Original Research Article

Trends in epidemiology, susceptibility pattern and serotyes of Salmonellae at a tertiary care hospital: An eight year study

Abstract:

Background- Enteric fever is a global disease. In India Enteric fever is endemic with Salmonella enterica serovar Typhi being the predominant etiological agent. Due to changing antimicrobial resistance patterns, knowledge of local epidemiology, antimicrobial resistance pattern helps in initiation of appropriate empiric therapy.

Methodology A prospective study on Salmonellae isolated from blood and stool specimens over 8 year period was conducted. Antimicrobial susceptibility was done as per CLSI guidelines. Serotyping was done by using commercial antisera and later confirmed at Central Research Institute, Kasauli.

Results Out of 52 salmonellae, 43 (82.6%) were from blood and 8 (15.3%) from stool and 1 (1.9%) from pus specimen. We observed a change in spectrum and susceptibility pattern of salmonellae the 8 year study period. In 2011, 2013, 2016 and 2018, Salmonella Typhi (serotype-9,12,vi:d:-) was the predominant etiological agent accounting for 81.8% , 66.6%, 51% and 80% of the total cases of salmonella respectively. Salmonella Paratyphi B (4,12:b:1,2) was predominant in 2012 (100% of cases). Salmonella Serotype Typhimurium (4,12;i:1,2) was predominant in 2014 (50%) while Salmonella Typhi and S. Paratyphi B contributed equally to infections in 2015 (40% each). From 2011, Non typhoidal salmonellae (NTS) steadily increased. 19 (36.6%).The most effective antimicrobials against typhoidal salmonellae were chloramphenicol, ceftriaxone and co-trimoxazole with all most 100% sensitivity from 2011 to 2018. Ciprofloxacin maintained good sensitivity in 2013, 2014 and 2015, 2016 and 2018 but ampicillin was ineffective in our set-up .

Conclusions- Due to changing trends in spectrum and sensitivity of salmonellae, continuous monitoring is essential.

Introduction-

Typhoid fever remains an important global public health problem accounting for 12-33 million cases worldwide.[1] Around 80% of these cases occur in Asia alone.[2] Many published Hospital based studies and outbreak studies suggests that Typhoid fever is a major public health concern in India with Salmonella enterica serovar Typhi (*Salmonella* Typhi) being main etiological agent. [3] Antimicrobial treatment is the mainstay of treatment of Typhoid and Paratyphoid fever.[4] Emergence of Antimicrobial resistance can pose a

challenge for effective management of typhoid fever,[5] especially the emergence and spread of multidrug resistant strains.

In India, drug resistant Salmonellae have been reported since 1960, first outbreak of multidrug resistant *Salmonella* Typhi occurring in Calicut. Since then multi drug resistant *Salmonella* Typhi have appeared throughout the world, especially in South America, the Indian sub continent, Africa and South East Asia.[6] Later an outbreak due to chloramphenicol resistant *Salmonella* Typhi was reported from Chandigarh.[7]

Subsequently, resistance to commonly used antibiotics such as chloramphenicol, ampicillin and cotrimoxazole has been reported from different parts of India.[5,7]

The present study was undertaken to know the trends in serotypes and antibiograms of Salmonellae isolates in a hospital setting over a 8 year period.

Methodology – A prospective study was conducted in the department of microbiology of Sassoon General Hospital Pune over a period of 8 years. Various clinical specimens like blood, stool, urine, and pus were processed for culture by routine methods and Salmonellae isolates were included in the study. Identification of salmonella was done by standard microbiological methods.[8] All the salmonella isolates were tested by commercially available(DENKA SEIKEN) Salmonella polyvalent antisera and group specific antisera (O9, O4 and O2). The isolates were preserved and also sent to CRI, Kasauli for serotyping. The antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method as per CLSI guidelines.[9] According to CLSI, antimicrobial sensitivity for non-typhoidal salmonellae is not recommended.So sensitivity was not analysed for non-typhoidal salmonellae The data was entered in WHONET.

