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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF SALMONELLA SPP. AMONG BLOOD CULTURE–POSITIVE ISOLATES IN A TERTIARY CARE PEDIATRIC HOSPITAL IN THE NATIONAL CAPITAL REGION DELHI DURING THE COVID-19 PANDEMIC.

Microbiology

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ABSTRACT

Background Drug resistant Salmonella poses a challenge for the treatment of typhoid fever globally. Understanding the current antimicrobial susceptibility pattern of Salmonella in the National Capital Region Delhi will help to understand the prevalent antibiotic resistance in this population in this region. **Methods** This study included a total of 366 children with suspected typhoid fever from 2020 to 2021. The automated BACT/ALERT system was used to perform blood culture. Antimicrobial susceptibility was determined with the Vitek 2 Compact. Serotyping was done by Salmonella antisera. **Results** Out of 366 isolates, 31 were identified as Salmonella spp., comprising 24 isolates of S.Typhi and seven isolates S.Paratyphi. Two isolates were multidrug-resistant, whereas the rest were susceptible to at least one first-line antibiotics (i.e., ampicillin, cotrimoxazole, or chloramphenicol). Among the fluoroquinolones, the rates of ciprofloxacin, pefloxacin, and nalidixic acid resistance were 70.9%, 80.6%, and 96.7%, respectively. Among the third-generation cephalosporins, ceftriaxone and cefotaxime were sensitive in 96.7% and 83.3% of isolates, respectively. Only 42 % of the isolates were sensitive to azithromycin, whereas 58% were resistant. Among the carbapenems, all but one isolate was found to be sensitive, as it was intermediate to imipenem. **Conclusions** Our results showed an increase in susceptibility for first-line antibiotics due to prolonged non-use. The higher rate of azithromycin resistance can be attributed to over-use during the COVID pandemic, which led to resistant strains being more prevalent in the study region. It requires the immediate attention of microbiologists, clinicians, and policymakers. The one imipenem intermediate isolate is a cause of concern, as Carbapenem Resistant Enterobaterales is already currently a big treatment challenge.

KEYWORDS

Salmonella spp., *Salmonella* Typhi, *Salmonella* Paratyphi A, antimicrobial resistance, typhoid fever, children, North India, Noida, azithromycin

INTRODUCTION

Typhoid is a public health problem in India [1]. It is one of the common causes of fever in the pediatric population [2], with the National Family Health Survey 2019 indicating that children bear the highest burden of the disease [3]. *Salmonella* has shown resistance to various classes of antibiotics in the past, such as fluroquinolone, ampicillin, and cotrimoxazole [4]. Resistance to cephalosporins and ciprofloxacin has also recently emerged, such as in Pakistan in 2016, with the increase of extensively drug-resistant (XDR) strains [5]. Increased resistance to carbapenem and macrolide is a worrisome situation, as these two drug classes are reserved for typhoid fever [6].

Globally, India bears the highest burden of disease [7]. Uttar Pradesh has the highest number of typhoid cases in India, with the Uttar Pradesh Noida-Ghaziabad region known for its water and air pollution. As typhoid is a communicable disease that spreads by the feco-oral route [8], children of this region are exposed to many risk factors. As per our literature review, there is no recent study in the Noida region on prevalent antimicrobial resistance patterns among *Salmonella* isolates, especially during the COVID-19 pandemic.

In this study, we aim to analyze the antimicrobial susceptibility pattern of *Salmonella* spp. isolates among pediatric blood culture–positive typhoid cases in the Noida-Ghaziabad region of Uttar Pradesh, India, during the COVID- 19 pandemic. In particular, we focus on the emergence of drug-resistant strains within the region during the COVID-19 pandemic. This article will provide an evidence-based analysis of the current situation concerning the prevalence of typhoid among children in the Uttar Pradesh region, and the associated antimicrobial resistance patterns. Additionally, as the article concerns pediatric typhoid cases, its outcomes can also be used to establish an improved knowledge base from which outbreak-preparedness strategies can be developed to curb the spread of the disease within the region.

