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A STUDY ON INCIDENCE OF ENTERIC FEVER AMONG FEBRILE PATIENTS IN A PRIVATE TEACHING HOSPITAL BY ANALYSIS OF CLINICAL AND MICROBIOLOGICAL PROFILES.

Microbiology

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ABSTRACT

Enteric fever or typhoid is caused by Salmonella enterica with multiple serotypes affecting all types of population, leading to morbidity and mortality. Some of the potential risk factors for enteric fever are consuming contaminated food and water, having close contact with recent enteric fever patients, poor housing, improper sewage system and lack of safe drinking water and increased use of broad-spectrum antibiotics. Of the 110 patients with fever included in the study, the mean duration of fever was 9.87 ±0.68 days with a male predominance and male to female ratio of 1.82:1 and the mean age of 44.5 ± 2.32 years. Blood culture yielded growth Salmonella enterica isolates in 32.7% of the subjects. Eight of the 36 isolates (22.2%) were MDR Salmonella. Widal test was significant in 48.2% (53) of patients. For correct diagnosis of enteric fever, rapid serological tests with better sensitivity and specificity are needed. Also, better living standards, health education, and proper treatment are required to control the endemicity of enteric fever.

KEYWORDS

Enteric Fever, Typhoid, Blood Culture, Widal Test, Antimicrobial Susceptibility, MDR Salmonella.

INTRODUCTION:

Enteric fever or typhoid is caused by *Salmonella enterica,* a gram-negative bacillus belonging family *Enterobacteriaceae*. Multiple serovars cause enteric fever, among which the most common in India are typhi and paratyphi A (S. Typhi and S. Paratyphi A). Improved sanitation and suitable public health measures have reduced the incidence and prevalence of this disease in developed nations. It may cause sporadic disease clusters in developed countries with occasional point-source epidemics. In underdeveloped and developing nations, it is one of the leading causes of morbidity and mortality. Enteric fever is an endemic disease with an incidence of about 214.2 per 100,000 persons /year. [1] S. typhi is the most common etiologic agent followed by S. paratyphi A, and its incidence is increasing.[2]

In 2014, the Indian Government launched a national programme called Swachh Bharat Abhiyan (SBA). This was introduced to modify municipal corporations, create awareness programs, change sanitation habits, and eliminate open defecation with 1.2 billion funds to build toilets for rural and urban people by 2019 (Swachh Bharat Mission, 2014). In August 2015, around 8,00,000 toilets were constructed but changing open defecation in rural population was challenging.[3]

Some of the potential risk factors for enteric fever are consuming contaminated food and water, having close contact with recent enteric fever patients, poor housing, improper sewage system and lack of safe drinking water supplies during monsoon, floods or droughts, and finally, inadequate personal hygiene, and increased use of broad-spectrum antibiotics.[4]

No single test is sufficiently sensitive, specific, and practical in resource-limited endemic area settings of developing and underdeveloped nations. Most of the infections caused by the *Salmonella* serotypes are diagnosed clinically without proper laboratory evidence and consequently treated presumptively with antibiotics.[5] Physicians is also facing problem with the increase in the number of drug-resistant strains of *Salmonella* Typhi. Antimicrobial susceptibility testing of all isolates is necessary to combat increasing resistance among *Salmonella* serotypes. Tests and assays of better sensitivity and specificity are employed to get accurate and specific diagnoses. [6]

This study was conducted to identify the incidence of enteric fever, do a comparative evaluation between blood culture and Widal test in the laboratory diagnosis of enteric fever, and determine the antimicrobial susceptibility patterns.

MATERIALS & METHODS:

This is a cross-sectional observational study conducted at the Department of Microbiology, NRI Institute of Medical Sciences, Visakhapatnam, attached to Anil Neerukonda Hospital, a private teaching hospital giving service to patients to the rural and urban areas nearby in Visakhapatnam and Vizianagaram. The study was conducted from March 2016 to December 2016 after obtaining approval from the Institutional Ethics Committee. Patients above 18 years who had attended medicine OPD with a presentation of fever for five days or more were included in this study to increase the sensitivity in identifying the disease among OPD attendees.

