

Incidence of Enteric Fever and their Resistant Pattern among School going Children in Ghaziabad, Uttar Pradesh- A Matter of Concern

SANJAY SINGH KAIRA¹, GEETA GUPTA², ANURADHA MAKKAR³

ABSTRACT

Introduction: Children still experience enteric fever as a severe health problem in developing countries like India. It is an infectious disease that exclusively affects humans. In the developing world, it is one of the most prevalent bacterial causes of acute febrile sickness and is spread by the consumption of unhygienic food or water.

Aim: To identify the incidence and resistance pattern of *Salmonella* Typhi and *Salmonella* Paratyphi isolated from school age children.

Materials and Methods: This cross-sectional study was conducted among children with *Salmonella enterica* infections in the Department of Microbiology, Santosh Medical College and Hospital in Ghaziabad, Uttar Pradesh, India. During the study period, (July 2021 to June 2022) a total of 776 blood samples were obtained from school going children (03-17 years old) and immediately inoculated into Bact/Alert aerobic blood culture bottle and incubated at 37°C for upto five days. After receiving a positive result from Bact/Alert, gram staining was done. Standard microbiological procedures were followed such

as different biochemical reactions, agglutination with different antisera for the identification of *Salmonella* serotypes. Antibiotic susceptibility testing was done using the Kirby-Bauer disk diffusion method. Data was analysed by software version 16.0 of the Statistical Package for the Social Sciences (SPSS).

Results: Total 88 *Salmonella* isolates were found, of which 21 (23.86%) were *Salmonella* Paratyphi A and 67 (76.14%) were *Salmonella* Typhi. Infection rate was more common in summer season. *S. Typhi* as well as *S. Paratyphi* A was found to be most resistance to nalidixic acid {58 (86.6%), 19 (90.5%)} followed by ciprofloxacin {42 (62.7%), 18 (85.7%)}. Ceftriaxone and azithromycin resistance pattern among *S. Typhi* isolates was 07 (10.4%) and 15 (22.4%), respectively and among *S. Paratyphi* A it was found to be 07 (33.33%) and 06 (28.6%), respectively.

Conclusion: This study found an increase in resistance to ceftriaxone and azithromycin, which are frequently thought of as the best medications for empiric therapy in children. Regular monitoring of the resistance pattern is necessary for doctors to select the appropriate empiric therapy due to the increasing resistance to the available class of antibiotics.

Keywords: Antibiotics sensitivity, Multidrug resistance, *Salmonella paratyphi*

INTRODUCTION

An important global health issue is "enteric fever" [1]. It is a severe infectious disease that exclusively affects humans. In the developing world, it is one of the most prevalent bacterial causes of acute febrile sickness and is spread by the consumption of tainted food or water [2,3]. This includes *Salmonella* Typhi causing typhoid fever and *Salmonella* Paratyphi causing paratyphoid fever. According to a recent estimate, there are more than 21 million cases of typhoid worldwide and more than 10 million deaths per year [4]. Children in school between the ages of 5-17 year experience the highest incidence of it as shown by Dasari R et al., (17.09%) [5]. Typhoid fever has the wide range of manifestation in the paediatrics age group, it can present as septicaemia in neonates and lower respiratory tract infections in older children. Consuming outside foods like junk food, ice cream, cut fruits, especially in the summer, is linked to a significant risk of typhoid in these kids [6]. The greatest typhoid outbreak in recorded history took place in Sangli (India) between December 1975 to February 1976 [7]. Typhoid continues to be a substantial health problem in the Indian subcontinent due to low living standards, overcrowding and subpar hygiene procedures, the prevalence has greatly decreased in the western countries as a result of improvements in sewage/water treatment and food handling [8]. Since, enteric fever's clinical symptoms are varied and challenging to distinguish from those of other common febrile disorders, it can be challenging to diagnose. Blood, feces, urine,

rose spot fluid and bone marrow samples must be cultivated in order to isolate *S. Typhi*/Paratyphi for a precise diagnosis. Bacteria can be isolated from blood in more than 90% of cases within the first week of sickness. Morbidity and death can be considerably reduced by early diagnosis, the administration of the right antibiotics and other supportive treatments.

