International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article.

Changing Resistance Pattern and Emerging *Salmonella* Paratyphi A Enteric Fever – New Challenges and New Trends: A Study from Tertiary Care Medical College and Hospital in North India

Rana Aditya[1], Chaudhary Anuradha[2*], Gupta Veeteeanveshna[1], Tamrakar Meenakshi[1]

[1]Junior Resident, [2*]Senior Resident, Department of Microbiology,
Dr. Rajendra Prasad Government Medical College, Kangra, Tanda, Himanchal Pradesh, India.

ABSTRACT

Background: Enteric fever or Typhoid fever is caused mostly by *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi is an important public health challenge for India, especially with the spread of antimicrobial resistance. The situation is further complicated by increased incidence in some parts of the country of *S.* Paratyphi A as a cause of enteric fever. This serovar is not prevented by currently available typhoid vaccines and represents an increasing threat to human health.

Purpose: The present study was undertaken to analyse the trend in prevalence of culture-positive typhoid fever during the last three years and to determine antimicrobial susceptibility profile of *Salmonella* Typhi and *Salmonella* Paratyphi A isolated from patients of enteric fever in this part of the country.

Material: This retrospective study incorporates a three years, (January 2015-December2018) laboratory data comprising 52isolates of Salmonella. Cultures were identified by standard methods. Antimicrobial susceptibility was done against chloramphenicol, amoxicillin, co-trimoxazole, ciprofloxacin, ceftriaxone, cefixime and azithromycin as per corresponding CLSI guidelines for each year.

Results: *Salmonella enterica* serotype Typhi (*S.* Typhi) was the more frequent serotype isolated i.e., 73% with the remaining 27% being *Salmonella enterica* serotype Paratyphi A (*S.* Paratyphi A). There was emergence of *S.* Paratyphi A serotype in 2017-18. Antimicrobial susceptibility for chloramphenicol, amoxicillin and co-trimoxazole, ciprofloxacin and ceftriaxone was found to be 97.2%, 88.5%, 90.5%, 66.8%, 99.4% for *S.* Typhi and 100%, 90.1% and 92.8%, 71.5%, 100% for *S.* Paratyphi A.

Conclusion: The present study confers *Salmonella* Paratyphi A as the rapidly emerging pathogen of enteric fever. The antibiogram of *Salmonella* Typhi and *Salmonella* Paratyphi A showed decreased susceptibility to fluoroquinolones and a notable decrease in the multi drug resistant strains of Salmonella isolates with re-emergence of susceptibility to first line antibiotics.

Keywords: Antimicrobial Susceptibility, Antibiogram, Resistance, *Salmonella* Paratyphi A Enteric Fever.

***Correspondence to:**

Dr. Anuradha Chaudhary,
Senior Resident,
Department of Microbiology,
Dr. Rajendra Prasad Government Medical College,
Kangra, Tanda, Himanchal Pradesh, India.

Article History:

Received: 06-02-2019, **Revised:** 03-03-2019, **Accepted:** 27-03-2019

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2019.5.2.013	

INTRODUCTION

Enteric fever is a major public health problem causing an estimated 11.9 to 26.9 million cases and 129 000 to 217 000 deaths worldwide each year The Indian subcontinent bears the brunt of the disease, both in terms of absolute case numbers and drug resistant strains.[1] It is caused mostly by *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi with *S.* Typhi predominating. Around 5 million cases occur annually in India.[2,3] There has been an upsurge in the occurrence of enteric fever due to *Salmonella* Paratyphi A and variation in the antimicrobial susceptibility pattern from various parts of India.[3-5] since 1996, the frequency of isolation of *Salmonella paratyphi* has been increased in comparison with *Salmonella typhi* in Indian scenario.[4] High degree of clinical suspicion leading to the evaluation of even a low grade fever, vaccination to *Salmonella* Typhi and the possible presence of carrier state, especially in food handlers could be the important contributing factors for the upsurge of paratyphoid fever.[2]

Since 1948, chloramphenicol usage decreased case fatality from 20% in pre antibiotic era to 1%.[1] Resistance was first reported from Britain (1950) and India (Kerala) since 1972.[5]