MATERIALS & METHODS

This was an observational, analytical, intramural, single-center study, which had both retrospective and prospective arms. We conducted the study at the Department of Microbiology, Post Graduate Institute of Child Health (formerly Super Specialty Pediatrics Hospital & Post Graduate Teaching Institute,), Noida, Uttar Pradesh. We included 104 cases from January 2020 to December 2020 in the retrospective arm based on certain inclusion and exclusion criteria.We retrieved patient files with permission from the Medical Records Department of the hospital. We performed the prospective arm of the study from January 2021 to December 2021 and included 262 cases, as given in Table-1.

Table-1:Total patients enrolled in the study by year.

Year	Enrolled Patients
2020	104
2021	262
Total	366

The study was approved by the hospital's institutional ethics committee, and we obtained informed consent from the patients or their guardians for prospective cases.

We included children <18 years of age with suspected typhoid fever (i.e., fever, white coated tongue, hepato-splenomegaly, and diarrhea/ constipation). We excluded neonates and children with a documented history of culture-positive typhoid fever in the recent past.

Blood samples were obtained from the ward and Out Patient Department as part of routine patient care. The blood was collected in a blood culture bottle under strict aseptic conditions. Blood culture was done using an automated method by incubating BACT/ALERT blood culture bottles in a BACT/ALERT automated culture system (bioMérieux). Automated identification and antimicrobial susceptibility determination was done using a Vitek 2 Compact (bioMérieux). Once identified by the Vitek-2 as *Salmonella* spp, bacteria serotyping was done by *Salmonella* antisera (Denka Seiken Co., Ltd., Japan).

All isolates were subjected to antibiotic susceptibility testing by the Vitek 2 Compact and an extended panel by Kirby Bauer disc diffusion

method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The extended panel of antibiotics included chloramphenicol; azithromycin and pefloxacin; and cefotaxime and ampicillin-sulbactam. Zone size was recorded and interpreted as per the CLSI guidelines.

RESULTS

Out of 366 samples from suspected typhoid patients were included during the study period, 31 (8.4%) were blood culture-positive. Of the 31 positive cultures, 24 (77.4%) were *Salmonella* Typhi and 7 (22.6%) were *Salmonella* Paratyphi A. In the retrospective arm (i.e., 2020), 14 patients were blood culture–positive, whereas 17 patients were blood culture–positive in the prospective arm (i.e., 2021) as given in Table -2.

Table 2 Blood culture-positive isolates by year.

Year	Blood Culture– Positive Isolates
2020	14
2021	17
Total	31

The age groups with the highest proportion of positive cases were <5 years and 6–10 years at 36 %, and 35 % of cases, respectively as given in Table-3, which was similar to the results of Mohanty et al. [9]

Table -3: Age distribution of culture- positive cases.

Age Group (years)	Number
≤5	11
6–10	11
11–15	7
16–18	2

Age group 11-15 years with culture positive 7 cases was 22.58%, whereas 16-18 years 6.45% as shown in Figure-1.

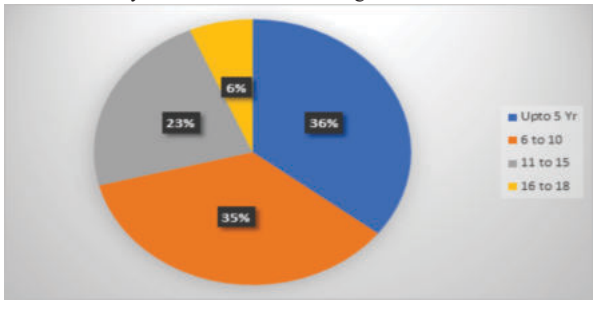

Figure -1 Age distribution

The gender distribution of our study population showed male preponderance. Among the 31 culture positive patients, 17 (55%) were male and 14 (45%) were female children, as given in Table-4.

Table - 4: Gender distribution of positive isolates.

	Male		Female	
	S. Typhi (n)	S Paratyphi (n)	S. Typhi (n)	S. Paratyphi (n)
2020	4	4	6	0
2021	7	2	7	1
Total	17	14		

The study found male preponderance among culture positive cases as given in Figure-2.

