

**Research Article**

**Study of multi drug resistant S.typhi and MIC in Ausa and Latur  
region in Maharashtra state of India**

**Vishwas Jagtap and Narendra Deshmukh**

Modern College of Biotechnology, Maharashtra, India

Correspondence: vishwa.jag@gmail.com

[Received: 02/06/2021; Accepted: 27/06/2021; Published: 25/07/2021] DOI: 10.5281/zenodo.5635606

**ABSTRACT:**

Enteric (typhoid) fever is a systemic disease characterized by fever and abdominal pain caused by dissemination of Salmonella Typhi or Salmonella Paratyphi type A, B, or C. Fever (38.8-40.5°C; 101.8-104.9°F) is documented at presentation in more than 75% of cases and is typically prolonged, continuing up to 4 weeks if untreated. Symptoms reported on initial medical evaluation include headache (80%), chills (35-45%), cough (30%), sweating (20-25%), myalgias (20%), malaise (10%), and arthralgia (2-4%). Gastrointestinal (GI) symptoms include anorexia (55%), abdominal pain (30-40%), nausea (18-24%), vomiting (18%), and diarrhea (22-28%) more commonly than constipation (13-16%). In a series in Delhi, India, only 527 isolates were obtained from 5,735 suspected cases (9.2%)(5).

This compares with 175 isolates in 243 cases (72%) in Rodesia(8) and 219 isolates from 298 clinical cases in a pediatric group in Natal(73%)(4).after removal of the serum and dissolution of the clot with streptokinase(6). Bile broth and Streptokinase broth were used. In the isolation both bill salt broth (broth culture) and streptokinase broth (clot culture) where used for the enrichment of blood samples.

**Keywords:** Clot Culture, Streptokinase, Bile broth, Entric fever, *Salmonella typhi*.

## **INTRODUCTION:**

Enteric (typhoid) fever is a systemic disease characterized by fever and abdominal pain caused by dissemination of Salmonella Typhi or Salmonella Paratyphi type A, B, or C. Fever (38.8-40.5°C; 101.8-104.9°F) is documented at presentation in more than 75% of cases and is typically prolonged, continuing up to 4 weeks if untreated. Symptoms reported on initial medical evaluation include headache (80%), chills (35-45%), cough (30%), sweating (20-25%), myalgias (20%), malaise (10%), and arthralgia (2-4%).

Gastrointestinal (GI) symptoms include anorexia (55%), abdominal pain (30-40%), nausea (18-24%), vomiting (18%), and diarrhea (22-28%) more commonly than constipation (13-16%).

Travelers to southern Asia are at highest risk for infections that are nalidixic acid-resistant or multidrug-resistant (resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole). Nalidixic acid (a non-fluorinated quinolone antibiotic)

is used as a marker for fluoroquinolone susceptibility. [1]

Emergence of multidrug resistant strains (MDRST) poses a risk to public health. In India, antibiotic resistance among s.typhi has been reported since 1960s and first outbreak of multidrug resistant s.typhi was reported in Calicut. Multidrug resistant s.typhi has appeared throughout the world, especially in South America, Africa and south Asia.

Children have a higher incidence of positive stool cultures compared with adults (60 vs 27%), and stool cultures may become positive during the third week of illness in untreated patients.

Thus, the optimal diagnostic approach in both children and adults is to culture blood, bone marrow, gastric or duodenal secretions, and stool. Using this approach, the diagnosis can be established in more than 90% of patients.[1]

We had done the research to observe the sensitivity pattern according to the different regions.