Multi-drug resistant (MDR) EF (enteric fever) subsequently emerged with epidemic of MDR *S.* Typhi in 1972 in Mexico and these strains are prevalent in India since 1989. Quinolones emerged as drug of choice. By the year 2000, clinical failure to ciprofloxacin was reported due to the nalidixic acid resistant *S.* Typhi (NARST) phenotypes causing enteric fever.[3,6] Since these strains were reported as ciprofloxacin susceptible by the laboratories as per the CLSI guidelines available that time, ciprofloxacin continued to be the first line of treatment. The culture-positive cases increased again during that time. When ciprofloxacin clinical failure and reports of NARST phenotypes causing enteric fever increased, cephalosporins (ceftriaxone in this case) became the choice of antibiotic to treat enteric fever. There were reports of gradual increase of mean MIC of the cephalosporins in the *S. Typhi* isolates from different parts of the world which later became obvious with clinical failures with ceftriaxone. A major problem in the last 2 decades of the 20th century has been the emergence of plasmid-encoded MDR, especially to the quinolones.[5,6] Gravity of problem is escalating in India. Changing resistance pattern, emergence of MDR and nalidixic acid resistant Salmonella t*yphi* (NARST) and switching serotype spectrum.[5-7]

The present study was carried out to study the rate of isolation and the antimicrobial susceptibility pattern of *Salmonella* Paratyphi A in comparison to *Salmonella* Typhi from cases of enteric fever in our region.

METHODS

A retrospective study was conducted from january2015 to December 2018 on blood samples received from suspected cases of enteric fever, septicaemia and PUO (pyerixia of unknown origin) in the Department of Microbiology, DRPGMC & Hospital, Kangra at Tanda.

Sample Processing

Clinical samples of blood were collected in brain heart infusion broth under sterile precautions and incubated aerobically at 37^0 C for 18-24 hours. Two subcultures were done on blood agar (BA) and Mac Conkey's agar (MA) after 24 hours and 72 hours, incubated aerobically at 37^0 C. Growth was identified by using standard methods.[8] On MacConkey agar, the nonlactose-fermenting translucent colonies were subjected for identification as per the standard procedures.[7] *Salmonella* spp. was identified as Gram-negative, motile, glucose, mannitol fermenting, sucrose and lactose nonfermenting, catalase positive, oxidase negative, indole negative, MR positive, citrate negative, urease negative, and H_2S and lysine positive in *S. typhi* and negative in *S* Paratyphi. *Salmonella* isolates were further confirmed by serotyping using group specific antisera (CSIR, Kasauli)).[8] Antibiotic susceptibility was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines for the corresponding years using commercially available disks (Hi-media, Mumbai):[9] ampicillin (10 µg), co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg) and ceftriaxone (30 µg). *Escherichia coli* ATCC 25922 was used as the quality control strain.

Statistical Analysis

The data was analysed by using the Chi Square test and the Fisher exact test depending whether the sample size was more than or less than five respectively.

RESULTS

During the three year study period, a total of 52 cases of enteric fever were culture confirmed. Out of these, 34(65%} were from male patients and 18{35%} from females, with a female to male ratio of 1:1.56.(Figure 1).

S. Typhi was the predominant serotype isolated in 38 (73%) cases whereas *S.* Paratyphi A accounted for the remaining 14 (27%) isolates. (Figure 2)

Enteric fever occurred in all age groups with a peak occurrence in the paedriatics age 23((44%), however *S.* Paratyphi A was detected more in middle age groups. (Table 1)

The emergence of *S.* Paratyphi A as the commonest isolate in the year 2018 was significant. (Figure 3) The rate of isolation of *Salmonella* Typhi were 1.98% in 2016 which fell to 0.34% in 2018, whereas, *Salmonella* Paratyphi A isolates were 0.37% in 2016 and raised to 3.66% in 2018. (Figure 3)

Salmonella Typhi were 88.45% sensitive to ampicillin and 99.4% to ceftriaxone and 97.2% sensitive to chloramphenicol and 90.5% to cotrimoxazole. Only 66.8% were sensitive to ciprofloxacin. Similarly, *Salmonella* Paratyphi A isolates were 90.1% sensitive to ampicillin and 92.8% sensitive to cotrimoxazole and 100% ceftriaxone and 100% sensitive to chloramphenicol and only 71.4% sensitive to ciprofloxacin to *S.* Paratyphi A. [Table 2] shows year wise per cent resistance pattern of *S.* Typhi and *S.* Paratyphi A to individual antibiotics.

