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Comparison of methods for the diagnosis of typhoid fever

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Abstract

Over five years the Bactec radiometric blood culture method yielded *Salmonella typhi* in 41 of 45 confirmed cases of typhoid fever, 90% of which were from the first culture set taken. Blood clot culture was positive in 18 (41%) of 44 confirmed cases and stool culture in 24 (59%) of 41. The yield from 2189 Widal clot cultures was only 0·03%. There were 68 positive results in 2258 unpaired Widal tests: 23 of them were falsely positive and 13 falsely negative, but in 11 out of 68 cases the Widal was the only positive laboratory test. It is concluded that routine clot culture is not cost effective if a sensitive blood culture method is used, and that the Widal test is useful only in selected patients.

The incidence of typhoid fever in Hong Kong is about 4·6 per 100 000 of the population.[1] The diagnosis is usually confirmed by culture of blood, blood clot, or stool, together with the results of the Widal agglutination test. The usefulness of the Widal test has been questioned.[2–7] In most cases only a single titre is obtained. Repeating the test may increase the number of positive results,[4] but there is often neither an initial increase nor a rise in titres, even in culture positive typhoid fever. Furthermore, the results of the test are invalidated by a previous history of vaccination or typhoid fever.[8] This study examines the relative contributions of each of these four laboratory investigations to the diagnosis of typhoid fever in Hong Kong over a five year period.

Methods

Results of Widal agglutination tests and *Salmonella typhi* cultures performed between April 1984 and December 1989 were obtained by retrospective analysis of laboratory records. There were very few paratyphoid infections, which were excluded from the study. Patients with a single positive Widal test with a previous history of typhoid fever or vaccination were also excluded. True positive, false positive, and false negative Widal results were determined by reviewing patient records, culture results, and notifiable disease returns.

Widal agglutination was performed using Wellcome reagents (Wellcome Diagnostics, Dartford) containing O and H antigens of *S typhi*. A dilution series of 1 in 50 to 1 in 400 of serum in 0·85% saline was made, and 0·3 ml of each dilution was added to 0·3 ml of antigen suspension. Positive and negative serum controls were included. The tubes were incubated in a 56°C water bath for 18 hours before reading. A titre of > 1/100 to either antigen in a single serum specimen was taken to be indicative of typhoid fever.[9] The laboratory participates in the external quality control programme of the Royal College of Pathologists of Australia and achieves consistently good results for Widal tests.

After removal of serum for the Widal agglutination test the blood clot was placed in 10 ml of 0·5% bile salt broth (Oxoid, Basingstoke) for culture. After two days of incubation at 37°C the broth was subcultured to DCA and MacConkey agar plates (Oxoid). Negative cultures were terminally subcultured on day 6.

Blood cultures were made with the Bactec 460 radiometric system (Johnson Laboratories, Towson, Maryland), using the 6B aerobic and 7D anaerobic broth media. The blood:broth ratio was about 1:6. The cultures were examined daily for six days before being discarded.

Stool specimens were plated directly on DCA and MacConkey agar (Oxoid) and inoculated into Selenite F broth (Oxoid) for enrichment culture.

Isolates were confirmed to be *S typhi* by their API20E profile (Anylab Products, Plainview, California) and by agglutination with salmonella agglutinating sera (Wellcome).

Results

There were 63 confirmed cases of typoid fever during the study. In 54 the diagnosis was confirmed by culture: 45 of these were blood positive, 18 clot positive, and 24 stool positive (table 1). In those patients in whom blood culture was performed, 45 of 49 (92%) yielded *S typhi*. The yield for blood clot culture was 18 of 44 (41%) and for stool culture 24 of 41 (59%). Of the 45 blood culture positive cases, 41 (90%) were diagnosed on the first culture performed. The modal time for the blood cultures to become positive was two days.

Over the same period 2258 assessable single Widal agglutination tests were performed. Sixty eight were positive (0·03%), of which 23 were false positive. There were 13 culture positive cases of typhoid fever in whom the Widal test was falsely negative. Eleven cases

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Table 1 Results of blood, stool, and clot culture and Widal agglutination in patients with bacteriologically confirmed typhoid fever

		Widal test								
		Positive			Negative			Not done		
		Stool culture								
Blood culture	Clot culture	+	−	Not done	+	−	Not done	+	−	Not done
+	−	6	2	1	2	1	1	1		
+	−	6	9	4	2		2		1	
+	ND		1	2	1			1	1	1
−	+		1	1		1				
−	−				1					
ND	+			1						
ND	−				1					
ND	ND				1			2		

ND = Not done.