Year of study	Year of publication	Author & Reference	No. of isolates	Study Design	Risk of Bias	
					QUIPS	JBI
2012	2017	Harichandran & Dinesh <sup>17</sup>	79	Retrospective	Low	No
2016	2016	Sharvani <i>et al</i> <sup>33</sup>	167	Retrospective	Low	No
2013-2014	2015	Misra <i>et al</i> <sup>26</sup>	50	Retrospective	Low	No
2015	2015	Narain & Gupta <sup>29</sup>	220	Prospective	Low	No
2012	2014	Srirangaraj <i>et al</i> <sup>34</sup>	16	Retrospective	Low	No
2014	2017	Dahiya <i>et al</i> <sup>33</sup>	380	Retrospective	Low	No
2010	2013	Choudhary <i>et al</i> <sup>12</sup>	322	Retrospective	Low	No
2012	2013	Venkatesh <i>et al</i> <sup>35</sup>	251	Retrospective	Low	No
2008-2010	2013	Gupta <i>et al</i> <sup>16</sup>	257	Retrospective	Low	No
2010-2012	2013	Jain & Chugh <sup>18</sup>	266	Retrospective	Low	No
2008	2011	Kumar <i>et al</i> <sup>22</sup>	128	Retrospective	Low	No
2011	2011	Adhikary <i>et al</i> <sup>9</sup>	2	Case Report	Low	Yes
2000-2006	2010	Verma <i>et al</i> <sup>36</sup>	159	Retrospective	Low	No
2008	2009	Kumar <i>et al</i> <sup>21</sup>	50	Retrospective	Low	No
1990	1992	Rodrigues <i>et al</i> <sup>31</sup>	74	Retrospective	Low	No
2004	2007	Joshi & Amarnath <sup>19</sup>	25	Retrospective	Low	No
2002	2007	Capoor <i>et al</i> <sup>11</sup>	178	Retrospective	Low	No
2003	2007	Banerjee <i>et al</i> <sup>10</sup>	60	Retrospective	Low	No
2004-2005	2006	Manchanda <i>et al</i> <sup>25</sup>	56	Retrospective	Low	No
2006	2006	Ray <i>et al</i> <sup>30</sup>	70	Cross-sectional	Low	No
1999-2004	2006	Mohanty <i>et al</i> <sup>27</sup>	629	Retrospective	Low	No
2001-2004	2006	Lakshmi <i>et al</i> <sup>23</sup>	60	Retrospective	Low	No
2003-2004	2005	Dutta <i>et al</i> <sup>14</sup>	379	Retrospective	Low	No
2004	2005	Senthilkumar <i>et al</i> <sup>32</sup>	6	Retrospective	Low	No
2002	2004	Madhulika <i>et al</i> <sup>24</sup>	157	Cross-sectional	Low	No
1997-2001	2002	Gautam <i>et al</i> <sup>15</sup>	436	Retrospective	Low	No
2001-2003	2005	Kadhiravan <i>et al</i> <sup>20</sup>	50	Retrospective	Low	No
2006-2007	2010	Nagshetty <i>et al</i> <sup>28</sup>	84	Retrospective	Low	No

QUIPS, Quality in Prognosis Studies tool<sup>6</sup>; JBI, Joanna Briggs Institute<sup>7</sup>

**Table 2:** Studies included in the systematic review in which phenotypic AMR trends of *S. Typhi* isolates were analysed

Thirty two studies [Table 2] [2-29] satisfied the inclusion criteria from which 49 yr-stratified summaries of *S. Typhi* antimicrobial-resistant isolates were obtained. For instance, Gautam *et al*<sup>181</sup>

#### MATERIALS AND METHODS:

The present study screened a total of 290 blood samples collected from the symptomatically suspected patients suffering from typhoid fever and attaining the various

private and public hospitals of this region from May 2020 to 2021 may and isolated a total of 71 s.typhi isolates.

*Enterobacteriaceae* inhabit a wide variety of niches that include the human gastrointestinal tract and various environmental niches. When blood samples from a patient with suspected enteric fever is submitted for the widal test, it is useful as a routine to culture the clot after separation of serum (30). Peptone serves as a source of nitrogen,

carbon, long chain amino acids and other essential amino acids. Sodium taurocholate would inhibit majority of Gram-positive species. Sodium chloride maintains the isotonicity of the medium. Whereas addition of streptokinase solution causes rapid clot lysis with release of bacteria trapped in the clot (31).

In the isolation both bill salt broth (broth culture) and streptokinase broth (clot culture) were used for the enrichment of blood samples. The enriched samples were incubated till visible turbidity. A medium destined to inoculate onto other less selective media. Streaked on the MacConkey and Wilson Blair bismuth sulfite agar. The isolates producing the characteristic colonies were identified by standard biochemical tests and confirmed serotypically by agglutination with salmonella O9, Vi specific and Hd antisera for *S.typhi* and paraA for the *S.paratyphi* A (Kings Institute of Preventive Medicine Guindy)

#### **Antimicrobial susceptibility testing:**

The antibiotic susceptibility testing of the isolates was done by Kirby-Bauer disk diffusion method according to CLSI guidelines as. Ampicillin (10µg/disk), chloramphenicol (30µg/disk), co-trimoxazole (25 µg/disk) ciprofloxacin (5µg/disk), tetracycline (30µg/disk) ceftriaxone (5 µg/disk) nalidixic acid (30µg/disk), cefotaxime (30µg/disk) Imipenem (10µg/disk). *Escherichia coli* ATCC.. Was

used as a negative control and *S.typhi* with MTCC was used as a positive control. Commercially available six mm disks (HiMedia Laboratories Mumbai) were used (1999, 2004).

MICs of isolates resistant to Ciprofloxacin, nalidixic acid, Ampicillin and co-trimoxazole were done by using HiComb strips (HiMedia Laboratories, Mumbai). MICs of ciprofloxacin were determined for both ciprofloxacin resistant and selected nalidixic acid resistant isolates. Isolates resistant to Ampicillin, chloramphenicol and co-trimoxazole were termed as MDR.

