International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 4, 2018, pp.-1123-1125.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

ANTIMICROBIAL RESISTANCE OF *Salmonella enterica* serovars *typhi* and *paratyphi* FROM BLOOD ISOLATES IN CHILDREN FROM A NORTH DELHI TERTIARY CARE HOSPITAL

JAIN S., SHREE N.*, SHARMA M., SATIJA S. AND KUMAR M.

Department of Microbiology, Hindu Rao Hospital, Malka Ganj, New Delhi, Delhi 110007
*Corresponding Author: Email-drneetushree@gmail.com

Received: February 12, 2018; Revised: April 04, 2018; Accepted: April 06, 2018; Published: April 30, 2018

Abstract- Background: *Salmonella typhi and paratyphi* are mainly responsible for causing enteric fever in India. Widespread use of some common antimicrobial agents led to emergence of *Salmonella* strains with reduced susceptibility and multidrug resistance. Susceptibility pattern keep changing and vary from place to place with time. Methods: A retrospective study was carried out to evaluate *Salmonella* blood isolates in children < 15 years, in relation to specific serotype, age distribution and prevalent antimicrobial resistance pattern. Results: *Salmonella typhi* was most common serotype isolated, accounting for 89.2% of total isolates. None of *Salmonella* isolates were MDR and all were resistant to nalidixic acid. Ciprofloxacin resistance was found in 34.2% of isolates. Low level resistance was seen towards first line antimicrobials including ampicillin (7.2%), chloramphenicol (5.4%) and cotrimoxazole (2.7%). Conclusion: Emergence of antimicrobial resistance in typhoidal *Salmonella*e constitutes a new challenge. Re-emergence of strains sensitive towards first line antimicrobial agents emphasizes concept of antibiotic recycling in hospitals.

Keywords- Multidrug resistance (MDR), nalidixic acid resistant *Salmonella typhi* (NARST), enteric fever

Citation: Jain S., *et al*., (2018) Antimicrobial Resistance of *Salmonella enterica serovars typhi* and *paratyphi* from Blood Isolates in Children from a North Delhi Tertiary Care Hospital. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 4, pp.-1123-1125.

DOI: http://dx.doi.org/10.9735/0975-5276.10.4.1123-1125

Introduction

Typhoid and paratyphoid fever, collectively known as enteric fever is an important health problem globally, especially in the developing and underdeveloped countries. In India also, enteric fever is endemic in many places due to lack of proper sanitation and hygiene for many socioeconomic reasons. Annual incidence of enteric fever has been reported as more than 100 per lakh population for South East Asia and as high as 10 per 1000 person in Delhi, India [1]. Enteric fever is mainly caused by typhoidal *Salmonella* including *Salmonella entrica serovars typhi* and *paratyphi* A [2]. In India, enteric fever is mainly seen in summers and predominantly caused by *Salmonella enteric serovar typhi*. Bacteremia with *Salmonella* may be serious and life threatening. Infection in children is reportedly more as compared to elderly [2-4].

Mainstay of enteric fever treatment includes fluoroquinolones, third generation cephalosporin and azithromycin [2-7]. Emergence of antimicrobial resistance was reported for *Salmonella* isolates from 1960 onwards including multi drug resistance [5]. Multi drug resistance (MDR) in *Salmonella* is defined as resistance for first line drugs *i.e*., ampicillin, chloramphenicol and cotrimoxazole [1]. This further worsens the situation making treatment difficult and complicating the management of enteric fever [1]. MDR in *Salmonella* strains is mainly due to plasmid mediated resistance whereas fluoroquinolone resistance is a result of chromosomal mutation of *DNA Gyrase* (*gyrA and gyrB*) and topoisomerase IV gene. Gene mutations encoding for extended spectrum beta lactamases confers resistance towards broad spectrum cephalosporins [1,3,8]. MDR *Salmonella* strains are more virulent in terms of morbidity and mortality, a troublesome fact concerning clinical management and prevention of these infections [1]. In India, first multi drug resistant *Salmonella typhi* (MDRST) outbreak was reported in 1960 from Calicut [6]. Increasing multi drug resistant isolates were being reported since then from all over the world. MDR incidence has been reported as high as 80 % during 1980 to 2004 from various parts of India [6]. A variable declining trend was observed thereafter from India and other countries [9-11]. Studies across the globe also documented an increase in number of quinolone resistant strains especially nalidixic acid resistant *Salmonella typhi* (NARST), apart from MDR isolates in typhoidal *Salmonella* [9-13]. Furthermore, various other studies across the globe documented an increase in resistance towards quinolones and third generation cephalosporins, commonly used for empirical treatment of enteric fever [8-13].