Results-

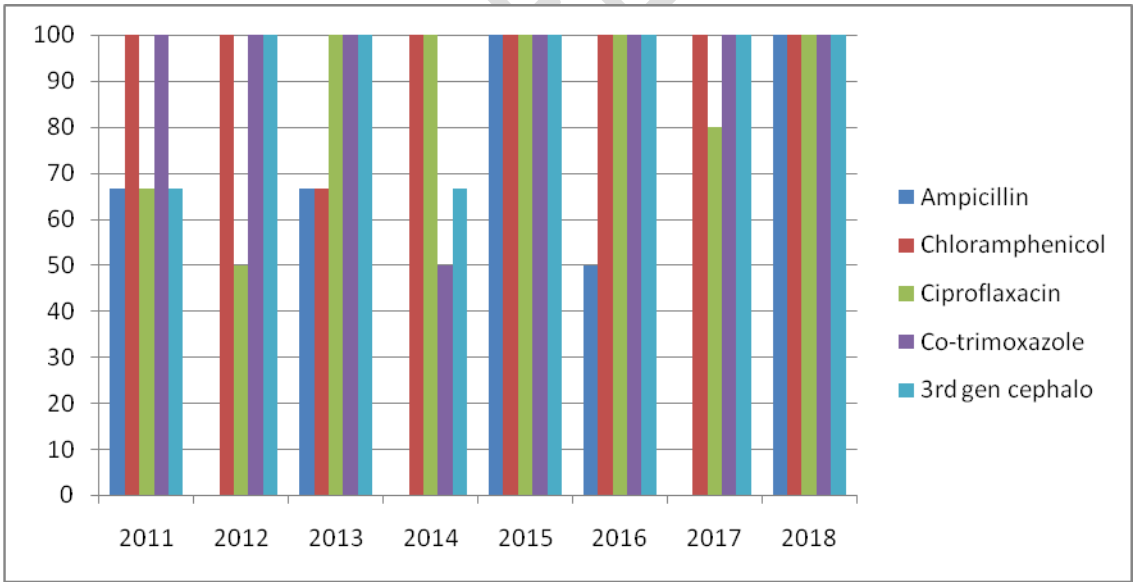
A total of 52 salmonellae were isolated over a period of 8 years. Out of the 52 isolates, 43 (82.6%) were obtained from blood cultures and 8 (15.3%) were obtained from stool specimens and 1 (1.9%) from pus. Demographic data revealed that males (56.4%) were more affected by Salmonella than females (43.6%).

Out of 52 salmonellae, 31 (59.6%) were *Salmonella enterica serovar* Typhi followed by *Salmonella* Paratyphi B. 9 (17.3%), *Salmonella* Typhimurium, 4 (7.6%), *Salmonella* Paratyphi A *3 (5.7%)* . *Salmonella* Jaffna and *Salmonella* Enteridis each were 3.8% of the isolates,. There was one isolate *Salmonella* Welteverden. Year wise distribution of the isolates revealed changing trends in etiology of typhoid fever. In 2011, 2013, 2016 and 2018, *Salmonella* Typhi (serotype-9,12,vi:d:-) was predominant etiological agent accounting to 81.8%, 66.6%, 51% and 80% of the total cases of salmonella respectively. *Salmonella* Paratyphi B was predominant in 2012 (100% of cases). *Salmonella Ser.* typhimurium was predominant in 2014 (50%) while *Salmonella* Typhi and *Salmonella.*Paratyphi B contributed equally in 2013, 2015 (40% each). In 2011 *Salmonella* Typhi was predominant isolate but after that non typhoidal salmonellae are steadily increasing.(Table 1)

Table 1-Yearwise distribution of Salmonellae from clinical samples (n=52)

Year	Isolates	No. of isolates	Serotypes
2011 (n=22)	*Salmonella* Typhi	18	9,12,vi:d:-
	Salmonella Paratyphi B	2	4,12:b:1,2
	Salmonella Typhimurium	2	4,12;i:1,2
2012 (n=2)	*Salmonella* Paratyphi B	2	4,12:b:1,2
2013 (n=6)	*Salmonella* Typhi	2	9,12,vi:d:-
	Salmonella Paratyphi B	2	4,12:b:1,2
	Salmonella Enteridis	2	9,12:g,m:-
2014 (n=4)	*Salmonella* Paratyphi A	1	2,12;a:-
	Salmonella Typhi	1	9,12,vi:d:-
	Salmonella Typhimurium	2	4,12;i:1,2
2015 (n=5)	*Salmonella* Typhi	2	9,12,vi:d:-
	Salmonella Paratyphi B	2	4,12:b:1,2
	Salmonella Welteverden	1	3,10:r:z6
2016 (n=6)	*Salmonella* Typhi	3	9,12,vi:d:-
	Salmonella Paratyphi A	1	2,12;a:-
	Salmonella Jaffna	2	9,12:d:Z39
2017 (n=2)	*Salmonella* Typhi	1	9,12,vi:d:-
	Salmonella Paratyphi A	1	2,12;a:-
2018 (n=5)	*Salmonella* Typhi	4	9,12,vi:d:-
	Salmonella Paratyphi B	1	4,12:b:1,2

Fig 1- Antibiogram of Typhoidal Salmonella showing % susceptibility. (n=35)



The most effective antimicrobial agent against typhoidal salmonellae was chloramphenicol with 100% sensitivity. Next drug found to be effective was Co-trimoxazole with again 100% sensitivity over all years except in 2014 (50%). Fluroquinolones like ciprofloxacin has maintained good sensitivity in 2013, 2014 and 2015, 2016 and 2018. Surprisingly in our

study we found very low resistance to 3rd generation cephalosporins in 2011 and 2014. However, ampicillin was ineffective in our set-up with almost 0% sensitivity in 2012, 2014 and 2017.