After fitting into the inclusion criteria, blood samples were collected aseptically from 110 patients after taking proper informed consent. In addition, clinical and socio-demographic data like age, sex, address, etc., were collected from every patient by standard questionnaires at the time of sample collection.

Blood was collected, and serum was separated. Widal test, a tube agglutination assay was performed with O antigens of S. Typhi and H antigens of S. Typhi, S. Paratyphi A and B antigens procured from Arkray diagnostics Ltd. A master serum sample was diluted by adding 1.9 mL fresh 0.95% saline to 0.1 mL serum to give a dilution of 1:20, and this was later serially diluted to 1:640 for anti O and anti H titre separately in Felix and Dreyer's tubes and incubated at 37°C for 16 – 20 hours, and then results were reported.

Blood from two different sites was collected aseptically half an hour apart and immediately introduced into a sterile blood culture bottle with a biphasic brain heart infusion (BHI) medium. For adults, 5 ml blood was added in 50 ml of BHI medium. The blood culture bottles were transported to the Microbiology laboratory and incubated aerobically at 37°C. Media are checked for signs of turbidity or growth on the slant in the biphasic medium for up to 7 days.

Blood culture bottles with turbidity or growth were sub-cultured on MacConkey agar (MA), xylose-lysine-deoxycholate agar (XLD), and deoxycholate citrate agar (DCA) plate and incubated aerobically at 37°C for 18 – 24 hours. A characteristic growth on XLD/ DCA was further identified by standard conventional phenotypic methods of identification using an array of biochemical tests to *Salmonella enterica*.[7] Isolation of S. Typhi and S. Paratyphi A were confirmed serologically by slide agglutination test with polyvalent O & H *Salmonella* group antisera, and then by serotype-specific O & H antisera procured from Difco Laboratories, (Difco-Thermofisher Scientific), USA. For S. Typhi 9-O and d-H antisera and 4-O and a-H antisera for the identification of S. Paratyphi A.[7]

Antimicrobial susceptibility testing was performed using Muller Hinton agar plate using modified Kirby-Bauer disc diffusion method following standard methods.[8] The antibiotic discs used were Nalidixic acid (30 µg), Ampicillin (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), and Trimethoprim/ sulfamethoxazole (1.25/ 23.75 µg), from HiMedia Laboratories Pvt Ltd. Antimicrobial Susceptibility Testing (AST) and interpretation was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines and

reported accordingly.[8]

We defined enteric fever among the cases presented with fever of 3 to 5 days and confirmed blood culture for *Salmonella* Typhi or *Salmonella* Paratyphi A. A positive serodiagnosis was made based on the interpretation of the Widal test. It was considered positive when titre was ≥ 1: 80 for TO and ≥1:160 for TH/AH/BH, or there was a four-fold rise in titre in paired sera taken after 10-14 days apart.[9]

Data obtained from questionnaires and laboratory investigations of the patients included in the study were analysed using a Microsoft Excel spreadsheet, descriptive biostatistics using SPSS software 16.0.

RESULTS:

Our study included 110 patients suspected of enteric fever during the study period. Of the 110 patients, 71 (64.5%) were males, and 39 (35.5%) were females, with the male to female ratio was 1.82:1 (Figure – 1). The age range in our study population was 19 to 67 years with a mean age of 44.5 ± 2.32 years.

