

RECURRENCE OF SUSCEPTIBILITY TO FIRST LINE DRUGS IN ENTERIC FEVER IN AN ERA OF ANTIMICROBIAL RESISTANCE: AN OLD WEAPON TO FIGHT AGAINST ENTERIC FEVER

Medical Microbiology

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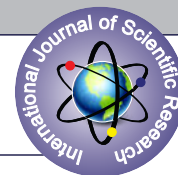
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ABSTRACT

Introduction- Enteric fever is still a major public health problem in developing countries including India despite the availability of drugs for the treatment. First line drugs ampicillin, co-trimoxazole and chloramphenicol have not been part of empirical therapy after the multidrug Salmonella stains emerged. The aim of this study was to evaluate the re-emergence of susceptibility of Salmonella stains towards first line drugs. **Methodology-** A cross sectional was conducted in a tertiary care hospital, Ghaziabad, Uttar Pradesh from July 2021 to August 2022. Standard Microbiological procedures were followed for collection, processing and identification. Susceptibility testing was determined as per CLSI guidelines. **Results-** A total of 228(12.28%) isolates were identified as Salmonella enterica. Out of this 164 (71.9%) S. Typhi and 64 (28.1%) were S. Paratyphi A. Only 37.8% S. Typhi and 20.3% S. Paratyphi A showed susceptibility to ciprofloxacin. In case of S. Typhi 20.12 % and in S. Paratyphi A 21.87% showed resistance towards ceftriaxone. Ampicillin, co-trimoxazole, chloramphenicol resistance detected in S. Typhi as 11.58%, 6.7% & 4.87% respectively & in S. Paratyphi A as 32.8%, 12.5 & 12.5% respectively. 07 S. Typhi isolates were emerged as Multi drug resistance (MDR). However, in case of S. Paratyphi A only 03 isolates were MDR. **Conclusions-** This study showed decreasing susceptibility pattern of fluoroquinolones and ceftriaxone. However, re-emergence of susceptibility to conventional first line drugs needs re-evaluation in the management of Enteric fever & supports the possibility of adding these drugs in empirical therapy.

KEYWORDS

MDR, First line drugs, Ceftriaxone, Re-emergence, Re- evaluation

INTRODUCTION-

Enteric fever is prevalent all over the world and continues to be a major health problem in developing countries including India, Nepal, Pakistan and African countries with inadequate cleanliness and safe drinking water.¹ Salmonella enterica serovar Typhi and Paratyphi A, B & C are mainly responsible which transmits the infection by contaminated food and water.² It is estimated that about 20 million new cases of typhoid fever occur worldwide annually. Annual mortality rate caused by Typhoid fever has between 1990 and 2020 is increased by 39%.³ In India, Salmonella Typhi is predominant species causing enteric fever but Salmonella Paratyphi A infection is also showing increasing trends as reported by Ashoka et al.⁴ In case of Enteric fever it is often necessary to give empirical treatment before the antimicrobial sensitivity report become available and in this respect, Ciprofloxacin has become first line of treatment after the emergence of resistance in late 1980s to traditional first line drugs like ampicillin, cotrimoxazole and chloramphenicol known as multi drug resistant Salmonella Typhi (MDRST)⁵ and this was linked to advent of the H58 haplotype that carried on IncHI1 plasmid with multiple resistance genes.⁶

However this switch to ciprofloxacin and indiscriminate use of fluoroquinolones both in human and animal therapeutics has led to emergence of Nalidixic acid resistant Salmonella Typhi (NARST) leaving ceftriaxone and azithromycin as last option.⁷ Still in developing countries, ciprofloxacin continues to be the mainstay for the treatment of enteric fever as it is orally effective and economical. Over the time, ceftriaxone resistant Salmonella stains (CRST) have started reported from various countries including India. Mahajan et al. in his study reported around 12.4 % isolates were ceftriaxone resistant. This is alarming as it leaves few therapeutic options for the management of enteric fever.⁸

In recent years, the re-emergence of susceptibility to ampicillin, chloramphenicol and co-trimoxazole has been reported from various parts of world including India.⁹ In this era where therapeutic option for the management of enteric fever is limited after the emergence of extended drug resistance (XDR) in some countries like Pakistan, US. However, notable reports indicating re-emergence of susceptibility towards conventional first line drugs have raised a hope in this antimicrobial resistance era.

This trend of downside of MDR Salmonella strains and revival of conventional first line drugs need to be re-evaluated to determine the therapeutic importance of these drugs. Therefore, this study was

undertaken to determine antibiotics susceptibility pattern of the Salmonella serovars isolated from blood so that appropriate steps could be adopted in the management of enteric fever.

MATERIALANDMETHODS –

This cross-sectional study was carried out from July 2021 to August 2022 in the Department of Microbiology at a tertiary care hospital in Uttar Pradesh. The ethical approval was received from Institute prior to the study ((Reference No: SU/2021/092[4]). The Salmonella enterica isolates recovered from the blood sample of patients with suspected enteric fever during the period of 14 months were included in this study. However duplicate samples and Salmonella enterica isolated from samples other than the blood were not included.