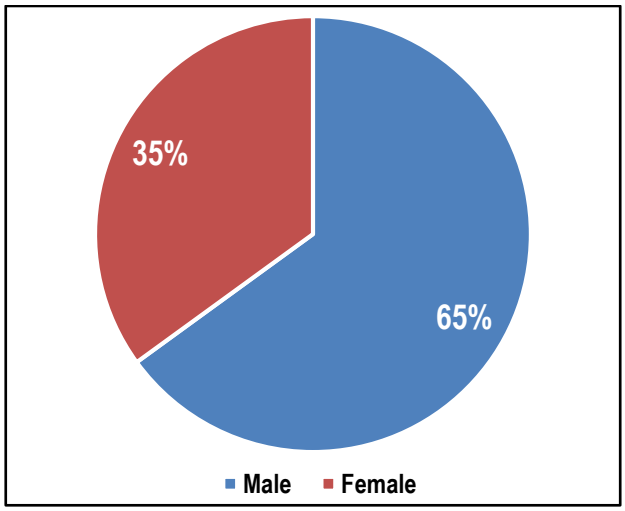

Figure 1: Gender wise distribution

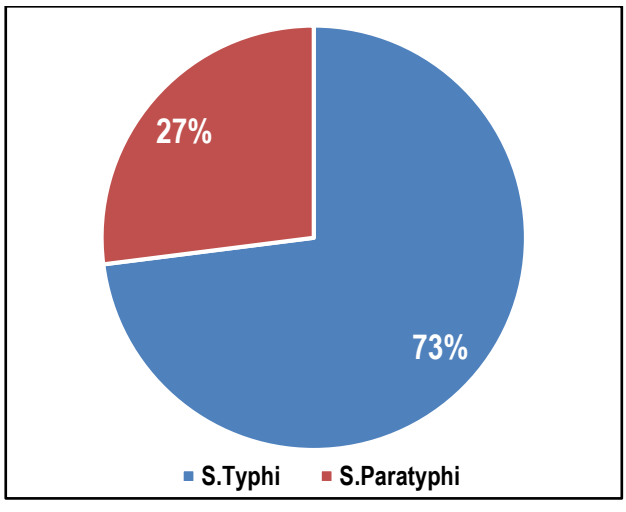

Figure 2: *S.*Typhi and *S.* Paratyphi detection in three years

Table 1: Age wise distribution of *S.* Typhi and *S.* Paratyphi.

Age wise distribution	*S.* Typhi=38	S. Paratyphi=14
1-17	19(50%)	2(14.2%)
18-35	12(31.5%)	7(50%)
36-53	5(13.1%)	4(28.7%)
54 onwards	2(5.2%)	1(7.1%)

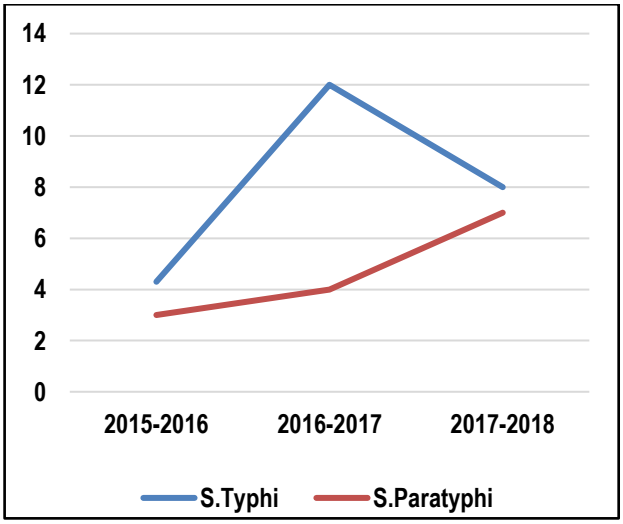

Figure 3: Shows rising trends of *S.* Paratyphi in comparison to *S.* Typhi

Table 2: Antibiotics resistance pattern in *S.*Typhi and *S.*Paratyphi

Antibiotics	*S.*Typhi	*S.*Parstyphi
Ampicillin	32(88.4%)	12(90.1%)
Co-trimoxazole	34(90.5%)	13(92.8%)
Chloramphenicol	36(97.2%)	14(100%)
Ciprofloxacin	25(66.8%)	10(71.4%)
Ceftriaxone	37(99.4%)	14(100%)