#### **RESULTS & DISCUSSION:**

A total of 71 *S.typhi* isolates were isolated from 290 samples showing the incidence of. Antibigram of these isolates revealed that all the isolates are sensitive to the Imipenem. (Table-2) highest resistance was observed equally in Ampicillin (89%) and nalidixic acid (89%) followed by chloramphenicol (76.12%). The *S.typhi* isolates showed low level resistance against the majority of remaining antibiotics.

MICs of the isolates, among the 61 Ampicillin resistant *S.typhi* isolates, 11 isolates showed an MIC of 64µg/ml while 30 isolates showed MIC of 128 µg/ml. and remaining 20 isolates showed, the MIC of 256 µg/ml. among the 60 chloramphenicol resistant *S.typhi* isolates, 20 have shown MIC of 32 µg/ml while the remaining 40 isolates

have shown the MIC of 256 µg/ml. among the 41 co-trimaxazole resistant isolates, 5 isolates have shown the MIC of 10 µg/ml and 11 isolates have shown the MIC of 30 µg/ml and remaining 25 isolates have shown the MIC of 240 µg/ml .out of 11 ciprofloxacin resistant

isolates 5 have shown the MIC 0.25 µg/ml and remaining 6 isolates have shown (Table-3) the MIC of 0.5 µg/ml. Among the 61 nalidixic acid resistant isolates chosen for the ciprofloxacin.

**Table 1:** Percentages of antibiotics are resistance to different antibiotics:

Antibiotics screened	No of isolates	Percentage
Ampicillin	61	89.00%
chloramphenicol	60	76.12%
Co-trimaxazole	41	67.60%
Nalidixic acid	61	89.00%
Ciprofloxacin	11	22.00%
Ceftriaxone	2	2.5%
Cefatidime	6	17.35%
Azithromycin	45	85.52%
Gatifloxacin	41	61.36%
Sparofloxacin	05	69.00%
cefotaxime	27	11.50%
MDR	37	76.25%

MDR resistant to Ampicillin, chloramphenicol and co-trimaxazole

**Table 2.** MIC value of resistant S.typhi to various antibiotics.

Antibiotic	No. of isolates	Range (µg/ml)
Ampicillin N = 61	11 30 20	64µg/ml 128 µg/ml 256 µg/ml
Chloramphenicol N=60	20 40	32 µg/ml 256 µg/ml
Cotrimoxzole N=41	5 11 25	10 µg/ml 30 µg/ml 240 µg/ml .
ciprofloxacin N=11	5 6	0.25 µg/ml 0.50 µg/ml

## CONCLUSION:

Despite public health efforts, typhoid fever is still a significant cause of morbidity and mortality worldwide. Sanitation management, public healthcare awareness, and nutritional programs boost both the control and prevention of disease should be maintained. We highlights that typhoid fever remains a major problem in most resource. Different seasons we collected samples and this disease is more occurs especially as per our studies in the month of July-Sept peoples suffering from this infection.

**Acknowledgements:** None Stated

**Conflicts of interest:** Authors declared No conflict of interest

## REFERENCES:

1. <https://www.infectiousdiseaseadvisor.com/home/decision-support-in-medicine/infectious-diseases/enteric-fever/>
2. Adhikary R, Joshi S. Dual Salmonella typhi Typhi infection. *Indian J Pathol Microbiol* 2011; 54 : 849-50.
3. Banerjee A, Kalghatgi AT, Singh P, Nagendra A, Singh Z, Handa SK. Epidemiological investigation of an outbreak of enteric fever. *Med J Armed Forces India* 2007; 63 : 322-4.
4. Capoor MR, Nair D, Aggarwal P, Mathys V, Dehem M, Bifani PJ. Salmonella enterica serovar Typhi: Molecular analysis of strains with decreased susceptibility and resistant to ciprofloxacin in India from 2001-2003. *Braz J Infect Dis* 2007; 11 : 423-5.
5. Choudhary A, Gopalakrishnan R, Nambi PS, Ramasubramanian V, Ghafur KA, Thirunarayan MA. Antimicrobial susceptibility of Salmonella enterica serovars in a tertiary care hospital in southern India. *Indian J Med Res* 2013; 137 : 800-2.
6. Dahiya S, Sharma P, Kumari B, Pandey S, Malik R, Manral N, 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. Dutta S, Sur D, Manna B, Bhattacharya SK, Deen JL, Clemens JD. Rollback of Salmonella enterica serotype Typhi resistance to chloramphenicol and other antimicrobials in Kolkata, India. *Antimicrob Agents Chemother* 2005; 49 : 1662-3.
8. Gautam V, Gupta NK, Chaudhary U, Arora DR. Sensitivity pattern of Salmonella serotypes in Northern India. *Braz J Infect Dis* 2002; 6 : 281-7.
9. Gupta V, Singla N, Bansal N, Kaistha N, Chander J. Trends in the antibiotic resistance patterns of enteric fever isolates - a three year report from a tertiary care centre. *Malays J Med Sci* 2013; 20 : 71-5.
10. Harichandran D, Dinesh KR. Antimicrobial susceptibility profile, treatment outcome and serotype distribution of clinical isolates of