All the blood culture samples of patients with febrile illness were processed in BacT/Alert blood culture system (Biomérieux, France) and incubated for a period of 5 days after which the sample was labelled sterile. A positive flagged bottle was sub-cultured on Blood agar and MacConkey agar subsequently and incubated overnight at 37°C. Non lactose fermenting colonies from MacConkey agar were further processed by standard Microbiological procedures and confirmed by agglutination with Salmonella polyvalent O and specific H antisera.

Antimicrobial susceptibility testing of isolated Salmonella serovars were determined by the Kirby-Bauer disk diffusion method in Mueller Hinton agar (Hi-media) against antibiotics disks of Ampicillin (10µg), chloramphenicol (30µg), co-trimoxazole (25µg), ciprofloxacin (25µg), nalidixic acid (30µg), pefloxacin (5µg), ceftriaxone (30µg), ceftazidime (30µg), azithromycin (30µg), cefoxitin (30µg) and imipenem (10µg). Results were interpreted as per Clinical and Laboratory Standard Institute guidelines (CLSI).

RESULT:-

During the study period, a total of 1856 blood samples were processed, out of which 228(12.28%) isolates were identified as Salmonella enterica. Out of this 164 (71.9%) S. Typhi and 64 (28.1%) were S. Paratyphi A. No Salmonella Paratyphi B and C were isolated. Salmonella isolates were mainly obtained from patients attending the OPD (58.8%) in comparison with patients admitted in the different wards (41.2%). Most of the Salmonella were isolated in the month of June and July with the majority being in the age group 11-20 years followed by 21-30 years with male majority as shown in fig 01.

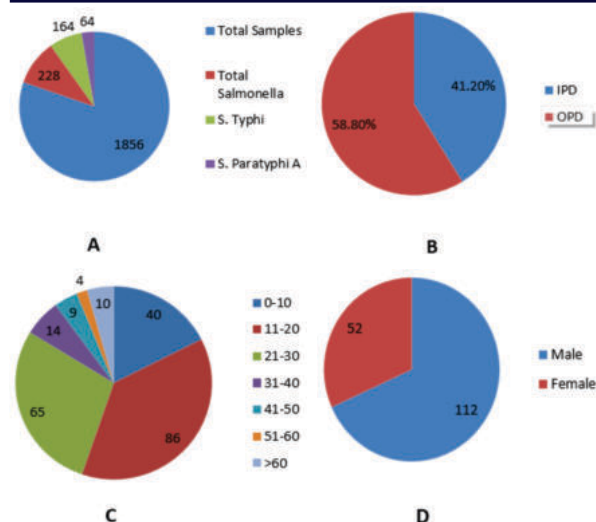


Fig 01.The distribution of Enteric fever cases according to (a) *Salmonella* serotype isolation (b) cases in OPD and IPD (c) age wise distribution (d) gender wise distribution

The antimicrobial susceptibility pattern of the *Salmonella* isolates showed decreased susceptibility to ciprofloxacin which is about 37.8% in *S. Typhi* and 20.3% in *S. Paratyphi A*. In case of *S. Typhi* 20.12 % and in *S. Paratyphi A* 21.87% showed resistance towards ceftriaxone. Ampicillin, co-trimoxazole, chloramphenicol resistance was detected in *S. Typhi* as 11.58%, 6.7% & 4.87% respectively & in *S. Paratyphi A* as 32.8%, 12.5 & 12.5% respectively (Table 1 & 2). *S. Paratyphi A* showed more resistance to first line drugs as compared to *S. Typhi*.

Table 01-Antibiotics sensitivity of *Salmonella Typhi* (n-164)

Antibiotics	Concentration in µg	Sensitive	Intermediate	Resistance
Ampicillin (AMP)	10 µg	142 (86.6%)	03 (1.8%)	19 (11.58%)
Cotrimoxazole (COT)	25 µg	153 (93.3%)	-	11 (6.7%)
Chloramphenicol (CH)	30 µg	155 (94.5%)	03 (1.8%)	08 (4.87%)
Ceftriaxone (CTR)	30 µg	122 (74.4%)	09 (5.5%)	33 (20.12%)
Ceftazidime (CAZ)	30 µg	126 (76.8%)	06 (3.66%)	32 (19.5%)
Azithromycin (AZM)	15µg	142 (86.6%)	-	22 (13.4%)
Nalidixic acid (NA)	30 µg	27 (16.5%)	-	137(83.5%)
Pefloxacin (PE)	5 µg	32 (19.5%)	-	132 (80.5%)
Ciprofloxacin (CIP)	5 µg	62 (37.8%)	17 (10.4%)	85 (51.83%)
Cefoxitin (CX)	30 µg	152 (92.68%)	-	12 (07.32%)
Imipenem (IMP)	10 µg	163 (99.4%)	-	01 (0.61%)

Table 02-Antibiotics sensitivity of *Salmonella Paratyphi A* (n-64)