Discussion-

Antimicrobial resistance is a major hindrance in successful treatment of typhoid fever. Environmental conditions like poor sanitation, bad personal hygiene, poor quality water aggravates the problem. In the present study, we analysed trends in etiology of typhoid fever and the susceptibility pattern of Salmonellae isolated from a tertiary care centre in western Maharashtra. Demographic data in our study revealed that males were affected more than females by salmonella which is in accordance to the finding by Saba et al [10] who also found that 90 (58%) isolates were obtained from male and 64 (42%) from female patients.

Salmonella Typhi was predominant pathogen isolated over the study period. It was also the commonest Samonella in 2011, 2013, 2016 and 2018 accounting for 81.8% ,66.6%, 51% and 80% of the cases respectively. Similar finding has been noted by V. Laxmi et al from Hyderabad, India in 2006.[11]

In the present study, from year 2012 onwards non-typhoidal salmonellae (NTS) emerged. And formed 32.6% of the isolates from 2012 to 2018. *Salmonella* Typhimurium, *Salmonella* Welteverden and *Salmonella* Enteridis, *Salmonella* Jaffna were the NTS detected. Similar findings have been reported by Suman Kanungo et al [12] [2008, kolkatta, India] in their review article. They have shown an increasing incidence of invasive salmonellosis due to Non-typhoidal salmonellae. In a study in Thailand, 135 cases of NTS bacteraemia have been reported.[13] But in contrast to this study only 2 cases of NTS from 1500 blood cultures has been reported by an Indian study.[2]

Drug resistance is a major challenge when treating typhoid fever. Chloramhenicol has remained the treatment of choice for typhoid fever for around six decades now. Chloramphenicol therapy is reduces mortality due to typhoid fever from 20% to 1% and duration of fever from 14-28 days to 3-5 days.[14] However, chloramphenicol has its own side effects like bone marrow toxicity, high carriage rates and emergence of drug resistance. In 1980s there was emergence of plasmid mediated chloramphenicol resistance in many countries including India.[15] In this scenario next options were ampicillin and Co-trimoxazole[16] This was followed by emergence of multidrug resistant (MDR) strains (combined resistance to chloramphenicol, ampicillin and co-trimoxazole) initially reported from India [karnataka,1999][17], Pakistan and Middle East and then from all over the world.[18]

In the present study good sensitivity to chloramphenicol and co-trimoxazole was observed over the 8 years. Similar findings have been mentioned by other Indian authors like shorey et al[Mumbai,1993] [19] and Nath et al from Varanasi,2003.[20] In the current study there was very low sensitivity to ampicillin almost 0% in 2012, 2014 and 2017. Increasing use of ampicillin seems to have decreased its efficacy in treatment of typhoid fever

Parenteral administration of 3rd generation cephalosporins specially ceftriaxone is often the treatment of choice for typhoid fever due to its short duration of therapy as compared to long duration of chloramphenicol. In the present study, in 2011 and 2014, we observed diminished sensitivity to ceftriaxone (66%) but it regained sensitivity in 2015 to 2018 (100%). This finding is similar to findings by saba et al, [10] who observed increased sensitivity to salmonella from 92% to 100%. Other Indian studies by Nath et al [20] and Gautam et al [6] also mentioned increasing sensitivity to 3rd generation cephalosporins in their studies.

Ciprofloxacin was used as a good alternative to chloramphenicol when it was initially introduced in 90s but because of overuse and misuse of the drug it also showed resistance.

Gautam et al reported diminished sensitivity to ciprofloxacin from 89% to 81% from 1997 to 2001.[6] In the present study, it was observed that there was diminished sensitivity to ciprofloxacin in 2011 (66.6%) and 2012 (50%) but it improved to 100% in 2013, 2015, 2016 and 2018. This could be due to the fact that resistance to this drug made bit ineffective and most clinicians stopped using this drug for the treatment of typoid fever. Hemlatha et al from Hyderabad also observed 95% sensitivity to ciprofloxacin in the year 1999.[21] In the present study, we observed a lot of variation in serotypes of salmonellae causing typhoid and also in susceptibility pattern of salmonella species. So continuous monitoring of Isolates causing enteric fever and their susceptibility to antimicrobials is recommended.

Conclusion- The study highlights the changing trends in etiology and susceptibility pattern of salmonellae causing typhoid fever. So, continuous monitoring of microorganisms causing enteric fever is important for optimum treatment of typhoid fever.