Multidrug Resistance Typhoid Fever (MDRTF), which is resistant to chloramphenicol, ampicillin and co-trimoxazole, was first identified in India in 1980 [9]. The rate of resistance has increased everywhere in the world, but it is especially high in underdeveloped countries. In addition, certain strains have demonstrated resistance to third generation cephalosporins, the preferred medication and fluoroquinolones [10]. Typhoid Conjugate Vaccines (TCVs) are being offered, and they are superior to polysaccharide vaccines in terms of effectiveness, displaying a persistently higher geometric mean titre of Immunoglobulin (Ig) G Vi antibodies 3-5 years after immunisation. The World Health Organisation (WHO) advises frequent use of TCVs in kids older than six months in typhoid endemic nations, focusing on those with a high burden or typhoid that is resistant to antibiotics [11].

This study's objective was to determine the overall incidence of enteric fever with culture confirmation (typhoid and paratyphoid) and their pattern of antibiotic resistance, especially in reference to ceftriaxone and azithromycin, in children aged 3-17 years in a tertiary care hospital, Ghaziabad, Uttar Pradesh, India.

MATERIALS AND METHODS

The cross-sectional study was conducted amongst the children (03-17 years of age) detected with *Salmonella enterica* infections in the Department of Microbiology, Santosh Medical College and Hospital in Ghaziabad, Uttar Pradesh, India, from July 2021 to June 2022. Prior to conducting the study, Institutional Ethics Committee (IEC) granted its approval for the project (Reference No: SU/2021/092(4)). Written informed consent was taken from all participants of the study.

Inclusion criteria: Children (03-17 years of age) detected with *Salmonella enterica* infection in blood culture.

Exclusion criteria:

1. Children with non typhoidal Salmonellosis.
2. Repeat isolates.
3. *Salmonella enterica* isolated from samples other than blood.

Sample size calculation: The sample size was done by using the formula $n = z^2 \frac{pq}{d^2}$, where 'p' is the prevalence, $q = 1 - p$, 'd' is the precision of the estimate (0.05), $Z = 1.96$ (95% confidence interval). The prevalence of culture positive *Salmonella* was taken to be 17.09% based on the study conducted by Dasari R et al., [5]. So, according to calculation a total of 776 school going children were enrolled in the study.

Study Procedure

Sample collection and transport: Strict aseptic procedures were followed for collecting blood samples in Bact/Alert blood culture vials using vein puncture method. The volume of blood sample was 2-4 mL. Collected blood samples were immediately transferred to BACTEC blood culture bottles (HiMedia).

Processing and identification of the isolates: Bottles inoculated with blood specimen were incubated at 37°C for upto five days. After receiving a positive result from Bact/Alert, subcultures on blood agar and MacConkey agar were performed and incubated for 24-48 hours at 37°C. Using common bacteriological techniques and conventional microbiology techniques, organism identification and antibiotic sensitivity testing were conducted. Antibiotic susceptibility testing was done using the Kirby-Bauer disk diffusion method, with interpretation performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) standards (2021) [12]. Pefloxacin was used a surrogate marker as per CLSI guidelines 2015 [13]. Antibiograms from non repeat positive cultures were included for profiling isolates and determining their susceptibility to various antibiotics.

All the isolates were therefore confirmed by slide agglutination test using the high titre sera of *Salmonella* Typhi O, H, *Salmonella* Paratyphi AH and *Salmonella* Paratyphi BH procured from National *Salmonella* and *Escherichia* Centre, Kasauli (HP), India. The colony to be tested was emulsified in two drops of normal saline on a sterile clean glass slide to form a uniform smooth milky white suspension. One emulsion was used as a control to check for autoagglutinable. Agglutination was done first with polyvalent O antisera and polyvalent H antisera. The slide was rotated thoroughly for few seconds. A positive result is indicated by visible clumping, whereas if the milky white suspension remains unchanged, it indicates a negative result. Further agglutination step was done with monovalent O antisera (O:9 antisera for *S. Typhi* and O:2 antisera for *S. Paratyphi A*) and monovalent H antiserum (anti-d serum for *S. Typhi* and anti-a for *S. Paratyphi A*) depending upon the isolates suspected [14].

