

Identification & Study on Antibigram of Salmonella Spp. Isolated through Clot Culture

Dr. Shubhangi Sharma¹, Dr. Bhumika Vageriya²

¹Med. Msc Microbiology, R.N.T. Medical College, Udaipur Affiliated by RUHS, Jaipur, Rajasthan, India

²M.D. Microbiology, R.N.T. Medical College, Udaipur Affiliated by RUHS, Jaipur, Rajasthan, India

Abstract: ***Aim:** To compare the effectiveness of the Widal screening & clot culture methods for typhoid /paratyphoid fever diagnosis in Widal positive patients. **Study design:** Analysis involved a experimental prospective study. **Place & duration of study:** The study took place at the Department of Medical Microbiology, at RNT Medical College between March 2018 –Sept. 2018. **Methodology:** A total of 306 samples from 143 male & 163 female aged 15-70yr. The isolation from samples & identification were carried out with clot culture methods, characterization was done using polyvalent sera. The rates of recovery of S. Paratyphi A, B, C and typhi D from clot culture of individuals positive at the various Widal antibody titres were evaluated. **Result:** Among the subjects positive for Widal titre of O & H variants, the isolation rate from clot culture 40 % at titre 1: 320, 14 % at titre 1: 160 & 10 %, p value is 0.045 indicates that isolation of organism are positively associated with increase ratio of antibody titre. **Keywords:** typhoid fever, widal, clot culture, Salmonella sp.*

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1. Background

The salmonellae are primarily intestinal parasites of vertebrates & which infect man, leading to enteric fever, gastroenteritis & septicaemia. The most important member is Salmonella typhi, the causative agent of enteric fever. Man is the only natural host for S. Typhi & S. paratyphi A whereas most of the other salmonellae are chiefly pathogenic in animal like poultry, pigs, cattle etc⁽¹⁾

Typhoid fever has largely disappeared from industrialized countries, but remains a serious public health problem in developing countries⁽²⁾

- According to conservative World Health Organization (WHO) estimates, 16 million cases occur each year, including about 600 000 deaths (WHO, 2000). US Centers for Disease Control and Prevention estimates that there are 21.6 million typhoid cases annually, with the annual incidence varying from 100 to 1000 cases per 100 000 population. The global mortality estimates from typhoid have also been revised downwards from 600 000 to 200 000, largely on the basis of regional extrapolations.⁽³⁾
- Salmonella infection (salmonellosis) is a common bacterial disease that affects the intestinal tract mainly. Salmonella bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food.⁽⁴⁾
- According to the most recent estimates by WHO, approximately 2, 00, 000 people die every year worldwide, bulk of the burden being borne by Asian countries as it accounts for 90% of this mortality.⁽⁵⁾

2. Methods

- It is a cohort prospective study were conducted in Department of Microbiology at RNT Medical College, Udaipur (Rajasthan), the study included patient name,

age, gender, geographical distribution and their clinical suspected enteric fever history.

- The samples received in a period of 1year by OPD & IPD were b processed in the microbiology lab. Blood Sample are included as a part of study.
- A prospective study on febrile patients was conducted in which patients were screened for typhoid fever and suspected patients were enrolled in the study.
- 8-10 ml of blood from each adult study subject was collected in a clean and dry tube by using all aseptic precautions. Allowed to clot then centrifuge & the serum performed the agglutination test (by precision biomed Kit).Qualitative slide agglutination and semi quantitative tube.
- Sample Processed in Lab. For Culture (7):The clot were lysed and dispensed into the culture medium bottle containing 20ml bile broth containing 0.8%concentration of sodium taurocholate (bile). Samples were subcultured on Blood agar, MacConKey agar,
- XLD agar media for isolation of Salmonella enterica serotype Typhi (S.Typhi) and S. enterica serotype Paratyphi (S. Paratyphi) A, B, C.
- Pic showing grth on xld.
- Isolates would be confirmed further with the help of various biochemical test, sugar fermentation test, amino acid decarboxylase test, and slide agglutination (by precision After 48 hours incubation subculturing was performed from the bile broth on XLD agar (microgen) Identification of Salmonella at genus level can be confirmed by slide agglutination using polyvalent O antisera (group A-G). Then the serotype can be identified by using type specific O antisera. (Especially agglutinates with O2 (group-A) antisera (DENKA SEIKEN Co. Ltd., JAPAN) in case of S.Paratyphi A (1).
- **Sensitivity Testing:** The isolates were tested for their susceptibility to 08 different antimicrobial agents Amoxicillin, Azithromycin, Ceftriaxone.
- Ciprofloxacin, Chloramphenicol, Co-trimoxazole, Nalidixic acid and
- Tetracycline. (HiMedia, Lab, INDIA) Antibigram assay were performed as per CLSI, 2017 & Ecoli. ATCC 25922 were used as a control strain.