Table 2 Results of single Widal agglutination titres

Clinical interpretation	Number (%)
True positive results:	45 (2·0)
Culture positive typhoid fever	34
Culture negative typhoid fever	11
False positive results:	23 (1·0)
Fever, non-typhoid	20
Salmonellosis	2
Liver disease	1
True negative results	2177 (97·0)
False negative results (culture)	13 (0·6)

Table 3 Forty two blood culture-blood clot culture pairs

Blood culture	Blood clot culture	Number (%)
+	+	14 (33)
+	−	25 (60)
−	−	3 (7)

were confirmed by Widal test alone. These results (table 2) give a sensitivity for the Widal test of 78%, a specificity of 99%, a positive predictive value of 66% and a negative predictive value of 99%.

There were 2189 blood clot cultures, of which 18 yielded *S typhi* (0·82%). In 42 patients both whole blood and blood clot cultures were performed (table 3). The blood culture was positive in 39 (93%), but the clot culture was positive in only 17 (40%). In three cases the clot culture was the only specimen which yielded *S typhi*, but in only one of these was the Widal test negative.

Discussion

In this study we obtained an isolation rate of 92% for blood culture with the Bactec 460 system using a blood:broth ratio of 1:6. This yield is comparable with those reported by other workers using 10% Oxgall.[10] The Bactec 460 media contain liquoid which probably contributed to the good recovery rates.[11 12] Some workers claim that bone marrow culture provides the highest yield of *S typhi* in the diagnosis of typhoid fever,[13–15] but the detection rate of 84–92% is no better than our result for blood culture. The invasive nature of bone marrow culture precludes its use as a routine method.

In our study clot culture had a yield of only 41%. In one out of 65 laboratory confirmed cases, the clot culture was the only test which gave a positive result. Some workers have found that the addition of streptokinase increases the yield of clot cultures, but others have failed to confirm this.[16] We did not use streptokinase, but many of our clots were already autolysed before inoculation into bile salt broth. In our laboratory the yield for routine clot culture is low (0·82%) and the cost is US$0·32 per test. In view of the limited cost effectiveness of this test we do not believe that the routine clot culture of specimens sent for Widal agglutination is justified except when good blood culture facilities are unavailable.

The low yield for stool culture in the initial week of typhoid fever is well known. In this study, however, 6% of cases were diagnosed by stool culture alone, and this test still has a role.

Only 3% of more than 2000 Widal tests gave a positive result, and only 2% were true positive. False negative results occurred in 0·6% of tests. Laboratory confirmation of typhoid fever, however, would not have been made in 11 of 65 (17%) cases had the test not been performed.

Quality control of Widal tests is important: a laboratory which consistently produces poor results in an external quality control programme should discontinue the test until technical problems are solved.[17] False positive results may be due to faulty technique or to poor quality of the antigen suspension. There is conflicting evidence as to the relative importance of somatic and flagellar agglutinin titres for the diagnosis of typhoid fever,[3 4] but in our study both agglutinins gave similar results.

The Widal test should be interpreted in the light of baseline titres in a healthy local population. This is especially important when there is a high local prevalence of non-typhoid salmonellosis.[4] The Widal test may be falsely positive in patients who have had previous vaccination or infection with *S typhi*.[7] Raised Widal titres have also been reported in association with the dysgammaglobulinaemia of chronic active hepatitis and other autoimmune diseases.[6 4 18 19] False negative results may be associated with early treatment, with "hidden organisms" in bone and joints, and with relapses of typhoid fever. Occasionally the infecting strains are poorly immunogenic.[4]

Our results suggest that modern blood culture techniques permit the bacteriological confirmation of typhoid fever in a high proportion of cases. Routine clot culture of specimens sent for the Widal test is not cost effective and the Widal test itself should be used more selectively. A single Widal test is not reliable for the diagnosis of typhoid fever because false positive and false negative results are common. In a patient strongly suspected to have typhoid fever it may be useful to perform the Widal test only if two blood cultures are negative.

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