Antibiotics	Concentration in µg	Sensitive	Intermediate	Resistance
Ampicillin (AMP)	10 µg	42 (65.6%)	1 (1.6%)	21 (32.8%)
Cotrimoxazole (COT)	25 µg	56 (87.5%)	-	08 (12.5%)
Chloramphenicol (CH)	30 µg	54 (84.4%)	02 (3.1%)	08 (12.5%)
Ceftriaxone (CTR)	30 µg	46 (71.9%)	04 (6.3%)	14 (21.87%)
Ceftazidime (CAZ)	30 µg	46 (71.9%)	02 (3.1%)	16 (25%)
Azithromycin (AZM)	15µg	56 (87.5%)	-	08 (12.5%)

Nalidixic acid (NA)	30 µg	02 (3.1%)	-	62 (96.8%)
Pefloxacin (PE)	5 µg	04 (6.25%)	-	60 (93.8%)
Ciprofloxacin (CIP)	5 µg	13 (20.3%)	10 (15.6%)	41 (64.1%)
Cefoxitin (CX)	30 µg	58 (90.6%)	-	06 (9.8%)
Imipenem (IMP)	10 µg	64 (100%)	-	0

07 *S. Typhi* isolates were emerged as Multi drug resistance (MDR). However, in case of *S. Paratyphi A* only 03 isolates were MDR.

DISCUSSION:-

Enteric fever is still a major public health concerns especially in developing countries like India, Nepal due to lack of safe drinking water supply, unhygienic practices, cross contamination of drinking water with sewage mainly in rainy seasons due to over flooding. It is essential to determine the susceptibility pattern of particular *Salmonella* isolates before prescribing drugs due to wide variation in the sensitivity pattern of different geographical areas. Therefore, we attempted to evaluate the sensitivity pattern of *Salmonella* serovar isolated from blood samples at a tertiary care hospital to understand the circulating trends of resistance pattern that would be helpful in rationalising proper treatment regime for enteric fever.

In this study, *S. Typhi* was the predominant serovar as compared to *S. Paratyphi A*. This is accordance with the study conducted in different parts of India.^{1,10,11} However, some study did not agree which showed *S. Paratyphi A* is more predominant.^{4,12}

Table 03 Shows Comparison Of Susceptibility Towards First Line Drugs & Ciprofloxacin In *S. Typhi* And *S. Paratyphi A*

Study	Year	Region	AMP		CH		COT		CIP	
			ST (%)	SPA (%)	ST (%)	SPA (%)	ST (%)	SPA (%)	ST (%)	SPA (%)
Taneja J et al. ¹⁰	2022	Haryana	76.8	0	74.3	82.6	78.5	95.6	12.4	17.3
Khanal PR e al. ¹	2017	Nepal	94.6	100	100	100	100	100	21.6	57.1
Veerara ghavan B et al. ¹¹	2021	Vellore	97.3	100	96.1	100	96.4	100	2.1	1.1
Behl P et al. ¹⁴	2017	Chandigarh	50	12.5	100	100	97.4	100	26.9	50
Kumar AP et al. ¹³	2018	Kerala	89.9	92.9	89.5	100	89.5	100	00	00
This study	2022	Ghaziabad	86.9	65.6	94.5	84.4	93.3	87.5	37.8	20.3

Table 3. AMP- Ampicillin, CH- Chloramphenicol, COT- Co-trimoxazole, CIP- Ciprofloxacin, ST- *Salmonella Typhi*, SPA- *Salmonella Paratyphi A*

Chloramphenicol, Ampicillin and co-trimoxazole were used to treat patient suffering from enteric fever till multi drug resistant *Salmonella* emerged in 1980s. After MDR emerged, fluoroquinolones became the drug of choice as it is cost effective and administered orally. They were uncontrollably used for treatment of enteric fever as well as for other infectious diseases in India & also in other countries. For this reason strain with reduced susceptibility to ciprofloxacin & other fluoroquinolones due to mutation in Quinolone resistant determining region (QRDR) emerged.¹⁵

In this study, 86.9 % of the *S. Typhi* isolates were sensitive to ampicillin, 94.5 % of them to chloramphenicol and 93.3 % to co-trimoxazole. Similarly, 65.6% of the *S. Paratyphi A* isolates were susceptible to ampicillin, 84.4 % to chloramphenicol and 87.5% to co-trimoxazole. Susceptibility of *Salmonella* isolates towards conventional first line drugs has increased over the time as also reported by other studies too (Table 3). However susceptibility towards fluoroquinolones and rate of multi drug resistant decreased over the years. Hence routine surveillance of resistant determinants is mandatory to understand the comeback of conventional first line drugs used for the treatment of enteric fever.

CONCLUSION-

The study shows a distressing increase of ceftriaxone resistant isolates. Incidence of Paratyphoid fever cases is also increasing hence future

vaccination strategies should include bivalent vaccines with protection against both *S. Typhi* as well as *S. Paratyphi A* & adhere to safe food and water practices. There is increasing in minimum inhibitory concentration (MIC) to ciprofloxacin ($\geq 4\mu\text{g/ml}$) & ceftriaxone ($\geq 32\mu\text{g/ml}$) and re-emergence of sensitivity to ampicillin, chloramphenicol and cotrimoxazole in northern India. This reemphasizes the necessity of continuous surveillance of antibiograms of *Salmonella* isolates to rationalize treatment protocols.

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