STATISTICAL ANALYSIS

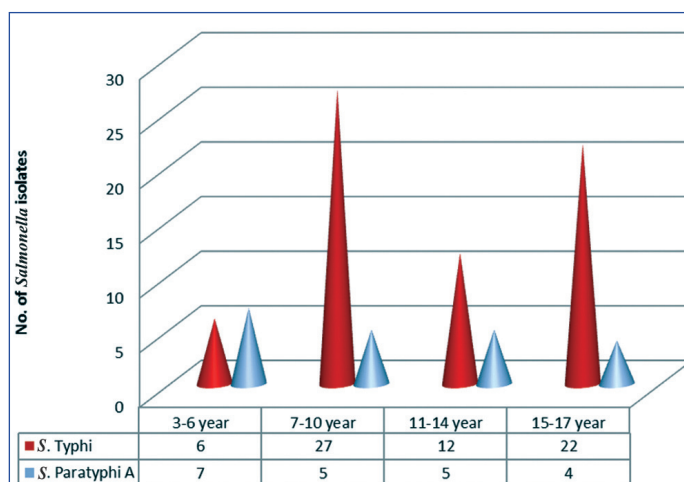
Software version 16.0 of the SPSS was used to conduct the statistical analysis. Data were displayed as proportions and percentages.

RESULTS

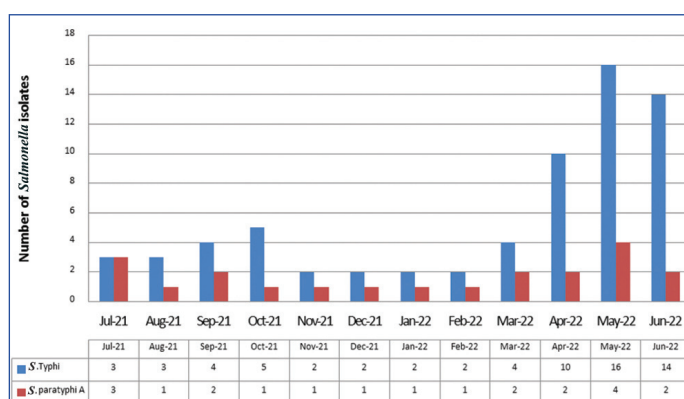
A total of 776 blood cultures from school going children were included in this study. Out of which 88 (11.3%) were culture positive

for *Salmonella*. The male:female ratio was 2.38:1, with 62 (70.45%) males and 26 (29.55%) girls in total. Among the 88 *Salmonella enterica* species isolates, 67 (76.14%) were classified as *Salmonella* Typhi, and 21 (23.86%) as *Salmonella* Paratyphi A. The most common serotype isolated was *Salmonella* Typhi. Throughout, this research period, no other *Salmonella* serotypes were found. The study's participants ranged in age from 3-17 years. Most number of enteric fever cases was found to be in 7-10 year age group 32 (36.4%) followed by 15-17 year age group 26 (29.5%) [Table/Fig-1].

The monthly distribution revealed that most of the positive cases appeared in May 2022 (n=20, 22.7%) followed by June 2022 (n=16, 18.2%) [Table/Fig-2].



[Table/Fig-1]: Distribution of *Salmonella* according to age groups.



[Table/Fig-2]: Month wise distribution of *Salmonella* isolates.

Salmonella Typhi showed the following resistance pattern-ciprofloxacin 42 (62.7%), chloramphenicol 6 (8.9%), ampicillin 8 (11.9%), cotrimoxazole 2 (2.9%), whereas the 58 (86.6%) of the isolated *S. Typhi* strains were resistant to nalidixic acid and to pefloxacin (a surrogate marker, as per CLSI 2015) (Refer [Table/Fig-3]) [13].

Antibiotics	S. Typhi (N=67)	S. Paratyphi A (N=21)
Ampicillin	08 (11.9%)	06 (28.6%)
Ceftriaxone	07 (10.4%)	07 (33.33%)
Imipenem	01 (1.5%)	0
Ceftazidime	05 (7.5%)	04 (19%)
Cotrimoxazole	02 (2.9%)	01 (4.8%)
Chloramphenicol	06 (8.9%)	04 (19%)
Cefoxitin	08 (11.9%)	01 (4.8%)
Pefloxacin	59 (88.1%)	19 (90.5%)
Nalidixic acid	58 (86.6%)	19 (90.5%)
Ciprofloxacin	42 (62.7%)	18 (85.7%)
Azithromycin	15 (22.4%)	06 (28.6%)

[Table/Fig-3]: Resistance pattern of *S. Typhi* and *S. Paratyphi A*.

