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Original Article

Antibiotic Susceptibility pattern of blood culture isolates of Enteric fever pathogens in a tertiary care center-A Retrospective study

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ABSTRACT

Objectives: This retrospective study was conducted to determine the prevailing antimicrobial resistance pattern amongst blood culture isolates from culture proven Enteric fever cases in a rural tertiary care teaching hospital.
Methods: This retrospective study was conducted in a rural tertiary care teaching hospital wherein the Laboratory data of blood culture positive cases of Enteric fever maintained over a period of 2 years from July 2015 to June 2017 was retriewed, reviewed and analysed to determine the antibiotic susceptibility pattern of *S.enterica* isolates.
Results: 512 blood samples processed for culture sensitivity yielded 30 strains of Salmonella typhi (86%) and 5 strains (14%) of Salmonella paratyphi A. S.typhi strains showed highest susceptibility towards Imipenem (100%) followed by 3rd generation cephalosporins (>90%), Aztreonam (90%), Cefepime (90%), Levofloxacin (86.67%) and Ciprofloxacin (70%). S.paratyphi A strains showed highest susceptibility towards Cefixime, Ceftazidime, Ceftriaxone, Amikacin & Imipenem.
Conclusion: This study emphasises the need for doing blood culture and antibiotic susceptibility testing for each and every suspected case of enteric fever. Irrational inappropriate indiscriminate usage of antibiotics must be strictly curbed through antimicrobial stewardship to prevent the emergence of multidrug resistant strains.

Keywords: Enteric fever, S.typhi, S.paratyphi A, Antimicrobial resistance pattern, Blood culture, Antibiotic susceptibility testing, Multi drug resistance.

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INTRODUCTION

Enteric fever is a potentially life threatening multisystemic infectious disease caused by Salmonella enterica, subspecies enterica seovar typhi and serovars paratyphi A,B & C[1]. It is a serious public health threat throughout the world with an annual incidence of over 16.6 million cases resulting in 6 lakh deaths every year as per WHO records[1].

The situation has further worsened by global emergence of multidrug resistant strains of enteric fever pathogens resistant to conventional first line drugs-Chloramphenicol, Ampicillin & Cotri-moxazole[2]. This may be due to selective pressures resulting from their indiscriminate usage and also due to a single large self-transferable plasmid responsible for such en-bloc resistance[3]. This has resulted in rampant usage of Fluoroquinolones esp.Ciprofloxacin in clinical settings leading to a gradual increase in Minimum inhibitory concentrations with emergence of Ciprofloxacin resistance causing therapeutic failure[3].This problem is aggravated with the advent of Nalidixic acid resistant S.typhi(NARST) as such strains are less susceptible to Fluoroquinolones[4,5].

In the current scenario, 3rd generation Cephalosporins like Ceftriaxone, Cefixime and macrolides like azithromycin has gained importance in Enteric fever therapeutics as appropriate alternatives in treatment of Multi drug resistant cases. But with their increasing usage in clinical settings the resistance against these antibiotics is increasingly reported amongst S.enterica strains[6].

Multidrug resistance has been emerging rapidly and consistently in Enteric fever pathogens driven by selection pressure due to inappropriate irrational drug therapy.This has resulted in treatment failures leading to extended hospital stay,health complications , increased potential for faeco oral transmission due to prolonged fecal carriage, significant rise in morbidity and mortality[7]. The study of antibiotic susceptibility pattern of S.enterica isolates is crucial in prompt & appropriate therapy of enteric fever in prevention and control of the disease. Continual consistent surveillance and monitoring of local antimicrobial resistance trends is a pre-requisite for implementing rational measures and to update the therapeutic guidelines [8].

In light of above facts,we had undertaken this retrospective study to evaluate the prevailing antimicrobial resistance pattern amongst blood culture isolates from culture proven Enteric fever cases in a rural tertiary care teaching hospital of western Uttarpradesh.