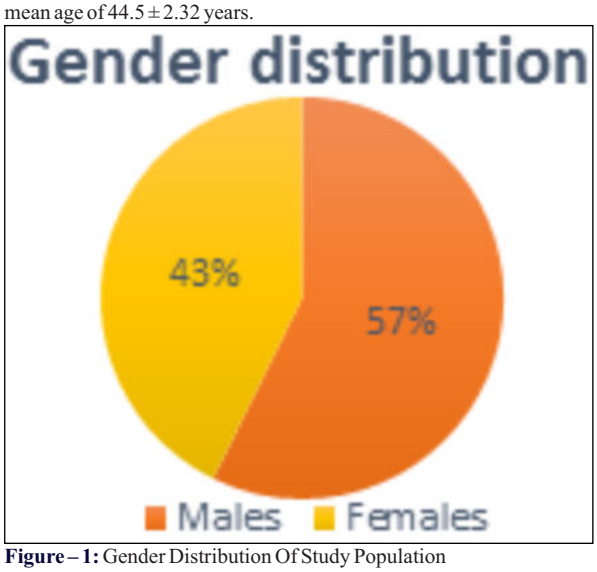

Figure – 1: Gender Distribution Of Study Population

The range of duration of fever in our study participants was 5 to 16 days with a mean of 9.87 ±0.68 days. Various clinical symptoms and signs associated with fever among the study population involved in the study are depicted in table – 1.

Table – 1: Clinical signs and symptoms of enteric fever suspects			
Sl no	**Parameter**	**Number**	**%**
1	Fever (> 100^{0}F)	110	100
2	Chills or shivers	64	58.2
3	Abdominal pain	46	41.8
4	Myalgias & arthralgias	43	39.1
5	Vomiting	22	20.0
6	Diarrhoea	11	10.0
7	Constipation	4	3.6
8	Bradycardia	59	53.6
9	Abdominal tenderness	26	23.6
10	Splenomegaly	24	21.8
11	Hepatomegaly	14	12.7

Among the 110 patients presented with fever and other varying symptoms, 42 (38.2%) blood cultures showed growth and were identified using standard biochemical tests and serological assays using antisera by slide agglutination. Of the 42 isolates, 22 (20%) were *Salmonella enterica* sub sps *enterica* serotype, 14 (12.7%) were *Salmonella enterica* sub sps *enterica* serotype Paratyphi A, and commensals were grown in 6 (5.5%) of the samples. *Salmonella enterica* sub sps *enterica* serotype Paratyphi B was not isolated in culture (Table – 2).

Table – 2: Outcomes of Blood cultures (n = 42, 38.2%)			
Sl no	Blood culture	Number	%
1	*Salmonella* Typhi	22	20.0
2	*Salmonella* Paratyphi A	14	12.7
3	*Salmonella* Paratyphi B	0	0.0
4	Skin Commensals	6	5.5
5	Culture negative	68	61.8

All the 22 *Salmonella* Typhi and 14 *Salmonella* Paratyphi A were subjected to antimicrobial susceptibility testing using standard CLSI guidelines following the Kirby-Bauer method. All 36 isolates were found to be susceptible to Ceftriaxone. Most resistance was seen with Cotrimoxazole (61.1%) and Nalidixic acid (52.8%). MDR *Salmonella* was isolated in 8 of the 36 isolates (22.2%). The resistance pattern of the isolates was detailed in figure – 2.

Widal tube agglutination test was performed on all patients using *Salmonella* Typhi 'O', Typhi 'H' and Paratyphi 'AH'. Seropositivity among the study subjects was 48.2% (n=53) with 53 patients positive for *Salmonella* Typhi 'O' > 1:80. Whereas *Salmonella* Typhi 'H' > 1:160 were 36 (32.7%) and *Salmonella* Paratyphi 'AH' > 1:160 were 17 (15.5%). Widal test was negative in 56 (51.8%) of the study patients (Table – 3). Widal was positive in 16 cases with significant titres who had positive blood cultures for *Salmonella* species.