Azithromycin, a broad-spectrum macrolide antimicrobial, although costly, introduced later for the treatment of enteric fever, as a very good option because of its property of intra cellular concentration and good clinical response [14-17]. Unfortunately, many researchers have reported emergence of resistance for azithromycin from various parts of the world [14-17].

This variable and changing susceptibility pattern worldwide among the isolates of *Salmonella typhi* and *Salmonella paratyphi* emphasizes upon the importance of continuous monitoring and evaluation of locally prevalent antibiogram pattern for making suitable treatment guidelines for enteric fever. Relevant microbiological and epidemiological data is required for planning prevention and management of these infections. Limited regional data is available regarding the current scenario of enteric fever focusing on susceptibility pattern of *Salmonella enteric serovars typhi* and *paratyphi* A. Therefore, the present study was planned to study the *Salmonella* blood isolates in children in relation to specific serotype, age distribution and the prevalent antimicrobial resistance pattern in Hindu Rao Hospital, a North Delhi tertiary care and referral hospital.

Material and methods

This retrospective study included a total of 4215 samples received for blood culture, admitted in the hospital over a period of one year from June 2015 to May 2016. The study population included children less than 15 years of age. The study was approved by the institutional ethical committee. Blood culture was done by automated BD BACTEC culture system. Blood culture bottles were incubated for a maximum of 5 days. Positive blood cultures were further streaked on blood agar and MacConkey agar for identification. Identification of isolates was done using standard phenotypic, biochemical and serological tests. Serological identification of isolates was performed by agglutination test using different types of polyvalent and monovalent antisera (Denka Seiken Co Ltd., Tokyo, Japan). Bacterial identification was additionally confirmed with VITEK 2C identification system. Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method as per CLSI guidelines, using commercially available 6 mm antibiotic disks from Himedia, Mumbai [18]. Various antibiotic disks tested includes Ampicillin (10ug), Chloramphenicol (30ug), cotrimoxazole (25ug), Ciprofloxacin (5ug), Nalidixic acid (30ug), Ceftriaxone (30ug), and Imipenem (10ug). Isolates resistant to ampicillin, chloramphenicol and cotrimoxazole were defined as multi drug resistant (MDR) strains. E coli strain, ATCC 25922 was used as standard strain for antimicrobial susceptibility testing.

Results

From a total of 4215 blood culture samples, 111 isolates of *Salmonella* were obtained with an isolation rate of 2.6% for *Salmonella*. 2499 (59.3%) samples were from male patients and 1716 (40.7%) were from female patients yielding 68 and 43 *Salmonella* isolates respectively. Isolation rate of *Salmonella* was 2.7% in males and 2.5% in females. Proportion of *Salmonella* isolates was maximum from the age group of less than 5 years (42.3%), followed by 5-10 years (32.4%) and more than 10 years (25.2%) with mean age as 6.48 years. [Table-1].

