REVIEWING ANTIBIOGRAM OF SALMONELLA TYPHI AS BLOOD CULTURE ISOLATE IN A TERTIARY CARE HOSPITAL, KOLKATA- REVERTING SUSCEPTIBILITY PROFILE

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ABSTRACT

BACKGROUND

Enteric fever is still a major contributor to the disease burden in India. According to National Health Profile 2018, a total of 493 deaths occurred in 2017 in India due to typhoid fever. Early diagnosis and treatment with proper drugs are the only option to reduce mortality. After development of resistance to fluoroquinolones (once the most frequently used drugs for treatment of multidrug resistant enteric fever), we are currently left with third generation cephalosporins and azithromycin. But irrational use of these drugs may lead to development of resistance in near future. So, it is time to review re-introduction of the age old first line drugs like ampicillin, chloramphenicol and co-trimoxazole, in the treatment of typhoid fever. The aim of our study was to review the susceptibility pattern of *Salmonella* Typhi based on their MIC and rational use of antibiotics to prevent further development of MDR strains.

METHODS

In this retrospective study, 4142 blood samples were collected and studied for one-year period. Isolated organisms and their drug susceptibility patterns were analysed based on their MIC.

RESULTS

Among the 1187 (28.65%) culture positive isolates, 1.85% of *Salmonella spp.* were isolated including 22 *Salmonella* Typhi and single isolate of *Salmonella* Paratyphi A. Most of the isolates were from male patients (68.18%) with less than 20 years of age (77.27%). *Salmonella* Typhi showed 100% sensitivity to ampicillin, chloramphenicol and co-trimoxazole. It also showed 100% resistance to nalidixic acid and 54.55% resistance to ciprofloxacin. However, 100% sensitivity to third generation cephalosporins, carbapenems, colistin and azithromycin was also observed. A single isolate of *Salmonella* Paratyphi A showed same resistogram as of *Salmonella* Typhi.

CONCLUSIONS

Delay in instituting effective antimicrobial treatment results in poor outcome in patients suffering from typhoid fever. As there are variations in the antibiotic susceptibility patterns of *Salmonella* isolates, periodic monitoring of resistance pattern will remain essential for effective empirical therapy which should correspond with patterns of antimicrobial susceptibility of local *Salmonella* strains; otherwise irrational use of antibiotics may again cause appearance of MDR strains in the near future.

KEY WORDS

Salmonella Typhi, MDR, NARST, DCS, Antibiogram

BACKGROUND

Enteric fever is a potentially fatal multisystemic illness caused primarily by *Salmonella enterica*, subspecies *enterica* serovar Typhi and to a lesser extent, related serovars Paratyphi A, B, and C. Enteric fever is a major public health problem causing an estimated between 11 and 21 million cases and 1,28,000 to 1,61,000 typhoid-related deaths occur annually worldwide (WHO 2018).

India bears the brunt of cases with an estimated 22, 21,190 cases and 493 deaths per year (2018).[1] The classical presentation of the disease includes fever, malaise, diffuse abdominal pain, occasional loose stool and constipation. The presenting signs and symptoms of typhoid fever in children differ significantly from those in adults. Abdominal distension more common in younger children whereas abdominal pain and headache were more common in older children.[2] The ever-changing resistance pattern of these causative agents makes the disease a true therapeutic challenge.

In India, antibiotic resistance has emerged whenever a drug has been widely used for the treatment of enteric fever. After its introduction in 1948, chloramphenicol resistance was seen within 2 years, although the first outbreak in India was not reported until 1972.[3] Subsequent widespread use of amoxicillin resulted in the emergence of TEM1, a plasmid borne β-lactamase capable of hydrolysing penicillin but not extended-spectrum cephalosporins. Use of co-trimoxazole promoted resistance through sul1 and sul2 and the dfra7 gene. By the end of the 1980s, multidrug resistant typhoid

