ORIGINAL RESEARCH

Identification and serotyping of *Salmonella* species from clinical suspected case of enteric fever in A Tertiary Care Centre in Central India- A prospective study

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ABSTRACT

Background and Objective: *Salmonella enterica* serovars Typhi (S. Typhi) andParatyphi (S. Paratyphi) A, B, and C are the pathogens that cause enteric fever, also known as typhoid and paratyphoid fever. Most cases of enteric fever are spread by the fecal–oral route. This study is to evaluate the isolation and identification of *Salmonella* spp. in suspected cases of typhoid fever. **Material method:** The current study was conducted in the Mahatma Gandhi Memorial Medical College's microbiology department in Indore, Madhya Pradesh, central India, between 2020 and 2021. Blood samples from suspected enteric fever caseswere received by the microbiology department. **Result:** Out of the 140 blood culture samples that were obtained, 26 strains of *Salmonella enterica* that were positive for culture were identified and isolated. All 26 isolates of the *Salmonella enterica* species were found to be serovar Typhi.We did not isolate S. Paratyphi in this investigation. **Conclusion:** It has been determined that younger age groups and men are more susceptible to enteric fever. This study indicates that a common cause of *Salmonella* infection is *Salmonella enterica* serovar typhi.

Key words: *Salmonella* typhi, BHI, Serotyping

INTRODUCTION

The human pathogen *Salmonella enterica* serovar Typhi causes a systemic infection called enteric fever. *Salmonella* Paratyphi A, *Salmonella* Paratyphi B and sometimes *Salmonella* Paratyphi C causes a similar illness but with less severity. [1] Typhoid fever is a global public health problem, epidemics of typhoid fever and high endemics rates reported in India and countries in South Asia [2, 3]. *Salmonella* typhi and non-typhoidal Salmonellae (NTS) serovars comprise the more than 2,600 distinct serovars that collectively make up the species *Salmonella enterica*. Typhoid fever is caused by the *Salmonella* typhi serovar. Between 128,000 and 161,000 people globally pass away from this illness each year [4]. In India, annual incidence of 102 to 2,219 cases, per 1,00,000 person per year was reported [5]. These organisms are facultative intracellular pathogens that cause systemic infections after consumption, colonisation of the small intestine, invasion of the gastrointestinal mucosal surface, and dissemination in the reticuloendothelial system throughout the body, encompassing the spleen, liver, and bone marrow [6]. Definitive diagnosis of S. Typhi is established when S. Typhi is isolated from blood, bone marrow, rectal swab, urine and duodenal aspirate [7]. *Salmonella enterica* serovar Typhi/ Paratyphi isolated from acute typhoid fever cases has been shown to exhibit excellent levels of sensitivity when cultured in either a large volume (~30 ml of peripheral blood) or high density (bone marrow). It has been found that the bacterial density in bone marrow of patients with acute typhoid fever is ten timeshigher than that in peripheral blood [8]. The Gold standard for diagnosis of enteric fever is still blood culture. The positivity in first week of illnessis about 80% in untreated cases and the rate declines to 20-

30% in the later course of disease [9]. Blood culture being the gold standard test, it is highly specific but sensitivity is affected by a number of factors like prior intake of antibiotics and stages of illness and thus, blood culture positivity varies [10].

AIMS AND OBJECTIVE

Current study is to evaluate the isolation and identification of Salmonella spp. in suspected cases of typhoid fever in a tertiary care centre.

MATERIAL METHOD

The present study was carried out from 2020 to 2021 in the department of microbiology at Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, central India. Blood samples were received from suspected cases of enteric fever in the department of microbiology. A total of 140 blood samples were taken, out of which 26 isolates found to be salmonella species were enrolledin this study.

Inclusion criteria

Clinical specimens of suspected cases of enteric fever received in the department.

Exclusion criteria

Non-typhoidal isolates.

Salmonella isolates from environmental samples like food, water, etc.

Withdraw 2 mL–5 mL of venous blood from children and 05 mL–10 mL of blood from adult patients suspected of enteric fever. Brain heart infusion (BHI) broth bottles (Bijou bottles) or automated blood culture bottles (BacTalert) containing blood samples were received in the laboratory. Incubate the BHI bottle at 37 oC for 18–24 hours and follow for 7 days. Perform the first blind subculture on solidagar media, namely blood agar and MacConkey agar, after 18–24 hours of incubation, and then on every alternate day until day 7. Examine the BHI broth bottle daily for any visible signs of growth like hemolysis, turbidity, gas formation, pellicle formation, clotting, etc. For automated blood culture bottles, look for flagging at least daily, and once it flags positive, do gram staining, culture, and identification. Automated blood culture bottles also need to be followed for 7 days. Identification of the organism was done by colony morphology, Gram staining of the marked isolated colony, motility testing (hanging drop), and standard biochemical tests. The serotyping was performed using specific antisera by the standard slide agglutination method.

STATISTICAL ANALYSIS

The data were analysed using SSPS version 22. Frequencies and percentages were used to describe the categorical variables in this study. The results were presented as proportion ratios with a 95% confidence interval. Statistical significance was set if p-value <0.05.

RESULT

In this study, blood in BHI broth was collected for culture and antibiotic sensitivity was performed and analysed from 140 clinically suspected enteric fever cases, which were received in the Department of Microbiology, M.G.M. Medical College, Indore. From the 140 blood culture samples received, 26 culture-positive *Salmonella enterica* strains were isolated and identified. The rateof isolation was 18.6%. (n=26) [Table 1] 100% (n = 26) of the isolates of *Salmonella enterica* species were identified as serovar Typhi. *S.* Paratyphi was not isolated in this study. The ratio of males and females presenting with symptoms of enteric fever was almost similar (1.1:1), but the ratio of males andfemales in blood culture positive was higher in males than females (2.1:1) [Table2]. The age group ranged between 1 year to >50 years and with predominant isolation of *Salmonella* Typhi in age group of children i.e., 11-20 years (30.8%) followed by young adults, i.e., 21-30 years age group (23.1%) and least in the agegroup of > 50 years age [Table 3].