Table-1 *Age wise distribution of Salmonella isolates*

Age group (Years)	Number (n= 111)	Percentage
<5	47	42.3%
5-10	36	32.4%
>10	28	25.2%

Of the 111 *Salmonella* isolates, 99 (89.2%) were *S. typhi* and only 12 (10.8%) were *S. paratyphi* A. All of the *Salmonella* isolates were susceptible to ceftriaxone and imipenem. All the 111 *Salmonella* isolates were resistant to nalidixic acid. After nalidixic acid, second highest resistance of 34.2% (38/111) was seen against ciprofloxacin followed by ampicillin 7.2% (8/111), chloramphenicol 5.4% (6/111) and cotrimoxazole 2.7% (3/111). None of the *Salmonella* isolates were MDR i.e. resistant to ampicillin, chloramphenicol and cotrimoxazole. [Table-2]

Table-2 *Antimicrobial resistance in Salmonella isolates*

Antimicrobial agent	Number of isolates resistant (n)	Percentage (%)
Nalidixic acid (30ug)	111	100%
Ciprofloxacin (5ug)	38	34.2%
Ampicillin (10ug)	8	7.2%
Chloramphanical (30ug)	6	5.4%
Cotrimoxazole (25ug)	3	2.7%
Ceftriaxone (30ug)	NIL	-
Imipenem (10ug)	NIL	-

Discussion

Enteric fever, a major infectious disease, is endemic in India constituting an important public health problem. Widespread use of some common antimicrobial agents led to emergence of antimicrobial resistance among *Salmonella* strains with reduced susceptibility. Important barriers to control are poorly immunogenic vaccines with short lasting immunity especially in very young children and emergence of multidrug resistance [3]. In the present study, the predominant *Salmonella* isolate was *Salmonella typhi* accounting for up to 89.2% of the total isolates which may be attributed to the endemicity of the organism in India. Some studies have reported replacement of *Salmonella typhi* by *Salmonella paratyphi* strains in enteric fever [3,6]. 10.8% of the isolates were *Salmonella paratyphi* A. Rate of isolation of *Salmonella* was almost comparable in both male and female patients (2.7 verses 2.5%). Most affected group was children less than 5 years of age as also documented by Mweu, *et al*. and others [3,4,6]. Though there are previous reports of cephalosporin resistant *Salmonella* isolates from different parts of the world associated with its clinical failure [8- 13], all the isolates in our study were fully susceptible to 3rd gen cephalosporins and carbapenems. None of the *Salmonella* isolates were MDR. Resistance towards the first line antimicrobials including ampicillin (7.2%), chloramphenicol (5.4%) and cotrimoxazole (2.7%) is comparatively very low as compared with previous studies pointing towards the reemergence of susceptibility for these agents [7,9-11]. Similar results were shown by 5 years review from Indonesia [17]. It clearly indicates the possible excellent scope for reconsideration of these antimicrobials back in the treatment of enteric fever. This reversal in the drug resistance pattern may be attributed to the restricted use of these antimicrobials in the recent past following the reports of emergence of MDR isolates from various parts of country and worldwide. Nalidixic acid resistance has been associated with reduced susceptibility towards fluoroquinolones even when the isolates may appear sensitive in vitro to ciprofloxacin[9-13, 19].Many authors have reported high resistance for nalidixic acid among *Salmonella* isolates, threatening efficacy of treatment and clinical failure of ciprofloxacin [7,9-13]. Ciprofloxacin resistance was found in 34.2% of isolates in our study. Increasing ciprofloxacin resistance is worrisome as it is an important oral antibiotic commonly used for empirical treatment of enteric fever. In our study, all the isolates tested were resistant to nalidixic acid (NAR), which is a very serious matter of concern posing challenge in effective treatment and management of these infections. Other treatment options for enteric fever includes azithromycin, a microcline antimicrobial, used widely now a day with good clinical response because of intra cellular accumulation of the drug [7, 14]. Unfortunately, some authors have reported resistance to azithromycin too from different parts of the world [14-17]. Based on the results of our study, especially low level resistance observed towards first line antimicrobials used in the treatment of enteric fever, it can be suggested that these can be recommended either alone or in combination with cephalosporin or azithromycin as part of empirical treatment regimen. Moreover, it will spare the frequently used drugs for which high antimicrobial resistance already exists. Furthermore, this will also help to reduce the antimicrobial resistance developed against these drugs out of selection pressure.

Conclusion

Emergence of MDR and NARST in typhoidal *Salmonellae* constitutes a new challenge limiting therapeutic options. Re-emergence of strains sensitive towards first line antimicrobial agents emphasizes the concept of antibiotic recycling in hospital settings unless contraindicated. A retrospective analysis of prevalent antibiogram pattern may therefore be very helpful for selection of optimal antibiotic regimen.