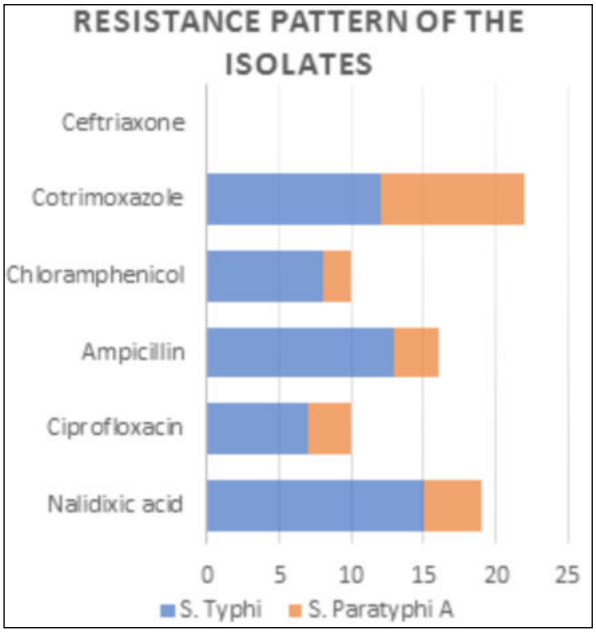

Figure – 1: Resistant Pattern Of The Isolates

Table – 3: Widal tube agglutination test (n = 53, 48.2%)			
Sl no	O & H antibodies	Number	%
1	*Salmonella* Typhi	53	48.2
2	*Salmonella* Typhi	36	32.7
3	*Salmonella* Paratyphi A	17	15.5
4	*Salmonella* Paratyphi B	0	0.0
5	Widal test – Negative	57	51.8

DISCUSSION:

Enteric fever is still a major public health problem, especially in tropical countries like India. There is a lack of available epidemiological data to project the actual situation in India.[2] Our study included 110 patients with febrile illness and are clinically suspected of having enteric fever has shown a male predominant with a ratio of 1.82:1. The mean age was 44.5 ± 2.32 years. This was slightly higher compared to studies conducted by others.[10,11]

The mean duration of fever in our study was 9.87 ±0.68 days. The most common presentation in our study was fever chills (58.2%) followed by abdominal pain (41.8%), arthralgias (39.1%), vomiting, diarrhoea and constipation. Most clinical signs seen were bradycardia (53.6%) followed by abdominal tenderness (23.6%), splenomegaly (21.8%) and hepatomegaly (12.7%). In the study by Gupta S. et al., all patients presented with fever with a mean duration of 8.8 (range 2 to 30) days. Pain abdomen, vomiting, diarrhoea, headache and cough were other common symptoms.[12]

Blood culture yielded growth in 42 cases, and *Salmonella* Typhi was the most common with 22 (20%) isolates, followed by *Salmonella* Paratyphi A (12.7%) with 14 isolates and commensals grown in 6 cases (5.5%). Culture positivity has a lower incidence in our study compared to other studies conducted in South India.[11] Among the 36 *Salmonella* isolates, Ceftriaxone was the sensitive among all the isolates (100%), followed by Ciprofloxacin and Chloramphenicol with 72.2%

sensitivity and Ampicillin with 55.6%.

Cultures are considered multi-drug resistant (MDR) when Ampicillin, chloramphenicol and trimethoprim-sulphamethoxazole. Eight of the 36 isolates (22.2%) were found to be MDR *Salmonella*. Enteric fever is becoming harder to treat due to the endemicity of resistance among bacterial isolates and the availability of over-the-counter antibiotics. Hence proper antimicrobial susceptibility testing and reporting of all cultures of confirmed enteric fever cases are compulsory.

Seropositivity among the study subjects was 48.2%, with a total of 53 patients with significant titres with the Widal tube agglutination test. Widal test was considered diagnostic for typhoid fever if titres of more than 1:160 or demonstration of a four-fold rise in antibody titre. Widal test showed raised titres indicative of typhoid fever in 36 cases (32.7%) and paratyphoid fever in 17 patients (15.5%). Widal was positive in 16 subjects with significant titres who had positive blood cultures for *Salmonella* species. Our study shows the current increasing trend of paratyphoid fevers in the community.

CONCLUSION:

The high MDR Salmonella Typhi isolation rate suggests the necessity for control and judicious usage of antibiotics and compulsory AST reporting. Strict measures to curtail the emergence & spread of XDR S. Typhi and allow enteric fever back into the Pre-antibiotic era are to be implemented immediately. Even though blood culture is the gold standard test for the diagnosis, the number of culture-confirmed enteric fever cases is low. This leads to dependency on serological tests for diagnosis. For correct & accurate diagnosis of enteric fever, rapid serological tests with better sensitivity and specificity are needed. Also, better living standards, health education, and proper treatment are required to control the endemicity of enteric fever.

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