(MDRT), defined as isolates resistant to amoxicillin, co-trimoxazole and chloramphenicol, was raging throughout India. Although there are no countrywide surveillance data from this period, individual studies from all regions of India showed the emergence and rise of MDRT during the late 1980s and 90s.[4-7] After the rise of MDRT, ciprofloxacin became the drug of choice. However, point mutations in the Quinolone Resistance Determining Region (QRDR) of DNA gyrase gave rise to decreased ciprofloxacin susceptibility (DCS), identified in vitro by nalidixic acid resistance. DCS strains were associated with delayed fever clearance and an increase in the rate of complications and clinical failure.[8] Most clinicians now favour azithromycin for uncomplicated disease and ceftriaxone for patients requiring intravenous therapy and this is recommended in the National Treatment Guidelines for Antimicrobial Use in Infectious Diseases, recently published by the National Centre for Disease Control in India (NCDC).[9] Cefixime, an oral third-generation cephalosporin, is also recommended by the NCDC guidelines and the Indian Association of Paediatricians for uncomplicated disease,[10] but is associated with delayed fever clearance times and clinical failure.[11] Similarly, even ceftriaxone resistant strains have been reported from various parts of India.[12] As there are variations in the sensitivity patterns of *Salmonella* isolates from time to time, continuous monitoring of antibiotic susceptibility pattern of locally prevalent strains is needed and the study was conducted to review the antimicrobial susceptibility of local *Salmonella* isolates against commonly used drugs.

METHODS

A retrospective study was done from the laboratory records and relevant data maintained over a period of one year, from April 2018 to March 2019 in a tertiary care hospital. Total 4142 blood samples were collected during the study period from febrile patients admitted in different wards by aseptic measures irrespective of age and sex. Approximately 10-15 ml of blood was collected from the adult patients and 5-10 ml from paediatric age group as adequate volume of blood is required for culture to become positive. Each blood samples were directly inoculated in BacT/ALERT (bioMerieux System) blood culture bottle. Subcultures were done on MacConkey's agar and Blood agar from the bottles which gave positive signals. All positive growths including non-lactose fermenting colonies were processed for identification, antibiotic susceptibility and MIC values with the help of Vitek2 (bioMerieux System) and interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations. To confirm the serotype slide agglutination test with specific antisera was also done. Vitek2 system gave sensitivities of the antibiotics used in typhoid fever with their MIC values except Chloramphenicol and Azithromycin. So, E-test (Hi media, Mumbai, India) strip was used for detection of MIC values of the aforementioned drugs. Clinical history was taken from the patients whose blood culture yield *Salmonella spp.* to find out the most common presenting symptoms. Clinical features were also studied in different age groups. Data were analysed and expressed in number and percentages.

RESULTS

Total 4142 blood samples were tested, out of which 1187 (28.65%) samples were "Culture positive". *Coagulase Negative Staphylococcus* (CONS) (34.71%) was most common among the isolates followed by *Staphylococcus Aureus* (30.92%), *Klebsiella spp.* (12.89%), *Escherichia coli* (9.43%), *Acinetobacter spp.* (6.07%), *Pseudomonas spp.* (3.12%), *Salmonella spp.* (1.85%) and *Candida spp.* (1.01%) (figure 1). Total 22 (21 *Salmonella* Typhi and 1 *Salmonella* Paratyphi A) *Salmonella spp.* were isolated with moderate male preponderance (68.18%) and increased (77.27%) predisposition in children and young adults (<20 yrs.) (Table 1).

Figure 1. % Distribution of Blood Culture Isolates
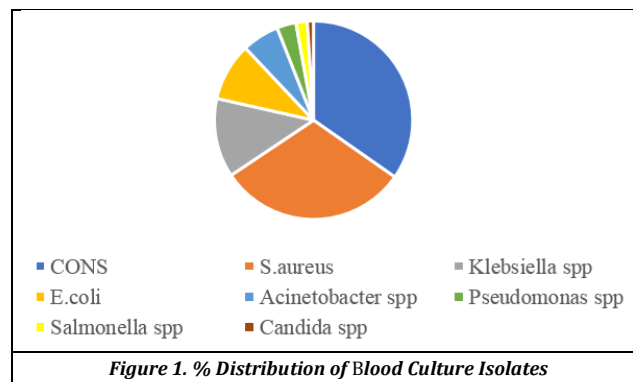
Age Group	<10 Yrs.	10-20 Yrs.	20-30 Yrs.
% of patients	27.27%	50%	22.73%

Table 1. Age Distribution of Patients with Enteric Fever

Age Group	<10 Yrs.	10-20 Yrs.	20-30 Yrs.
Diarrhoea	68.75%	60.0%	33.33%
Constipation	31.25%	40.0%	66.67%