DISCUSSION

Salmonella enterica serotype Typhi and *Salmonella enterica* serotype Paratyphi A are the predominant cause of enteric fever globally. It is widespread throughout the tropics and is one of the leading causes of morbidity and mortality.[1,2] In a systematic review and meta-analysis of the published studies in India between 1950 and 2015 by John J et al, the incidence and prevalence of typhoid and paratyphoid fever was 377 and 105 per 100,000 person years. This reflects the burden of the disease in India.[1-3]

In our study, in the retrospective analysis done during the 3 year study period (2015-2018), a total of 52 cases of enteric fever were culture confirmed, from the blood culture samples received in the department of Microbiology, DRPGMC & Hospital, Kangra at Tanda.

Out of 52 *Salmonella* isolates, 38(73%) were *Salmonella* Typhi and 14(27%) were *Salmonella* Paratyphi A. *Salmonella* Paratyphi A cases increased from 13.4% in 2015-2017 to 29 %in 2018 among culture confirmed enteric fever, with a significant p value of < 0.05. [Figure 3]. This is an alarming increase conferencing *Salmonella* Paratyphi A as the rapidly emerging pathogen of enteric fever. This is in concordance with the reports from Nagpur (46.15%),[10] Sevagram (53.33%)[11] during 2001-2003 and Chandigarh (34.18% and 40.63%)[12] during 2006 and in the early months of the year 2007 which showed a dramatic rise in the number of *Salmonella* Paratyphi A isolates. High degree of clinical suspicion leading to the evaluation of even a low grade fever, vaccination to *Salmonella* Typhi and the possible presence of carrier state, especially in food handlers could be the important contributing factors for the upsurge of paratyphoid fever.

In our study, endemic throughout the year, cases were concentrated during the rainy season contamination. However, the occurrence of enteric fever was throughout the year, as stated by the World Health Organization.[1] The disease is more prevalent in the tropics affecting the young and paediatric age groups.

Emergence of drug resistance has become a confounding factor in the treatment of EF and is a problem that confronts the medical fraternity. The drug resistance pattern of *S.* Typhi in Shimla[13] as documented in 1993 denoted resistance to the tune of 48.40% for ampicillin, 40.82% for cotrimoxazole, 13.6 1% for nalidixic acid, 13.38% for gentamicin and 3.67% for ciprofloxacin.[12] In one study by Jayavarthinni et al[14] *Salmonella* Typhi were 98.4% fully susceptible to ampicillin, chloramphenicol and cotrimoxazole. *Salmonella* Paratyphi A isolates were 96.9% fully susceptible to ampicillin, chloramphenicol and cotrimoxazole. In our study also *Salmonella* Typhi were 88.4%, 97.2%, 90.5% fully susceptible to ampicillin, chloramphenicol and cotrimoxazole. *Salmonella* Paratyphi A isolates were 90.1, 100%, 92.8% fully susceptible to ampicillin, chloramphenicol and cotrimoxazole. This reveals a trend of increased susceptibility to first line antibiotics compared to previous studies done from Pondicherry (2005-2009); *Salmonella* Typhi isolates showed 65.9% and *Salmonella* Paratyphi A showed 76.4% susceptibility to first line antibiotics.[13] Only one (1.9%) of the *Salmonella* Typhi isolate was multi-drug resistant. However, it was sensitive to ceftriaxone. This is also in concordance with the other studies done from various parts of India showing a low percentage of MDR isolates 10% (2006-2007) in Karnataka and 4.7% (2008-2009) in Himachal Pradesh.[13,14]

