agglutination tests.

Blood-culture supernates gave positive results in co-agglutination tests for 74 (67.3%) patients. For the 28 blood culture-positive patients, 26 (92.9%) gave positive co-agglutination results with 24-h blood-culture supernates. Of 82 blood culture-negative patients, 48

Table III. Detection of *S. typhi* by co-agglutination tests with blood culture supernates and serum

Blood culture result	Number of patients	Number of patients that gave positive results in co-agglutination tests with					
		Blood culture supernate			serum		
		O9	d	Vi	O9	d	Vi
Positive	28	26 (92.9)	26 (92.9)	25 (89.3)	27 (96.4)	27 (96.4)	26 (92.9)
Negative	82	48 (58.5)	48 (58.5)	48 (58.5)	51 (62.2)	51 (62.2)	50 (60.9)
Total	110	74 (67.3)	74 (67.3)	73 (66.4)	78 (70.9)	78 (70.9)	76 (69.1)

Figures in parentheses indicate percentages.

(58.5%) gave positive co-agglutination results with 24-h blood-culture supernates (table III).

Co-agglutination tests gave uniformly negative results with the 90 serum samples from the three control groups.

Discussion

Typhoid fever is a serious public health problem in India. Rapid laboratory diagnosis is essential for early therapy to reduce morbidity and mortality. However, isolation of *S. typhi* can be difficult, particularly when patients present late in the course of the disease, or after inadequate or inappropriate antibiotic therapy, as frequently happens in India.

In this study, the isolation rate of *S. typhi* from blood cultures was low; only 28 (25.5%) of 110 clinically diagnosed patients (table II) were culture-positive. This low isolation rate may be due to prior antibacterial therapy or late presentation. The majority of patients in this study presented 2 weeks after onset of symptoms and after antibiotic therapy. Similar low isolation rates have been reported by various Indian authors.^{6,7}

In contrast, serum co-agglutination tests gave positive results in 96.4% of the blood culture-positive patients and 62% of the blood culture-negative patients. John *et al.*,⁸ in a study in Vellore, have also reported that serum co-agglutination tests gave positive results in 97% of blood culture-positive and 85% of blood culture-negative patients with clinically diagnosed typhoid fever.

Co-agglutination tests with blood-culture supernates in this study gave positive results with 67.3% of patients. Of the 28 blood culture-positive supernates, 92.2% were co-agglutination-positive. Even among samples from 82 blood culture-negative patients, the co-agglutination test gave positive results in 58.5% of cases. Clearly, non-viable or antibiotic-suppressed

organisms were present in many of these negative blood cultures.

Co-agglutination tests with serum showed maximum positivity for those patients in the first week of illness (85.3%) reducing through the second and third weeks to 0% in the fourth week of illness.

The effects of prior antibacterial therapy on the results of serum co-agglutination tests and blood culture were significant. Of 25 cases that had not received antibacterial agents before sample collection, 24 (96%) gave positive results in serum co-agglutination tests and 88% were blood culture-positive (table II). In patients who had already taken antibacterial agents, 63.5% were co-agglutination-positive whereas only 7.1% gave positive results by conventional blood culture. Clearly the results of blood culture are more seriously affected than those of the co-agglutination test by the prior intake of antibacterial agents ($\chi^2 = 66.76$; $p < 0.001$).

In the present study, co-agglutination has shown promise as a rapid, sensitive and specific method for the detection of *S. typhi* infection. The test is economical and the reagents maintained their activity for 3 months when kept at 4°C.

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