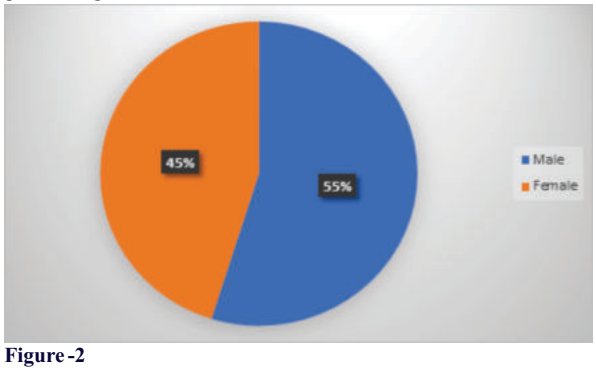

Figure -2

In this study, two isolates (6.45%) demonstrated resistance to all three first-line antibiotics (i.e., ampicillin, chloramphenicol, and cotrimoxazole); therefore, they were labelled as MDR strains. The remaining isolates were found to be susceptible to at least one of these three antibiotics. 80.7% of the isolates were sensitive to ampicillin, and 12.9% were resistant. 87.1% of the isolates were sensitive to cotrimoxazole, and 12.9% were resistant. 80.7% of the isolates were sensitive to chloramphenicol, and 6.4% were resistant as given in Table-5.

Table -5: Antimicrobial Susceptibility Pattern Of Culture-positive Isolates.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin	25 (80.7%)	2 (6.4%)	4 (12.9%)
Cotrimoxazole	27 (87.1%)	0	4 (12.9%)
Chloramphenicol	25 (80.7%)	4 (12.9%)	2 (6.4%)
Ciprofloxacin	5 (16.2%)	4 (12.9%)	22 (70.9%)
Nalidixic Acid	0	1 (3.3%)	30 (96.7%)
Pefloxacin	5 (16.2%)	1 (3.2%)	25 (80.6%)
Ceftriaxone	30 (96.7%)	1 (3.3%)	0
Cefotaxime	26 (83.8%)	5 (16.2%)	0
Azithromycin	13 (42%)	0	18 (58%)
Ertapenem	31 (100%)	0	0
Imipenem	30 (96.7%)	1 (3.3%)	0
Meropenem	31 (100%)	0	0
Ampicillin sulbactam	28 (90.4%)	3 (9.6%)	0
Amoxicillin-clavulanic acid	31 (100%)	0	0
Piperacillin-tazobactam	31 (100%)	0	0
Cefoperazone-sulbactam	31 (100%)	0	0
Cefepime	31 (100%)	0	0

Of the positive isolates, 16% were sensitive to both ciprofloxacin and pefloxacin, whereas 96.7% were resistant to nalidixic acid. Among the *S.* Typhi isolates, 95.8% (23/24) were resistant to nalidixic acid. All seven isolates of *S.* Paratyphi A were resistant to nalidixic acid. 70.9% of the isolates were resistant to ciprofloxacin, and 80.6% were resistant to pefloxacin.

No XDR isolates were detected in our study; however, one isolate was found to be intermediate to ceftriaxone and resistant to ciprofloxacin. There were also five isolates found to be intermediate to cefotaxime. Ceftriaxone sensitivity was found in 96.7% of isolates, and cefotaxime sensitivity was found in 83.8% of isolates.

Azithromycin and carbapenem are World Health Organization (WHO)-reserved category (AWaRe Classification of essential drugs) drugs for typhoid fever. In our study, carbapenem proved to have 100% sensitivity in isolates of typhoid fever, with the exception of one isolate, which was intermediate to imipenem, resulting in an overall imipenem sensitivity of 96.7%. 42% of isolates were sensitive to azithromycin, with the remaining 58% showing resistance. As there is no CLSI zone diameter breakpoint provided for *S.* Paratyphi A, the zone diameter for *S.* Typhi was taken to interpret susceptibility testing.

The sensitivity of ampicillin-sulbactam among the Beta Lactam-Beta Lactam Inhibitor combinations was 90.4%, whereas that of amoxicillin-clavulanic acid, piperacillin-tazobactam, and cefoperazone sulbactam was 100%. Cefepime, a fourth-generation cephalosporin, showed 100% sensitivity.