Future Perspective

The study would have been better with periodic future studies including more number of samples to notice the changing antimicrobial resistance trend along with clinical and molecular correlation.

Application of research

Antimicrobial resistance keeps changing for typhoidal *Salmonellae*. A knowledge of prevalent antibiogram pattern is very crucial for selecting appropriate treatment regimen and to prevent emergence or spread of drug resistance in enteric fever.

Research category: Antimicrobial resistance, *Salmonella enterica*.

Abbreviations:

MDR: Multidrug resistance

NARST: Nalidixic acid resistant *Salmonella typhi*

Acknowledgement/ Funding: Author thankful to Department of Microbiology, Hindu Rao Hospital, Malka Ganj, New Delhi, Delhi 110007.

***Principle Investigator: Dr Sanjay Jain**
Organisation: Department of Microbiology, Hindu Rao Hospital, Delhi, India.
Research project name or number: NIL

Author contributions: All authors equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of interest: None declared.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors

References

[1] Eng S.K., Pusparajah P., Mutalib N.S.A., Ser H.L., Chan K.G. and Lee L.H. (2015) *Frontiers in Life Science*, 8(3), 284-93.
[2] Harish B.N. and Menezes G.A. (2011) *Indian J Med Microbiol,* 29, 223-9.
[3] Wain J., Hendriksen R.S., Mikoleit M.L., Keddy K.H. and Ochiai R.L. (2015) *Lancet*, 385, 1136-45.
[4] Mweu E. and English M. (2008) *Tropical medicine and International health*, 13, 532-40.
[5] Montville T.J. and Matthews K.R. (2008) Food microbiology: an introduction. 2nd edition. Washington, USA: ASM press.
[6] Dutta S., Das S., Mitra U., Jain P., Roy I., Ganguly S.S., *et al*. (2014) *PLoS One*, 9(8), E101347.
[7] Patel S.R., Bharti S., Pratap C.B. and Nath G. (2017) *J ClinDiag Res*, 11(6), DM01-03.
[8] Gokul B.N., Menezes G.A. and Harish B.N. (2010) *Emerg Infect Dis*, 16(7), 1170-71.
[9] Nath G. and Maurya P. (2010) *Int J Antimicrobe Agents*, 35(5), 482-5.
[10] Pratap C.B., Patel S.K., Shukla V.K., Tripathi S.K., Singh T.B. and Nath G. (2012) *Int J Antimicrobe Agents*, 40(3),277-85.
[11] Nath G., Tikoo A., ManochaH., Tripathi A.K. and Gulati A.K. (2000) *J Antimicrobe Chemother*, 46(1), 149-50.
[12] Geetha V.K., Yugendran T., Srinivasan R. and Harish B.N. (2014) *Indian J Med Microbiol*, 32, 31-4.
[13] Capoor M.R. and Nair D. (2010) *J Globe infect Dis*, 2(3), 258-62.
[14] Molloy A., Nair S., Cooke F.J., Wain J., Farrington M., Lehner P.J., *et al*. (2010) *J ClinMicrobiol*, 48(12), 4655-7.
[15] Rai S., Jain S., Prasad K.N., Ghosal U. and Dhole T.N. (2012) *Indian J Med Microbiol*, 30(1), 30-3.
[16] Choudhary A., Gopalakrishnan R., Nambi P.S., Ramasubramanian V., Gafur K.A. and Thirunarayan M.A. (2013) *Indian J Med Res*, 137(4), 800-2.
[17] Lugito NPH and Cucunawangsih (2017) *International Journal of Microbiology*, article ID 6215136.
[18] Performance standards for antimicrobial susceptibility testing. CLSI document (M100-S20), 30, Clinical and Laboratory Standards institute: Wayne, Pennsylvania. 2010.
[19] Bhagra S., Kanga A., Ganju S.A. and Sood A. (2014) *Int. J. Pharm. Bio. Sci.,* 5(4), 1-9