Our study reveals a higher susceptibility to all first line drugs, < 2% MDR rate, which is indeed welcoming. Fluoroquinolones had become the drug of choice in the early 1990's, following the evolution of Multidrug resistant (MDR) strains of *Salmonella* spp conferring resistance to ampicillin, cotrimoxazole and chloramphenicol.[6] Consequent to the widespread use of ciprofloxacin, especially in the community, resistance to same and failure of treatment were reported. Capoor RM et al also has documented high MICs (8-≥512 µg/ml) to ciprofloxacin in their isolates.[15] In another study by Jayavarthinni et al, *Salmonella* Typhi showed 49.9% susceptibility while *Salmonella* Paratyphi A showed 55% susceptibility to ciprofloxacin by disc diffusion method. In our study also 66.8% of *Samonella* Typhi and 71.5% of *Salmonella* Paratyphi A showed susceptibility to ciprofloxacin.. It reveals that most of the isolates of *Samonella* Typhi and *Salmonella* Paratyphi A are resistant to ciprofloxacin. To have a clinical relevance it might be required of us to further determine the exact MICs of all our *Salmonella* isolates to ciprofloxacin and document its true susceptibility pattern.

Ceftriaxone, an injectable drug, is now considered to be the first drug of choice for the treatment of enteric fever.[5,6] In a study from North India the sensitivity was 95% to ceftriaxone.[12] In this study, only two isolates of *Salmonella* Typhi showed intermediate levels of resistance to ceftriaxone with 99.4% sensitivity. However, both the patients responded clinically. *Salmonella* Paratyphi A showed 100% sensitivity to ceftriaxone. On the basis of above

observations, as the emergence of resistance to ciprofloxacin is slowly increasing, it may be suggested that the conventional drugs e.g., co-trimoxazole, ampicillin and chloramphenicol should be prescribed and ceftriaxone and azithromycin may be kept on hold for a while with the expectation of their come back in the treatment. As susceptibility pattern varies in different places of India, continuous monitoring in this regard seems to be the need of the hour. We have to generate actual data from the developing countries where typhoid is an endemic problem along with antibiotic resistance. In absence of facilities for blood cultures available or even undertaken in most of the healthcare facilities, the empirical antibiotics used to treat all fevers in the community could be responsible for only the complicated cases presenting to the tertiary care centres. Thus, we might be identifying the cases not responding to first line. Although this is purely conjectural, we feel that the decrease in culture-positive cases may actually be driven by the empirical antibiotic choices to treat fever in the community and hospitals.

CONCLUSION

Salmonella Paratyphi A has emerged as the predominant isolate in the year 2018. However, it did not show much variation in the susceptibility pattern in comparison to *Salmonella* Typhi isolates. Thus there is a need for continuous surveillance to inform clinicians for better management of infections, availability of blood cultures in the peripheral hospitals to understand the actual burden of the disease and for government policymakers for vaccine strategies, especially in light of the increase in *S.* Paratyphi A prevalence. Safe supply water, personal hygiene and effective antibiotic policies can help us to control the disease and hence reduce antimicrobial resistance.

