Original Article

Microbiology Section

The Study of Salmonellosis with Reference to *Salmonella Typhi* in Enteric Fever Patients

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ABSTRACT

The present study was carried out at the College of Medical Sciences and K J Mehta Hospital, Amargadh, Dist. Bhavnagar, Gujarat, during the period from January 2010 to December 2010, to detect Salmonella (*S. typhi*) cases and carriers by various methods, in clinically suspected cases of enteric fever. The sensitivity of each microbiological test for the diagnosis of salmonella was also evaluated.

A total of 300 blood samples, 50 controls and 100 stool samples were processed, among which 90 cases were of clinically suspected enteric fever and 10 were of gastroenteritis. One hundred stool samples from healthy individuals as the controls were processed and urine was processed in 10 cases, while CSF and pus were processed in one case each. S. typhi was the commonest isolate (75%), followed by S. Paratyphi A (15 %) and S. Paratyphi B (2.5 %) from blood, S. enteritidis (2.5 %) was isolated from pus and S. typhimurium (5 %) was isolated from the stool samples. The Widal test positivity was 38.66 % and the blood culture positivity was 12.33 %. The coagglutination test is a valuable adjuvant for the diagnosis of enteric fever. The Quinolone group of drugs is an alternative drug of choice for multi drug resistant salmonellosis. The incorporation of this drug in the base line restoration, if technically feasible, has to be advised.

Key Words: Salmonellosis, Enteric fever, Coagglutination, Widal test

INTRODUCTION

Typhoid fever is an example of infectious diseases which lead to death. It causes more than 5 % deaths in the areas of high transmission [1].

As per the health statistics of India, in children, it is one of the ten major causes of mortality [2].

This is because the infection is spread through contaminated water or food in rural areas because of poor water supply, poor sanitation and unhygienic practices, which is complicated by overcrowding; the incidence of salmonellosis is higher [3].

Due to the occurrence of drug resistant salmonella, which is mostly due to the misuse of antibiotics by unqualified practitioners, often without laboratory support in the antibiotic sensitivity test of organisms [4], an efficient and rapid technique of identification is essential for proper therapy [5].

The present investigation was undertaken with the salmonella being isolated from blood and stool samples, along with evaluation by Widal and coagglutination tests.

The main objectives were to detect the salmonella (*S. Typhi*) by various methods in clinically suspected cases of enteric fever, to determine the sensitivity and specificity of each microbiological test for the diagnosis of salmonella and to detect the carriers of salmonella.

MATERIALS AND METHODS

The study group comprised of patients of all age groups with a provisional clinical diagnosis of enteric fever. Various samples viz blood, stool, urine and pus were processed by using standard protocols (Mackie and Mcartney 1996).6 Simultaneously, 100 stool samples and 50 blood samples from patients who had symptoms other than fever, were processed as the controls. The blood was inoculated in bile broth and was incubated and sub cultured on nutrient Maconkey's and XLD agar after 24, 48 and 96 hours. A final subculture was done at the end of 7 days. The growth of *S. typhi* was confirmed by standard biochemical tests and slide agglutination tests by using salmonella polyvalent 0 antisera and the 09, Hd and Vi antisera which were obtained from CRI, Kasauli. The faeces were first passed in Selenite F broth and were then sub-cultured by following the above mentioned procedure. Urine and pus cultures were also screened wherever applicable.

The Widal test was confirmed to be positive by the tube agglutination method by following the standard procedure. A coagglutination test by using the Cowan 1 Staphylococcus strain, was done. One drop of each of the coagglutinable reagents was placed on a clear glass side. A drop of serum was added and both were mixed thoroughly. "The result was observed at the end of 2 minutes for any visible agglutination" [7],[8]. Forty strains were sent to Kasauli for serotyping, while 18 strains were sent to the Phage Typing Center, Department of Microbiology, Lady Hardinge Medical College, New Delhi.

Clot Culture: Five ml of blood was received in a sterile test tube and it was allowed to clot. The serum was used for the Widal test. The clot was ground aseptically with a sterile glass rod. 5 ml of bile broth was added to the clot. The clot was cultured and the results were interpreted after 24, 48 and 96 hrs respectively. The faecal culture was done by passing a loopful of the stool in the Selenite F broth and by incubating the broth at 37° C for 8 to 10 hrs. This was then sub cultured on XLD and Maconkey's agar plates.

Coagglutination Test: The Cowan 1 Staphylococcus aureus NCTC (8530) strain was grown in BHI broth at 37°C for 4 hrs. BHI agar slopes were inoculated with this broth culture and were incubated

Serotypes	Clinical Specimens					Per-centage
	Blood	Stool	Urine	Pus	CSF	
S. typhi	30	–	–	–	–	75
S. Paratyphi A.	6	–	–	–	–	15
S. Paratiphi B	1	–	–	–	–	2.5
S. enteritidis	–	–	–	1	–	2.5
S. typhimurium	–	2	–	–	–	5

[Table/Fig–1]: Species of salmonellae from different clinical specimens

at 37°C for 18 to 24 hrs. The cells were harvested in a minimal volume of 0.03 M Phosphate buffered saline (PBS), washed 4 to 5 times and resuspended in PBS containing 0.5 % formaldehyde for 3 hrs at room temperature. The cells were again washed in PBS 3-4 times. The suspension was heated at 80°C for 10 minutes with frequent agitation. After 3 washings in PBS, a 5 % cell suspension was prepared by sensitizing 1ml of the 5 % formalin stabilized cowan 1 Staphylococcal cells with 0.1 ml of different antisera, poly A-G, 0-9, H-d and Vi and normal rabbit serum (NRS) for 3 hrs at room temperature. These final suspensions were called polycoag, d-coag, vicoag and NRS-coag. The reagents were stored at 4°C until use.[7],[8]

OBSERVATION AND RESULTS

A majority (12.3 %) of the salmonella stains were isolated from blood, followed by their isolation from stool (5%) and pus (2.5 %). No stains were isolated during the present study from urine and CSF. S. typhi was the commonest isolate (75 %), followed by S.paratyphi A (15 %), *S.typhimurium* (5%), S. paratyphi B (2.5 %) and S. enteritidis (2.5 %) [Table/Fig-1].