In the current study, only 2 (2.9%) strains out of the 67 isolates of *Salmonella* Typhi were found to be MDR. A 10.4% of *Salmonella* Typhi isolates were resistance to ceftriaxone. Only one isolate of *S. Typhi* was imipenem resistance. The overall resistance pattern of *Salmonella* Paratyphi A is shown in [Table/Fig-3]. The maximum resistance was found in ciprofloxacin 18 (85.7%) followed by pefloxacin and nalidixic acid 19 (90.5%). Total 7 (33.33%) isolates showed resistance to ceftriaxone.

DISCUSSION

One of the primary causes of illness and mortality worldwide is enteric fever [15]. It is the most prevalent public health issue in developing nations, including India, and is mostly brought on by rapid population expansion, a lack of adequate water supply, poor sanitation, overcrowding, inappropriate waste disposal and unsanitary practices. With the appearance of Nalidixic Acid Resistant *Salmonella* Typhi (NARST), the issue grew worse, making ciprofloxacin a dubious treatment option. However, recent investigations have indicated that *Salmonella* has returned in response to first-line medications. With this fluctuating pattern of antibiograms, it is essential to continuously track the drug resistance pattern and comprehend the underlying mechanism to prevent treatment failure [16]. The incidence of enteric fever documented from various studies of India is shown in [Table/Fig-4] [5,17-21].

Author name	Place of study, year of publication	Incidence	<i>Salmonella</i> Typhi	<i>Salmonella</i> Paratyphi A
Sinha A et al., [17]	Delhi, 1999	11.7%	41 (65%)	22 (34.9%)
Prajapati B et al., [18]	Nepal, 2008	2.38%	195 (83%)	40 (17%)
Ghosh T et al., [19]	West Bengal, 2018	20.92%	38 (76%)	12 (24%)
Budhathoki S et al., [20]	Nepal, 2020	7.8%	61 (76%)	10 (12%)
Dasari R et al., [5]	Hyderabad, 2021	17.1%	18 (32.17%)	37 (67.3%)
Dudeja N et al., [21]	New Delhi, 2022	6.67%	68 (85%)	10 (12.5%) (02- co-infection)
Present study	Ghaziabad, Uttar Pradesh 2023	11.3%	67 (76.14%)	21 (23.86%)

[Table/Fig-4]: Comparison of incidence of enteric fever [5,17-21].

The incidence rate of *Salmonella enterica* in present study was 11.3% which was higher to 2.38% as reported by Prajapati B et al., 7.8 % by Budhathoki S et al., and 6.67% by Dudeja N et al., [18,20,21]. However, Dasari R et al., (17.1%) Sinha A et al., (11.7%) and Ghosh T et al., (20.92%) obtained the higher incidence rate [5,17,19]. Over the course of the study's 12 month timeframe, 88 different *Salmonella* isolates were isolated. The male to female ratio was 2.38:1, which is almost identical to research done by Saraswat S et al., (2.8:1) and Tewari R et al., (2.57:1) [16,22]. Gender preference may be linked to patriarchy, in which males are more likely to be valued than girls and are hence more likely to be taken to the hospital. This claim, as well as the likelihood that boys and girls use the restroom differently, behave differently, or are exposed to the outdoors, have not yet been confirmed [23].

This study also revealed that incidence was higher in the summer, particularly during the rainy season (54.5%), which was consistent with the findings of Prajapati B et al., (47.7%) [18]. This occurs because the sewage system overflowed during the rainy season, which may contaminate the environment and drinking water with fecal matter, leading to infection. The majority of the isolates were detected in children aged 7 to 10-year-old, which was almost identical to the findings of study by Judio MP et al., (59.1%) and Saleem S et al., (52.2%), which revealed that those aged 5 to 10-year-old were most commonly affected [23,24]. This age group