Table 2. % Distribution of G.I. Symptoms with Age of Enteric Fever Patients

Drugs	Sensitive			Intermediate SDD= Susceptible Dose Dependent			Resistant		
	% sensitivity	MIC value μg/ml	Break point MIC value μg/ml (According to CLSI)	% sensitivity	MIC value μg/ml	Break point MIC value μg/ml (According to CLSI)	% sensitivity	MIC value μg/ml	Break point MIC value μg/ml (According to CLSI)
Ampicillin	100	≤ 2	≤ 8	0	-	16	0	-	≥ 32
Chloram-phenicol	100	≤4 (27.27%) ≤8 (72.73%)	≤8	0	-	16	0	-	≥ 32
Co-trimoxazole (Trimethoprim-sulphame-thoxazole)	100	≤20	≤2/38	0	-	-	0	-	≥4/76
Azithromycin	100	≤4 (13.64%) ≤8 (63.63%) ≤16 (22.73%)	≤ 16	0	-	-	0	-	≥ 32
Ceftriaxone	100	≤1	≤ 1	0	-	2	0	-	≥ 4
Cefo-sulbactam	100	≤8	–	0	-	–	0	-	–
Cefepime	100	≤1	≤ 2	0	-	4-8(SDD)	0	-	≥ 16
Pip-tazo	100	≤4	≤16/4	0	-	32/4-64/4	0	-	≥ 128/4
Ertapenem	100	≤0.5	≤ 0.5	0	-	1	0	-	≥ 2
Imipenem	100	≤0.25	≤ 1	0	-	2	0	-	≥ 4
Meropenem	100	≤0.25	≤ 1	0	-	2	0	-	≥ 4

Colistin	100	≤0.5	_	0	-	_	0	-	_
Nalidixic Acid	0	--	≤16	0	-	-	100	32	≥ 32
Ciprofloxacin	0	--	≤ 0.06	54.55	= 0.5 (33.33%) ≤ 0.25 (66.67%)	0.12-0.5	45.45	>1 (40.0%) >4(60.0%)	≥ 1

***Table 3. A**ntibiotic Susceptibility Pattern of Salmonella*

Ciprofloxacin MIC≤0.25	Ciprofloxacin MIC=0.5	Ciprofloxacin MIC >1	Ciprofloxacin MIC > 4
36.36%	18.18%	18.18%	27.27%

Table 4. MIC Value Distribution of Ciprofloxacin

Parameters	Present Study	Other Studies		
		Study 1	Study 2	Study 3
Proportion of S. Typhi among culture positive isolates in blood	1.85% (April 2018-March 2019)	20.30% Lunguya et al[13] (2007-2011)	4.47% Singh Lavan et al[14] (2012-2014)	10.3% Tack et al[15] (2007-2017)
Age distribution	72.72% (<20 yrs.) 27.27% (>20 yrs.)	81%(<30yrs) Sharvani et al[16] (Jan-Dec. 2013)	60%(10-19yrs) 32.8% (<10yrs) Lunguya et al (2007-2011)	56.52% (<16 yrs) 43.47% (>16 yrs) Singh Lavan et al (2012-2014)
Sex distribution	Male 68.18%	Male 63% Sharvani et al (Jan-Dec 2013)	Male 62.5% Bhattacharya et al[17] (2016)	Male 54.23% Dutta & Afroj et al[18] (Jan-Dec. 2012)

% of Susceptibility of *Salmonella spp.* to Commonly Used Drugs				
	Present study (April 2018-March 2019)	Shrirangaraj et al[19] (Jan 2012-June 2013)	Sharvani et al (Jan-Dec 2013)	Chaudhury et al[20] (May 2009-June 2011) / S.V.Wankhede et al[21] (Jan 2015 to May 2018)
Chloramphenicol	100%(S)	93.75%(S)	95.2%(S)	100%(S) / 100% (S)
Ampicillin	100%(S)	93.75%(S)	94.6%(S)	90.68% (S) / 72.66% (S)
Co-trimoxazole	100%(S)	87.5%(S)	95.8%(S)	95-03%(S) / 100% (S)
Nalidixic acid	100%(R)	100%(R)	90.5%(R)	74%(R) / 90.90% (R)
Ciprofloxacin	54.55%(IS) 45.45%(R)	93.75%(IS) 6.25%(R)	62.2%(S) 37.85(R)	54.34%(S) / 21.22% (S) 48.48% (R) 30.30% (IS)
Ceftriaxone	100%(S)	93.75%(S)	97%(S)	100%(S) / 90.90% (S)

