Original Research Article

Trends in epidemiology, susceptibility pattern and serotypes of Salmonellae at a tertiary care hospital, India: An eight-year study (2011 – 2018)

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 the initiation of appropriate empiric therapy.

Methodology: A prospective study on Salmonellae isolated from blood and stool specimens over an 8 year period was conducted. Antimicrobial susceptibility was done as per The Clinical & Laboratory Standards Institute (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 suggest that Typhoid fever is a major public health concern in India with Salmonella enterica serovar Typhi (*Salmonella* typhi) being the main etiological agent. [3] Antimicrobial treatment is the mainstay of treatment of Typhoid and Paratyphoid fever.[4] The 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, the first outbreak of multidrug-resistant *Salmonella typhi* occurring in Calicut. Since then multidrug-resistant *Salmonella t*yphi has appeared throughout the world, especially in South America, the Indian subcontinent, Africa and South-East Asia.[6] Later an outbreak due to chloramphenicol resistant *Salmonella t*yphi 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 an 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 Salmonella polyvalent antisera (DENKA SEIKEN) 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-

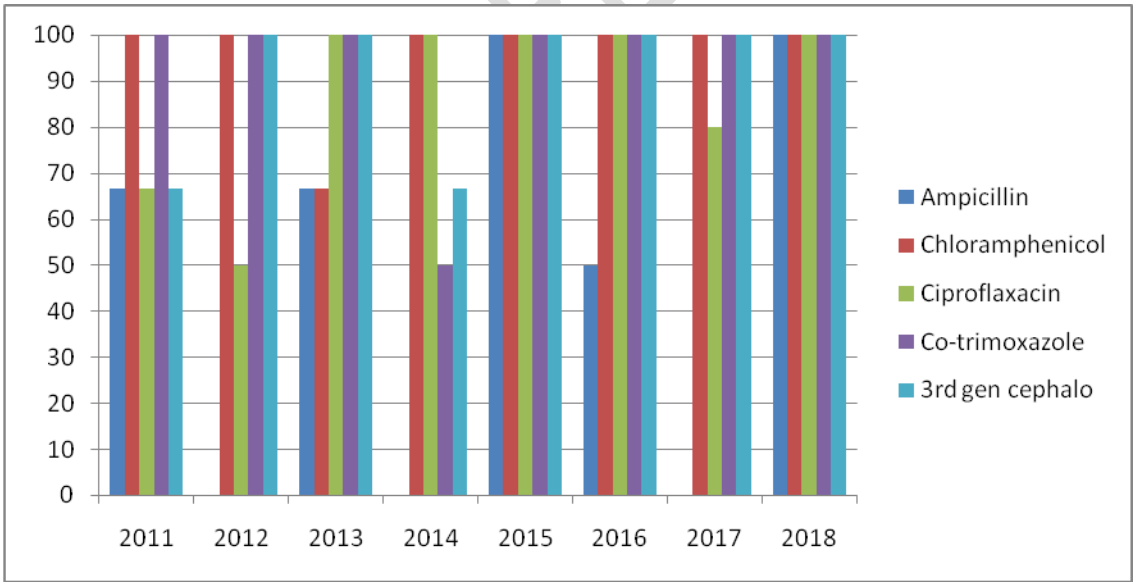
A total of 52 salmonellae were isolated over a period of 8 years. Out of the 52 isolates, 43 (82.6%) were obtained from blood cultures and 8 (15.3%) were obtained from stool specimens and 1 (1.9%) from pus. Demographic data revealed that males (56.4%) were more affected by Salmonella than females (43.6%).

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 the aetiology 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 overall 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 the successful treatment of typhoid fever. Environmental conditions like poor sanitation, bad personal hygiene, poor quality water aggravate the problem. In the present study, we analysed trends in the aetiology 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. A similar finding has been noted by V. Laxmi et al from Hyderabad, India in 2006.[11]

In the present study, from the year 2012 onwards non-typhoidal salmonellae (NTS) emerged. And formed 32.6% of the isolates from 2012 to 2018. *Salmonella t*yphimurium, *Salmonella* welteverden and *Salmonella* enteridis, *Salmonella* jaffna were the NTS detected. Similar findings have been reported by Suman Kanungo et al [12] [2008, Kolkata, 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. Chloramphenicol has remained the treatment of choice for typhoid fever for around six decades now. Chloramphenicol therapy 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 the emergence of drug resistance. In the 1980s there was the 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 the emergence of multidrug-resistant (MDR) strains (combined resistance to chloramphenicol, ampicillin and co-trimoxazole) initially reported from India [karnataka,1999][17], Pakistan and the 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 the treatment of typhoid fever Parenteral administration of 3rd generation cephalosporins especially ceftriaxone is often the treatment of choice for typhoid fever due to its short duration of therapy as compared to a long duration of chloramphenicol. In the present study, in 2011 and 2014, we observed diminished sensitivity to ceftriaxone (66%) but it regained sensitivity from 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 the 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 a bit ineffective and most clinicians stopped using this drug for the treatment of typhoid 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 aetiology and susceptibility pattern of salmonellae causing typhoid fever. So, continuous monitoring of microorganisms causing enteric fever is important for optimum treatment of typhoid fever.

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