A majority of the patients were within the age group of 10-20 years, followed by the age group of more than 20 years. Males were found to be commonly affected than the females. The maximum numbers of isolations were in the month of July, followed by a lower number of isolations in August. Out of 300 samples, 37 showed blood culture positivity, while 116 were slide and tube Widal positive. The clot culture was positive in 30 (10 %) out of 300 samples. Both the blood and clot cultures were positive in 30(10 %) samples [Table/Fig-2].

Out of the 300 samples, 225(75%) were co- agglutination positive. Out of the total 37 salmonellae which were isolated from blood, 30 were *S.typhi*, 6 were S. paratyphi A and one was S. paratyphi B. One S.enteritidis strain was isolated from the pus of a patient who was suffering from osteomyelitis.

Two S. typhimurium strains were isolated from the stool samples of patients with acute gastro enteritis. The commonest phage type of *S.typhi* was E1 and that of S.paratyphi A was [1], while all other salmonella were untypeable.

All the quinolone groups were 100 % sensitive to the salmonella isolates. The most resistant pattern was observed with respect to Chloramphenicol and Tetracycline.

DISCUSSION

It was observed in the present study, that *S.typhi* was the commonest serotype which was isolated, followed by S. parathyphi A. and *S.typhimurium* from the blood and stool samples. S. paratyphi B was isolated from the blood samples. S.enteriditis was isolated from the pus sample from a case of osteomylitis and sickle cell anaemia. Braude also described cases of sickle cell anaemia, which were susceptible to infections which were caused by S. enteriditis [9]. Blaser and Feldman (1981), in their study, found that *S. typhimurium* was the commonest isolate, followed by *S.typhi* from the samples blood and stool [10]. The maximum incidence of salmonellosis was seen in the month of July, followed by a lower incidence in August. Sen and Saxena (1968) observed a high incidence of salmonellosis in the months of June to September [11].

Salmonellosis was reported most commonly in the patients who were less than 20 years of age and the incidence was higher in males than in females during the present study.

The commonest age group which was affected was the 11-20 years age group and there was no incidence in the age group of below one year. The male to female ratio was approximately 2:1. The same results were observed by Padmanabhan [12] (1970) and Sharma Gathwala [13] (1993).

Two methods were used for the isolation of salmonellae, clot culture and blood culture. In the present study, as compared to the blood culture (37 positive out of 300), the clot culture showed a relatively low isolation rate i.e. 30 out of 300 patients. This low isolation rate in the clot culture may be due to the improper lysis of the clot by mechanical methods, as the costs limited the use of streptokinase for the same.

The Widal test was positive in 116 cases (38.66 %). The higher numbers of Widal positivity in comparison to those of the blood culture may be due to the delay by the patients in seeking medical advice, which is very common in the rural population. A titre of 100 was taken as a significant titre, thus indicating a positive Widal test for the O antigen and 1:160 for the H antigen.

The serum of normal (uninfected) persons agglutinates the salmonella suspensions at dilutions up to about 1:50 and so that the titres cannot be taken as significant unless they are greater than 1:100 (Mackie and Mcartney 1996).

A circulating antigen of *S.typhi* was found in 75% of the sera which were tested by co agglutination, whereas in the blood culture, the positivity was only 12.33 % and the Widal positivity was 38.66%. Shetty et al (1985) found that 81 % of the sera tested positive by coagglutination [14]. Mukherji et al (1993), in another study of coagglutination with the serum of patients, found positive results in 67.3 % of the patients [15]. Mathai and Jesudason evaluated the specificity and sencitivity of the coagglutination test to be 97 % and 88 % respectively [7]. Shobharam et al found coagglutination as an acceptable alternative for serotyping [16].

	Total Samples	Coaglutionation		Widal		Blood Culture		Clot Culture		Both blood culture & clot culture positive
		+ve	–ve	+ve	–ve	+ve	–ve	+ve	–ve	
Patient	300	225 (75 %)	75 25%	116 (38.66 %)	184 (61.33 %)	37 (12.33 %)	263 (87.66 %)	30 (10 %)	270 90%	30 (10 %)
Controls	50	50		50		50		50		

[Table/Fig-2]: Comparison of coagglutination test, widal test, blood culture, and clot culture

False positive rates of the coagglutination test in the febrile control groups in our study were found to be nil. The results which were obtained, suggested that the coagglutination test of serum had the advantages of simplicity, easy reproducibility, rapidity and higher sensitivity, as compared to the blood culture and the Widal test. It is therefore a promising test for the diagnosis of typhoid.

Chloramphenicol is one of the drugs of choice in the treatment of typhoid. Fever is losing its efficacy and 4 quinolones, ciprofloxacin, ofloxacin, pefloxacin, and sparfloxacin were 100 % sensitive to the isolates. Gupta and Mena (1992) found that all their isolates were 100 % sensitive to ciprofloxacin [17].

Thus, it can be concluded that coagglutination is one of the most useful and easy methods because of its higher sensitivity and low cost. Quinolone is a useful alternative for the treatment of enteric fever.

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