Table 1: Percentage of Blood Culture Positives

	No. of Cases	Percent
S. Typhi	26	18.6
Sterile	114	81.4
Total	140	100.0

Table 2: Gender wise distribution of total samples received.

Gender	No. of cases	Culture positive
Male	74 (52.86%)	18 (69.2%)
Female	66 (47.14%)	8 (30.8%)
Total	140 (100%)	26 (100%)

Table 3: Age & Sex Distribution according to Culture Positive

	SEX				Total	
	Female		Male			
Age (years)	No.	%	No.	%	No.	%
1-10	1	12.5%	1	5.6%	2	7.7%
11-20	4	50.0%	4	22.2%	8	30.8%

21-30	2	25.0%	4	22.2%	6	23.1%
31-40	0	0.0%	6	33.3%	6	23.1%
41-50	0	0.0%	3	16.7%	3	11.5%
>50	1	12.5%	0	0.0%	1	3.8%
Total	8	100.0%	18	100.0%	26	100.0%

Pearson Chi-Square = 8.005, df = 5, p value = .156, Not Significant

Table 4: Characterization of *Salmonella* isolates based onserotyping.

S. No	IsolateId	Aggluti- nation withPoly "O" antisera	Aggluti- nation with 0-9antisera	Aggluti- nation with H-dantisera	Serogroup	Serovar
1	S-1	+	+	+	D	Typhi
2	S-2	+	+	+	D	Typhi
3	S-3	+	+	+	D	Typhi
4	S-4	+	+	+	D	Typhi
5	S-5	+	+	+	D	Typhi
6	S-6	+	+	+	D	Typhi
7	S-7	+	+	+	D	Typhi
8	S-8	+	+	+	D	Typhi
9	S-9	+	+	+	D	Typhi
10	S-10	+	+	+	D	Typhi
11	S-11	+	+	+	D	Typhi
12	S-12	+	+	+	D	Typhi
13	S-13	+	+	+	D	Typhi
14	S-14	+	+	+	D	Typhi
15	S-15	+	+	+	D	Typhi
16	S-16	+	+	+	D	Typhi
17	S-17	+	+	+	D	Typhi
18	S-18	+	+	+	D	Typhi
19	S-19	+	+	+	D	Typhi
20	S-20	+	+	+	D	Typhi
21	S-21	+	+	+	D	Typhi
22	S-22	+	+	+	D	Typhi
23	S-23	+	+	+	D	Typhi
24	S-24	+	+	+	D	Typhi
25	S-25	+	+	+	D	Typhi
26	S-26	+	+	+	D	Typhi

DISCUSSION

Worldwide, *Salmonella* infections pose a threat to public health. The exact incidence of the disease in India is understated due to limited laboratory diagnosis. To determine the disease's prevalence, information on typhoid cases with positive cultures and identification by serotyping is needed [11].

In this study, 140 clinically suspected cases of enteric fever were included, and 26 (18.6%) of them were found to be blood culture positive for *Salmonella* typhi serovar. A similar result was shown by Ruchi Girotra *et al.* (2016) and Suresh K.and Balachandran C.S. *et al.* (2017) [12, 13].

This difference in the isolation rate is attributed to a number of factors, like different geographical areas with different endemicities, antibiotic intake before blood culture, a lower count of bacteria in the blood, and a smaller amount of blood taken for blood culture, which leads to the difficult isolation of *Salmonella* [12, 13, 14,15].

The isolation of *Salmonella* species was shown to be higher in males than in females in this study, with a ratio of 2.3:1, despite the fact that the number of males and females presenting with symptoms of the illness is the same, at 1.1:1. Most researcher have discovered an almost similar ratio of isolation salmonella species (Shoorashetty Manohar Rudresh *etal*, PGIMSR.(2015), Varsha Gupta *et al* (2013) [16, 17]

In this study, *Salmonella* typhi species were isolated in blood culture, with predominant isolation in children in the age group of 1–19 years (38.5%), followed by young adults aged 21–30 years (23.1%). And the isolation rate was low in the age group > 50 years (3.8%). This result correlates with various studies by and Shoorashetty Manohar Rudresh *et al.* (2015), Varsha Gupta *et al.* (2013), Sarika Jain *et al.* (2013) [16,17,18]. While a study by Lovely Akter *et al.* (2021) showed a higher prevalence amongst younger adults [19].

The increase in prevalence in males and in the young adult age group could be because males are more frequently found to be staying away from home for varied reasons like job, study, and are more likely to

eat outside contaminated food. Thus, proper sanitation and personal hygiene need tobe taken care of to prevent these situations and thus lower the occurrence of enteric fever caused by eating contaminated food and water.

Based on serotyping employing slide agglutination, 26 isolates from the current investigation were identified; all of them were determined to be S. typhi. Random isolates were sent to CMC, Vellore, for serotyping in order to obtain confirmation. The study's findings are consistent with the serotyping of the isolates that were sent to CMC, Vellore. This finding was in accordance with the previous reported study by Balaji Veeraraghavan et al. (2016) [20].

CONCLUSION

We have come to the conclusion that males and younger age groups are more likely to contract enteric fever. This study shows that *Salmonella* enterica serovar typhi is frequently involved in *Salmonella* infection. Early isolation and identification of organisms required for proper management of patients.

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Conflicts of interest: none declared

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