DISCUSSION

Studies conducted worldwide in the 1990s reported an incidence of MDR S.Typhi of up to 80% [10], during which India saw an incidence of MDR S.Typhi of 7 to 55% [11]. In recent years, the prevalence of MDR strains has been declining, possibly due to discontinued use of these drugs (Ampicillin, Chloramphenicol, Cotrimoxazole) for treating typhoid fever. Another reason could be the loss of plasmids (e.g., R-plasmid) that confer multi-drug resistance, or the emergence of *de novo* susceptible strains. In our study, the prevalence of MDR S.Typhi was 6.45%, which was similar the prevalence of 6% reported by Dahiya et al. [12]. Based on this result, using these antibiotics again for *Salmonella* may be tempting, but this would risk the recurrence of typhoid infection, as phenotypic sensitivity may quickly vanish in presence of resistance-coded bacterial genes. Therefore, genotypic absence of such genes should be confirmed on a regional basis before using these drugs.

Fluoroquinolone was the drug of choice after the emergence of MDR

isolates [13]. However, rampant use led to resistance to this drug class since the 1990s, as described by Rahman et al. [14]. Quinolone resistance is mediated by chromosomal mutations in quinolone resistance-determining regions (QRDR) genes- *gyrA*, *gyrB*, *parC*, and *parE* [15]. In 2015, Fang et al. studied fluoroquinolone resistance in *Salmonella* and the utility of pefloxacin disc diffusion; they reported that pefloxacin disc diffusion provided better separation of ciprofloxacin susceptible and non-susceptible strains than other disk diffusions, including that of ciprofloxacin itself [16]. In our study, 80.6% and 70.9% of isolates were resistant to pefloxacin and ciprofloxacin, respectively. Overall, 96.7% of isolates were resistant to nalidixic acid, and 95.8% were nalidixic acid– resistant S.Typhi isolates.

Third-generation cephalosporins have been the drug of choice for the last decade for enteric fever. In our study, ceftriaxone was found to be sensitive in 96.7% of isolates. 83.8% of the isolates were sensitive to cefotaxime, and none was resistant to these drugs. These findings were similar to those of Sarika et al. [17]. This requires close monitoring of drug resistance against third- generation cephalosporins—not only by phenotypic methods but also genotypic methods—to predict near-future sensitivity patterns, as these are drugs of choice in treating typhoid fever in children, as per Indian Association of Pediatrics Standard Treatment Guidelines 2022 [18]. There have been reports of XDR isolates from Pakistan since 2016, resistant to ceftriaxone and quinolones. In our study, we found no isolate to be XDR.

Carbapenems are another WHO-reserved category (AWaRe classification) of antibiotics. In this study, 100% of the isolates were sensitive to ertapenem and meropenem, whereas 96.7% were sensitive to imipenem, as one isolate was found to be intermediate. No isolate was found to be resistant. The CRE is a bigger picture,. as it is currently one of the greatest treatment challenges. *Salmonella* is a member of the family Enterobacteriaceae, various members of which harbor resistance against carbapenems. Ain et al. reported S.Typhi isolates harboring vim and ges genes for carbapenem resistance from Faisalabad, Pakistan [20]. The emergence of carbapenem-resistant isolates would pose a challenge to treating typhoid in this region in the future. Therefore, we recommend continuous monitoring of carbapenem resistance among *Salmonella* in this region.

Our results showed that only 42% of isolates proved to be sensitive to azithromycin, and the remaining 58% were resistant. Similarly, Khan et .al. reported azithromycin resistance of 16 % [21], which was contrary to studies by Butler et al. [22] and Frenck et al. [23]. This level of resistance could be a result of the soaring usage of azithromycin due to the COVID-19 pandemic, which most likely enabled the proliferation of azithromycin-resistant strains. Epidemiologically, all seven isolates of S.Paratyphi A were found to be resistant to azithromycin, as compared to only 11 out of 24 isolates of S Typhi. Recently, there have been numerous studies on rising azithromycin resistance among *Salmonella*. The rampant over-use of this drug during the COVID-19 pandemic poses a challenge to treating typhoidal infection as azithromycin resistant strains may have emerged in this region. This emergence is worrisome, as azithromycin is one of only two available oral drugs for *Salmonella* infection. Therefore, we strongly recommend continuous genotypic and phenotypic analysis of all *Salmonella* isolates in the Noida Ghaziabad region.