***Table 5. Comparison of Various Parameters B**etween Present **A**nd other Related Studies*

[Sensitive→(S), Intermediate Sensitive →(IS), Resistant→(R)]

The common presenting symptoms of patients with enteric fever were fever (100%), malaise (85%), anorexia (84%), headache (80%) followed by pain abdomen (60%), diarrhoea (52%), skin rashes (40%), constipation (27%) and clinically it was found that adults were more prone to constipation in comparison to children, who usually presented with diarrhoea (Table 2). Other clinical symptoms did not differ with age.

Salmonella Typhi showed 100% sensitivity to Ampicillin, Chloramphenicol and Co-trimoxazole, the age old first line drugs which eventually lost their efficacy in the previous years (1980s). MIC values of chloramphenicol varied between 4-8 µg/ml with increased (72.73%) tendencies to ≤8 µg/ml. It also showed 100% sensitivity in third generation Cephalosporins, Carbapenems, Colistin and Azithromycin. The single isolate of *Salmonella* Paratyphi A showed the same sensitivity pattern. 100% resistance was seen with Nalidixic acid. Resistance to ciprofloxacin was among 45.45% isolates and Intermediate sensitivity to ciprofloxacin was seen in 54.55% isolates (Table 3). According to CLSI 2019 MIC of ciprofloxacin ≤ 0.06 µg/ml is sensitive, 0.12 to 0.5 µg/ml is intermediate-sensitive and ≥1 µg/ml is resistant.

As all the isolates have decreased ciprofloxacin susceptibility, determination of MIC value in ciprofloxacin is necessary (Table 4).

DISCUSSION

In our study among the blood culture positive isolates, 1.85% was *Salmonella spp.* and fever was the common clinical presentation (100%) followed by anorexia (84%), pain abdomen (60%). In a study by Sarswat S, et al,[22] fever (100%), anorexia (87.65%), abdominal pain (75.34%) and vomiting (47.9%) were the most common clinical symptoms. We compared our study with others which is shown in the following table (Table 5) to get a comprehensive data on current trends in prevalence of *Salmonella spp.*, age-sex distribution of enteric fever cases and antibiotic profile of *Salmonella* Typhi.

Azithromycin was 100% sensitive in our study whereas it was 99.5% sensitive in a study by Christopher M. Parry et al (2015).[23] In another similar study by Mishra et al (2016)[24] also showed more than 99% susceptibility to Azithromycin and majority of isolates (75%) were with MIC values between 4 and 8 µg/ ml as in our study (>76%). In this study there was no Multidrug resistant (MDR) isolates. Similar finding was observed in Pune by S. V. Wankhede, et al [22] and in Puducherry by Bhat K. S. et al.[25] Multidrug resistant (MDR) strains was observed in Delhi (14.9%) by R Raveeendran et al[26] and in Karnataka which was even lower (10%) by Kavita Nagshetty et al[27] while 3.7% was reported in a study by S Rai et al[28] and 1.7% was reported by Sharvani et al on 2013.[17]

CONCLUSIONS

The present study indicates that the currently prevalent strain in Kolkata is Nalidixic acid resistant *S.* Typhi (NARST) with decreased ciprofloxacin susceptibility (DCS). As no isolates were MDR, reuse of the first line antibiotics can be considered for treating enteric fever cases. To avoid treatment failure, it is necessary to determine MIC levels for ciprofloxacin before use. Third generation cephalosporins still remain a drug of choice for treatment of MDR enteric fever cases. As in some cases, it causes delayed fever-clearance or relapse; azithromycin can be considered with third generation cephalosporins. In case of enteric fever, it is advisable to start empirical antibiotic therapy as it decreases the mortality rate of 30% to as low as 0.5% [29]. Due to variations in the antibiotic susceptibility pattern of *Salmonella* isolates, periodic monitoring of resistance pattern will remain essential for deciding rational empirical treatment as overuse or irrational use of antibiotics may again cause emergence of MDR strains.

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