REFERENCES

1. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ. 2004; 82:346–53.
2. Mogasale V, Maskery B et al. Burden of typhoid fever in low-income and middle-income countries: A systematic, literature-based update with risk-factor adjustment. Lancet Glob Health2014;2:570-80.
3. Ochiai RL, Acosta CJ et al. A study of typhoid fever in five Asian countries: disease burden and implications for controls. Bull World Health Organ. 2008; 86:260-68.
4. John J, Van Aart CJC, Grassly NC. The Burden of Typhoid and Paratyphoid in India: Systematic Review and Metaanalysis. PLoS Negl Trop Dis. 2016; 10.
5. Bhattacharya SS, Das U, Choudhury BK. Occurrence & antibiogram of Salmonella Typhi & S. Paratyphi A isolated from Rourkela, Orissa. Indian J Med Res. 2011; 431-33.
6. Dahiya S, Sharma P et al. Characterisation of antimicrobial resistance in Salmonellae during 2014-2015 from four centres across India: An ICMR antimicrobial resistance surveillance network report. Indian J Med Microbiol 2017;35:61-8.
7. Menezes GA, Harish BN, Khan MA, Goessens W, Hays JP. Antimicrobial resistance trends in blood culture positive Salmonella Paratyphi A isolates from Pondicherry, India. Indian J Med Microbiol. 2016;34: 222-27.
8. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. London: Churchill Livingstone; 1996.;131-49.
9. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
10. Mendiratta DK, Deotale V, Thamke D, Narang R, Narang P. Enteric fever due to S. paratyphi A-an emerging Problem. Indian J Med Microbiol. 2004; 22 :196.
11. Menezes GA, Harish BN, Khan MA, Goessens W, Hays JP. Antimicrobial resistance trends in blood culture positive Salmonella Typhi isolates from Pondicherry, India, 2005–2009. Clin Microbiol Infect. 2012; 18:239-45.
12. Varsha Gupta, Jaspal Kaur & Jagdish Chander. An increase in enteric fever cases due to Salmonella Paratyphi A in & around Chandigarh. Indian J Med Res. 2009; 129: 95-8.
13. Verma S, Thakur S, Kanga A, Singh G, Gupta P. Emerging Salmonella Paratyphi A enteric fever and changing trends in antimicrobial resistance pattern of salmonella in Shimla. Indian J Med Microbiol. 2010; 28: 51-3.
14. Jayavarthinni M, Madhusudhan NS, Dhanaleha P, Sivagamasundari D. Emergence of Salmonella Paratyphi A and Submergence of Salmonella Typhi and Their Trend over Four Years. Ann. Int. Med. Den. Res. 2016;2:07-10.
15. Capoor RM, Nair D, Deb M, Aggarwal P. Enteric fever perspective in India: emergence of high-level ciprofloxacin resistance and rising MIC to cephalosporins. J Med Microbiol.2007; 56:1131-32.
16. Tankiwale SS, Agarwal G, Jalgaonkar SV. An unusually high occurrence of Salmonella enterica serotype Paratyphi A in patients with enteric fever. Indian J Med Res. 2003; 117:10-2.
17. Sur D, von Seidlein L et al. The malaria and typhoid fever burden in the slums of Kolkata, India: data from a prospective community based study, Trans R Soc Trop Med Hyg. 2006;100.
18.Nagshetty K, Channappa S, Gaddad S. Antimicrobial susceptibility of Salmonella Typhi in India. J Infect Dev Ctries. 2010; 4:70-3.
19. Kumar Y, Sharma A and Mani KR. Antibiogram Profile of Salmonella enterica Serovar Typhi in India – A Two Year Study. Tropical Life Sciences Research. 2013;24:45–54.
20. DuPont HL. Quinolones in Salmonella typhi infection. Drugs. 1993;45:119-24.
21. Lakshmi, Ashok R, Susmita J, Shailaja VV. Changing trends in the antibiograms of salmonella isolates at a tertiary care hospital in Hyderabad. Indian J Med Microbiol. 2006; 24:45-8.
22. 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;134:961-6.
23. Balaji V, Sharma A, Ranjan P, Kapil A. Revised ciprofloxacin breakpoints for Salmonella Typhi: Its implications in India. Indian J Med Microbiol 2014;32:161-3.
24. Gautam V, Gupta NK, Choudhary U, Arora DR. Sensitivity Pattern of Salmonella serotypes in Northern India. Braz J Infect Dis. 2002;6:281–7.

Source of Support: Nil. **Conflict of Interest:** None Declared.

Copyright: © the author(s) and publisher. IJMRP is an official publication of Ibn Sina Academy of Medieval Medicine & Sciences, registered in 2001 under Indian Trusts Act, 1882. This is an open access article distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cite this article as: Rana Aditya, Chaudhary Anuradha, Gupta Veeteeanveshna, Tamrakar Meenakshi. Changing Resistance Pattern and Emerging *Salmonella* Paratyphi A Enteric Fever – New Challenges and New Trends: A Study from Tertiary Care Medical College and Hospital in North India. Int J Med Res Prof. 2019 Mar; 5(2):55-58. DOI:10.21276/ijmrp.2019.5.2.013