Among the BL-BLI combinations, ampicillin-sulbactam showed sensitivity in 90.4% of the isolates. Others such as amoxicillin clavulanic acid, piperacillin-tazobactam, and cefoperazone-sulbactam showed sensitivity in 100% of the isolates. Fourth-generation cephalosporin cefepime showed a sensitivity of 100 %.

We acknowledge certain limitations of our study. During the COVID-19 pandemic, our hospital was declared a COVID hospital, which reduced the number of patients coming to our hospital at that time. This effect led to a small sample size in our study. Further research is required to verify our assumption that over- use of azithromycin during the COVID-19 pandemic gave rise to resistant strains. Furthermore, we could not perform geotropic analysis for drug resistance on our isolates. CLSI does not provide Minimum Inhibitory Concentration and zone diameter breakpoint for azithromycin in *Salmonella*, nor for Paratyphi serotypes.

CONCLUSION

This study provides insight into the antimicrobial susceptibility pattern of *Salmonella* isolates among pediatric patients in the Noida region during the COVID-19 pandemic. Resistance to azithromycin in this region must be addressed immediately, and resistance to carbapenem and third-generation cephalosporins should be continuously monitored. To ensure that real-time information on drug resistance is accurately captured, we advocate for an integrated reporting and surveillance system for *Salmonella* spp. in the Noida region, as this may facilitate timely intervention from microbiologists, clinicians, and policy-makers in response to this public health issue.