References-

1) Miller SI, Pegues DA. Salmonella species, including *Salmonella Sertype Typhi*, chapter 210. *In*: Mandell GL, Bennett JE and Dolin R, *Principles & Practice of infectious diseases*, 5th edn. (Churchill Livingstone, London); 2000. p. 2344–63.
2) Ochiai RL, Acosta CJ, Danovaro-Holliday MC , Baiqing D, Bhattacharya SK,Agtini MD et al . A study of typhoid fever in five Asian countries: disease burden and implications for controls. Bull World Health Organ 2008; 86: 260-8.
3) AS Damle, RP Karyakarte, AB Deshmukh, MP Bansal. Salmonella phage types prevalent at Aurangabad. Indian journal of medical microbiology 1988; 6, 151-154.
4) Preeti Behl, Varsha Gupta, Atul Sachdev, Vishal Guglani & Jagdish Chander. Patterns in antimicrobial susceptibility of Salmonellae isolated at a tertiary care hospital in northern India.Indian J Med Res 145, January 2017, pp 124-128.
5) Jacob John, Carola J. C. Van Aart, Nicholas C. Grassly. The Burden of Typhoid and Paratyphoid in India: Systematic Review and Meta-analysis. PLOS Neglected Tropical Diseases | DOI:10.1371/journal.pntd.0004616 April 15, 2016.
6) Gautam V, Gupta NK, Choudhary U, Arora DR. Sensitivity Pattern of Salmonella serotypes in Northern India. *Braz J Infect Dis* 2002;**6:**281–7.
7) Kapil A, Ayyagiri A, Garg RK, Agarwal KC. *Salmonella Typhi* with transferable Chloramphenicol Resistance Isolated in Chandigarh during 1983–87. *Indian J Pathol Microbiol* 1994;**38**:179–83.
8) Forbes BA, Sahm DF, Weissfeld AS. In: Bailey and Scott's Diagnostic Microbiology. 12th ed. Missouri: Mosby Elsevier; 2007. p. 779.
9) CLSI performance standards for antimicrobial susceptibility testing. Twelfth international supplement. NCCLS document M100-S12 [ISBN1-56238-454-6]. NCCLS, 940 WEST VALLEY Road, suite 1400, Wayne Pennsylvania 19087-1898 USA, 2002.
10) Saba Qaiser, Seema Irfan, Erum Khan, Tanwir Ahsan, Afia Zafar. In Vitro Susceptibility of typhoidal Salmonellae against newer antimicrobial agents: A search for alternate treatment options. J Pak Med Assoc, May 2011; Vol. 61(5):462-465.
11) V Lakshmi, R Ashok, J Susmita, VV Shailaja . Changing trends in the antibiograms of *Salmonella* isolates at a tertiary care hospital in hyderabad**.** *Indian Journal of Medical Microbiology, (2006) 24 (1):45-8.*
12) Suman Kanungo, Shanta Dutta, and Dipika Sur1.Epidemiology of typhoid and paratyphoid fever in India. *J Infect Developing Countries* 2008; 2(6): 454-460.

13) Kiratisin P (2008) Bacteraemia due to non-typhoidal *Salmonella* in Thailand: clinical and microbiological analysis. Trans R Soc Trop Med Hyg 102(4):384-8. Epub 2008 Mar 5.
14) Mandal S, Mandal MD, Kumar NP. Reduced minimum inhibitory concentration of chloramphenicol for *Salmonella enterica* serovar typhi. *Indian J Med Sci* 2004;**58:**16–23.
15) Srivastava L, Aggarwal P. Multidrug resistant *Salmonella Sertype Typhi* in Delhi. *Indian J Med Microbiol* 1994;**12:**102–5.
16) Parry CM. The treatment of multi–drug resistant & Nalidixic acid–resistant typhoid fever in Vietnam. *Trans Roy Soc Trop Med Hyg* 2004;**98**:413–22.
17) Ciraj AM, Seetha KS, Gopalakrishna BK, Shivananda PG .Drug resistance pattern and Phage types of Salmonella typhi isolates in Manipal, South Karnataka. Ind J Med Sci 1999 ;53(11): 486-89
18) Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. BMJ 2006; 333: 78-82.
19) Sheorey HS, Kaundinya DV, Hulyalkar VS, Deshpande AK. Multidrug Resistant *Salmonella Sertype Typhi* in Bombay. *Indian J Pathol Microbiol* 1993;**36**:8–12.
20) Nath G, Tikoo A, Manocha H, Tripathi AK, Gulati AK. Drug resistance in *Salmonella Sertype Typhi* in North India with special reference to Ciprofloxacin. *J Antimicrobial Chemother* 2003;**46**;145–53.
21) Hemalatha R, Vijayalakshmi P, Gyaneshwari, Rao MV, Ramani A. Multidrug resistant salmonella typhi in Hyderabad. *Indian J Med Microbiol* 1999;**17**:39–41.