was impacted, maybe as a result of eating unhygienic street food, using school water, or not knowing how to properly wash their hands. *Salmonella* Typhi was most sensitive to imipenem (98.5%) followed by cotrimoxazole (97%), ceftazidime (92.5%) and ceftriaxone (89.5%). A study conducted by Prajapati B et al., which showed 93.5% isolates were sensitive to cotrimoxazole, 98.5% isolates sensitive to ceftriaxone which was higher than present study [18]. In case of *S. Paratyphi A*, resistance to ceftriaxone was higher (33.33%) as compared with *S. Typhi*. Azithromycin can be a drug of choice. Tiwari R et al., and Gautam V et al., reported 90.8% and 100% sensitivity to azithromycin, respectively [22,25]. Azithromycin sensitivity in this investigation ranged between 77.6% and 71.4% for *S. Typhi* and *S. Paratyphi A*, respectively. Although, fluoroquinolones have been restricted in children due to potential cartilage damage, is still the drug of choice for treating enteric fever and widely used due to their moderate cost, oral intake benefit and tolerance. This led to declines in their sensitivity and poor clinical efficacy, which compelled doctors to employ third generation cephalosporins [21]. A 86.6% of the isolates of *S. Typhi* and 90.5% of the isolates of *S. Paratyphi A*, respectively, were nalidixic acid resistance, which was used as a surrogate marker for predicting low level ciprofloxacin resistance among *Salmonella* species. This finding was somewhat similar to a study by William A et al., which showed 98.4% *S. Typhi* isolates and 99.1% *S. Paratyphi A* isolates were nalidixic acid resistance [15].

Different geographic regions or population genetic variability may be the cause of the variance in the research of antibiotic susceptibility [15,26]. The persistence of clinical symptoms or a delayed response to treatment may be linked to the resistant strains of ciprofloxacin, nalidixic acid and pefloxacin. In the event, that a ciprofloxacin, levofloxacin Minimal Inhibitory Concentration (MIC) test cannot be performed pefloxacin disc diffusion may be used as a surrogate test [15]. Although, the precise mechanism of fluoroquinolone resistance in *Salmonella* isolates is not entirely understood, numerous findings indicate that ciprofloxacin resistance is at a high level. According to some research, a single mutation in the gyr A gene is enough to cause nalidixic acid resistance and reduced susceptibility to ciprofloxacin [15]. Ceftriaxone was suggested by the Indian Academy of Paediatrics as the first-line of treatment for complex typhoid fever. For *S. Typhi* and *S. Paratyphi A*, respectively, around 10.4% and 33.33% of the population showed resistance in this study which was higher than the study conducted by, Jha G et al., (10%) Saraswat S et al., (0%), Prajapati B et al., (1.1%, 2.6%) [7,16,18]. Concerns are raised about their use without proper antibiotics susceptibility testing. It is obvious that it is time to review our treatment plans in view of the emergence of strains that are resistant to ceftriaxone and azithromycin. Patients who have such strains of infection are more likely to develop complications and require antibiotic therapy with more modern drugs.

Limitation(s)

This study was an entirely on culture positive enteric fever cases where consideration of the actual therapeutic intervention and clinical outcome of the patients were not taken into account. The samples were obtained from a tertiary care hospital only, not from peripheral health centres. Therefore, the results may not be applicable to other geographical areas. Also, molecular characterisation of ceftriaxone resistant strains could not be determined due to limited resources.

CONCLUSION(S)

In developing nations, enteric fever is still a serious public health issue that primarily affects school aged children. Good personal hygiene practises, appropriate sanitation and knowledge of the disease and its transmission should all be observed. The drinking water supply in schools, especially in government institutions, should be checked on a regular basis. Students need to understand the advantages of proper hand washing. Parents should encourage their kids not

to eat any junk food outside. Additionally, typhoid vaccination and prudent antibiotic administration based on the culture sensitivity pattern will aid in lowering the disease's impact.

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PARTICULARS OF CONTRIBUTORS:

1. PhD Scholar, Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India.
2. Professor, Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India.
3. Professor and Head, Department of Microbiology, Army College of Medical Sciences, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Mr. Sanjay Singh Kaira,
PhD Scholar, Department of Microbiology, Santosh Medical College and Hospital,
Ghaziabad, Uttar Pradesh, India.
E-mail: sanjay.singh4055@gmail.com

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