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REFERENCES

1. Cao Y, Karthikeyan AS, Ramanujam K, Raju R, Krishna S, Kumar D, Ryckman T, Mohan VR, Kang G, John J, Andrews JR, Lo NC. Geographic Pattern of Typhoid Fever in India: A Model-Based Estimate of Cohort and Surveillance Data. J Infect Dis. 2021 Nov 23;224(224 Supple 5):S475-S483. doi: 10.1093/infdis/jiab187. PMID: 35238365; PMCID: PMC8892532.
2. Crump JA, Luby SP,Mintz ED.2004.The global burden of typhoid fever.Bull World Health 82: 346-353.
3. National Health Profile 2019, DGHS, Ministry of Health and Family Welfare, New Delhi.
4. Rowe B,Ward LR,Threlfall EJ.Multidrug-resistant Salmonella typhi :a worldwide epidemic .Clin Infect Dis.1997;24:106-9.
5. Klemm EJ, Shakoor S, Page AJ, et al. Emergence of an extensively drug-resistant Salmonella enterica serovar typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio 2018; 9:e00105-18.
6. Sajib MSI, Tanmoy AM, Hooda Y, et al. Tracking the emergence of azithromycin resistance in multiple genotypes of typhoidal salmonella. MBio 2021; 12: 1–12.
7. John J, Bavdekar A, Rongsen-Chandola T, Dutta S, Gupta M, Kanungo S, Sinha B, Srinivasan M, Shrivastava A, Bansal A, Singh A, Koshy RM, Jinka DR, Thomas MS, Alexander AP, Thankaraj S, Ebenezer SE, Karthikeyan AS, Kumar D, Njarekkattuvalappil SK, Raju R, Sahai N, Veeraraghavan B, Murhekar MV, Mohan VR, Natarajan SK, Ramanujam K, Samuel P, Lo NC, Andrews J, Grassly NC, Kang G; NSSEFI Study Team. Burden of Typhoid and Paratyphoid Fever in India. N Engl J Med. 2023 Apr 20;388(16):1491-1500. doi: 10.1056/NEJMoa2209449. PMID: 37075141; PMCID: PMC10116367.
8. https://www.cdc.gov/typhoid-fever/health-professional.html accessed on 17-12-22
9. Mohanty S,Renuka K,Sood S,Das BK,Kapil A.Antibiogram pattern and seasonality of Salmonella serotypes in a North Indian tertiary care hospital.Epidemiol Infect.2006;134:961-3.
10. Rowe B, Ward LR, Threlfall EJ. Multidrug-resistant Salmonella typhi: a worldwide epidemic. Clin Infect Dis. 1997 Jan;24 Suppl 1:S106-9. doi: 10.1093/clinids/24.supplement_1.s106. PMID: 8994789.
11. Renuka K,Mohanty S,Sood S,Das BK,Kapil A.Antibiogram pattern and seasonality of Salmonella serotypes in a North Indian tertiary care hospital.Epidemiol Infect 2006 ; 14;1-6.
12. Dahiya S, Kapil A,Lodha R,Kumar R,Das BK,Sood S et al.,Induction of resistant mutants of Salmonella enterica serotype Typhi under ciprofloxacin selective pressure.Indian J Med Res2014; 139: 746-53.
13. R Leon Ochia , Camilo Acosta , M Carolina Danovaro – Holiday ,et al . A Study of typhoid fever in five Asian countries : disease burden and implications for controls . Bulletin of the World Health Organisation .
14. Emergence of multidrug-resistant Salmonella enterica serotype Typhi with decreased ciprofloxacin susceptibility in Bangladesh. Epidemiol Infect 2006;134: 433-438.
15. V eeraraghavan B,Anandan S,Sethuvel DP,Ragupathi NK.Pefloxacin as a surrogate marker for fluroquinolone susceptibility for Salmonella Typhi : Problems and prospects. J Clin Diagn Res 2016; 10: DLO1-2.
16. Fang FC,Fluroquinolone resistance in Salmonella and the utility of Pefloxacin Disk Diffusion.J Clin Microbiol, 2015;53:3401-04.
17. Jain S, Das Chugh T. Antimicrobial resistance among blood culture isolates of Salmonella enterica in New Delhi. J Infect Dev Ctries. 2013 Nov 15;7(11):788-95. doi: 10.3855/jidc.3030. PMID: 24240035.
18. https://iapindia.org/pdf/Ch-008-Enteric-Fever.pdf IAP STANDARD TREATMENT GUIDELINES 2022 accessed on 20.05.2023
19. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, Wong VK, Dallman TJ, Nair S, Baker S, Shaheen G, Qureshi S, Yousafzai MT, Saleem MK, Hasan Z, Dougan G, Hasan R. Emergence of an Extensively Drug-Resistant *Salmonella enterica* Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins. mBio. 2018 Feb 20;9(1):e00105-18. doi: 10.1128/mBio.00105-18. PMID: 29463654; PMCID: PMC5821095.
20. Ain Q, Tahir M, Sadaqat A, Ayub A, Awan AB, Wajid M, Ali A, Iqbal M, Haque A, Sarwar Y. First Detection of Extensively Drug-Resistant *Salmonella* Typhi Isolates Harboring *VIM* and *GES* Genes for Carbapenem Resistance from Faisalabad, Pakistan. Microb Drug Resist. 2022 Dec;28(12):1087-1098. doi: 10.1089/mdr.2022.0094. PMID: 36534486.
21. Khan S, Kurup P, Vinod V, Biswas R, Pillai GK, Kumar A. Reconsidering azithromycin disc diffusion interpretive criteria for *Salmonellae* in view of azithromycin MIC creep among typhoidal and nontyphoidal salmonella. J Lab Physicians. 2019 Jan-Mar;11(1):39-44. doi: 10.4103/JLP.JLP_99_18. PMID: 30983801; PMCID: PMC6437832.
22. Butler T, Sridhar CB, Daga MK, Pathak K, Pandit RB, Khakhria R, Potkar CN, Zelasky MT, Johnson RB. 1999. Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicentre trial in India. J Antimicrob Chemother 44:243–250.
23. Frenck RW, Nakhla IA, Sultan Y, Bassily SB, Girgis FY, David J, Butler TC, Girgis NI, Morsy M. 2000. Azithromycin versus ceftriaxone for the treatment of uncomplicated typhoid fever in children. Clin Infect Dis31:1134–1138.