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3. Result

A total 5121 serum samples processed within 5 month of duration, in 600 serum samples showing positive result for widal test & among 600 positive widal serum samples 306 serum samples, found to be eligible according to inclusion criteria (patient having pyrexia less than a week & diagnosed for widal test.)

A. Distribution of isolates found in culture according to geographical area

N=306

Rural	Urban	Tribe
104	156	46

Prevalence of Clot culture positivity among patient having significant Widal titer.

Isolates	Urban 156	Rural 104	Tribe 46	Total 306	P Value
S.Typhi	05	11	05	21	.036
S.Paratyphi A	05	01	00	06	.257
Other bacteria	79	52	19	150	.522
Negative culture	67	40	22	129	.541

Prevalence of Clot culture positivity among patient having significant Widal titer.

Significant Widal titer	Clot culture positive	Clot culture negative	Total N=258	P Value
Only TO>1:80	11	107	118	0.045
Only TH>1:160	02	62	64	
Both TO >1:80 TH>1:160	13	57	70	
Only AH>1:160	00	03	03	
Only BH>1:160	00	03	03	

C. Antibiogram of cot culture isolated Salmonella species. N=27

Antibiotics	Salmonella Typhi	Salmonella paratyphi A
	Sensitive	Resistance
1.Ampicillin	21(100%)	0% 06(100%)
2.Ceftriaxone	21(100%)	0% 06(100%)
3.Ciprofloxacin	18(85.71%)	03(14.28%) 06(100%)
4.Nalidixic acid	20(95.23%)	01(4.76%) 06(100%)
5.Azithromycin	21(100%)	0% 06(100%)
6.Chloramphenicol	21(100%)	0% 06(100%)
7.Pefloxacin	18(85.71%)	03(14.28%)

4. Discussion

- Among the 306 cases studied, clot culture were positive in 27 patients of which 21 (77.77%) isolates were

Salmonella typhi and only 06 (22.22%) were *Salmonella paratyphi A*. Culture positivity was 8.8%. Significant Widal titers were seen in 258 (84.3%) samples. In a similar study done by Krishnan P et al⁴⁶ in Chennai, 70% of

- Isolates were *Salmonella typhi* and 30% were *Salmonella paratyphi A*, which was in accordance with our study.
- In our study among 27 isolates of *Salmonella* (21 *S.typhi* and 06 *S.paratyphi A*) showed that 100% susceptibility for ampicillin, ceftriaxone, cotrimoxazole, azithromycin, chloramphenicol, nalidixic acid and pefloxacin while as for ciprofloxacin and nalidixic acid 14.28% and 4.76%. *Salmonella typhi* were showing resistant pattern respectively.
- Our study is in accordance with Smitha et al⁽⁶⁾ found that Strains (both *S. typhi* and *S. paratyphi A*) were sensitive to drugs like Ampicillin, Chloramphenicol, Cotrimoxazole, Gentamicin, Ceftriaxone and 17% of *S. Typhi* and 40% of *S. paratyphi A* were intermediately sensitive to ciprofloxacin. The strains were 100% resistant to nalidixic acid. Nalidixic acid resistance is considered a marker of low level resistance to ciprofloxacin among *Salmonella* and also an indicator of treatment failure with ciprofloxacin.⁽⁷⁾
- Out of 306 serum samples, 27 samples had clot culture positive, And among 27 clot culture positive samples, twenty six samples had significant titer of Widal test, this shows that Widal can be used as a useful testing tool for screening enteric fever, unless done in paired sera.
- 27 isolates of *Salmonella* (21 *S.typhi* and 6 *S.paratyphi A*) showed that 100% susceptibility for ampicillin, ceftriaxone, cotrimoxazole, azithromycin, chloramphenicol and pefloxacin, while as for ciprofloxacin and nalidixic acid 14.28% and 4.76% *S.typhi* were showing resistant pattern respectively, Hence clinician should consider oral drugs Ampicillin and Cotrimoxazole as their first line of treatment of enteric fever.
- In Indian setting, low socio-economic conditions, rapid population growth limited safe water, overcrowding, poor sanitary conditions facilitated the cases are more likely to be seen, Hence low cost testing for screening is very useful like Widal test for enteric fever.

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