- Salmonella enterica subspecies enterica: A 2-year study from Kerala, South India. *Infect Drug Resist* 2017; 10 : 97-101.
11. Jain S, Chugh TD. Antimicrobial resistance among blood culture isolates of Salmonella enterica in New Delhi. *J Infect Dev Ctries* 2013; 7 : 788-95.
  12. Joshi S, Amarnath SK. Fluoroquinolone resistance in Salmonella Typhi and S. Paratyphi A in Bangalore, India. *Trans R Soc Trop Med Hyg* 2007; 101 : 308-10.
  13. Kadiravan T, Wig N, Kapil A, Kabra SK, Renuka K, Misra A. Clinical outcomes in typhoid fever: adverse impact of infection with nalidixic acid-resistant Salmonella Typhi. *BMC Infect Dis* 2005; 5 : 37.
  14. Kumar Y, Sharma A, Mani KR. High level of resistance to nalidixic acid in Salmonella enterica serovar Typhi in Central India. *J Infect Dev Ctries* 2009; 3 : 467-9.
  15. Kumar Y, Sharma A, Mani KR. Re-emergence of susceptibility to conventionally used drugs among strains of Salmonella Typhi in central west India. *J Infect Dev Ctries* 2011; 5 : 227-30.
  16. Lakshmi V, 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.
  17. Madhulika U, Harish BN, Parija SC. Current pattern in antimicrobial susceptibility of Salmonella Typhi isolates in Pondicherry. *Indian J Med Res* 2004; 120 : 111-4.
  18. Manchanda V, Bhalla P, Sethi M, Sharma VK. Treatment of enteric fever in children on the basis of current trends of antimicrobial susceptibility of Salmonella enterica serovar typhi and paratyphi A. *Indian J Med Microbiol* 2006; 24 : 101-6.
  19. Misra R, Prasad KN, Amrin N, Kapoor P, Singh S, Ghar M. Absence of multidrug resistance in Salmonella enterica serotypes Typhi and Paratyphi A isolates with intermediate susceptibility to ciprofloxacin. *Trans R Soc Trop Med Hyg* 2015; 109 : 538-40.
  20. 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.
  21. Nagshetty K, Channappa ST, Gaddad SM. Antimicrobial susceptibility of Salmonella Typhi in India. *J Infect Dev Ctries* 2010; 4 : 70-3.
  22. Narain U, Gupta R. Emergence of resistance in community-acquired enteric fever. *Indian Pediatr* 2015; 52 : 709.
  23. Ray P, Sharma J, Marak RSK, Garg RK. Predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance in Salmonella enterica var Typhi. *Indian J Med Res* 2006; 124 : 105-8.
  24. Rodrigues C, Mehta A, Mehtar S, Blackmore PH, Hakimiyani A, Fazalbhoy N, et al. Chloramphenicol resistance in Salmonella typhi. Report

- from Bombay. J Assoc Physicians India 1992; 40 : 729-32.
24. Senthilkumar B, Prabakaran G. Multidrug resistant Salmonella typhi in asymptomatic typhoid carriers among food handlers in Namakkal district, Tamil Nadu. Indian J Med Microbiol 2005; 23 : 92-4.
26. Sharvani R, Hemavathi, Dayanand DK, Shenoy P, Sarmah P. Antibiogram of Salmonella Isolates: Time to consider antibiotic salvage. J Clin Diagn Res 2016; 10 : DC06-8.
27. Srirangaraj S, Kali A, Charles MV. A study of antibiogram of Salmonella enterica serovar Typhi isolates from Pondicherry, India. Australas Med J 2014; 7 : 185-90.
28. Venkatesh BM, Joshi S, Adhikary R, Bhaskar BH. Antibiogram of Salmonella typhii and Salmonella paratyphi A in a tertiary care hospital in 2012. Indian J Pathol Microbiol 2013; 56 : 484-5.
29. 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.
30. Colle, J.G., Duguid J.P., Fraser A.G. and Marmion, B.P. (Eds.) 1989 Mackie and McCartney Practical Medical Microbiology, Vol. 2, p:134 Longman Group, UK.
31. Watson, K.C. 1955, Isolation of *Salmonella* Typhi from the blood stream. J. of Lab and Clinical Medicine 46:128-134.