MATERIAL AND METHODS

This retrospective study was conducted in the Department of Microbiology of a rural tertiary care teaching hospital of western Uttarpradesh, India wherein the Laboratory data of blood culture positive cases of Enteric fever maintained over a period of 2 years from July.2015 to June 2017 was retriewed, reviewed and analysed to determine the prevalence of culture proven Enteric fever cases and the antibiotic susceptibility pattern of *S.enterica* isolates. This study was undertaken after seeking approval for conduct of study from Institutional Ethics Committee.

Collection of Blood samples: Venous Blood samples (10ml from adults and 5ml from children) were drawn aseptically from clinically suspected cases of Enteric fever before starting any antibiotic therapy and inoculated into Adult & Paediatric blood culture bottles containing BHI Broth with SPS (Microexpress, India) respectively and transported immediately to Microbiology Lab.[9]

Blood Culture/Sensitivity: The inoculated blood culture bottles were incubated aerobically at 37^0C for 24 hrs. Subcultures were made on blood agar and MacConkey agar plates every alternate day till 7th day[9]. Non Lactose fermenting (NLF) colonies grown over the culture plates were further processed using standard microbiological techniques. Isolation, Identification and characterisation of Salmonella sp. was done as per standard protocol through biochemical tests followed by Slide agglutination using antisera [10,11]. Antimicrobial susceptibility testing was performed for all isolates using Kirby Bauer Standard Disc diffusion method as per CLSI 2016 guidelines on Mueller Hinton Agar plates[12] using following antibiotic discs(Himedia Lab.Pvt Ltd,Mumbai,India):
Ampicillin(10µg), Azithromycin(15µg) Ciprofloxacin(5µg), Levofloxacin(5µg), Ceftriaxone(30 µg), Cefixime(5µg), Cefotaxime(30µg), Ceftazidime(30µg), Cefipime(30µg), Chloramphenicol(30µg), Cotrimoxazole(1.25/23.75µg), Nalidixic acid(30 µg), Tetracycline(30µg), Amikacin(30µg), Imipenem(30 µg), Aztreonam(30 µg).

Detection of Extended spectrum beta lactamase: Isolates that were found to be resistant to at least two 3rd generation Cephalosporins {Cefotaxime(30µgm),Ceftazidime(30µgm), Ceftriaxone((30 µgm)etc.} were considered to be probable ESBL producers and further screened for ESBL production by Combined Disc diffusion Test

as per CLSI 2015 guidelines using Antibiotic discs of Cefotaxime (30µg) and Ceftazidime (30µg), Cefotaxime clavulanate (30/10µg) and Ceftazidime clavulanate (30/10 µg). ≥ 5 mm increase in diameter of the inhibition zone of the Cefotaxime clavulanate and Ceftazidime clavulanate disc compared with the respective cefotaxime and ceftazidime disc alone was interpreted as phenotypic evidence of ESBL production [12].

RESULT AND DISCUSSION

A total of 512 blood samples were processed for culture sensitivity during a period of 2 years from July 2015 to June 2017 out of which 35 clinical isolates of Salmonella spp (0.07%) were obtained. Salmonella typhi being predominant with 30 strains(86%) followed by Salmonella paratyphi A with 5 strains(14%).Similar findings were also reported by previous studies (Mohanty S et al 2009,Gupta V et al 2013, Behl P et al 2017,Ali A et al 2017) [4,13-15].

Table-1 depicts agewise distribution of Salmonella isolates.The majority of culture confirmed cases of enteric fever were in 21-30 yrs. age group and least from 51-60 & >60 yrs. age groups.Similar findings were also reported by Acharya A et al 2012, Bhagra S et al 2014, Afroz H et al 2014, Sharvani R et al 2016 and Gurung B et al 2017 [8,10,16-18].

In this study male preponderance was observed in Enteric fever cases with male:female ratio=2.2:1 for all Salmonella isolates with 2:1 for S.typhi and 4:1 for S.paratyphi A. A number of similar studies in past had also reported male preponderance in culture confirmed cases of enteric fever (Acharya A et al 2012, Gupta V et al 2013, Bhagra S et al 2014, Paudel KR et al 2016, Das S et al 2016, Porwal A et al 2016, Sharvani R et al 2016, Duggal S et al 2016, Ali A et al 2017) [8,10,13,15,17,19,20].This may be due to increased vulnerability of males due to more outdoor exposure esp.in countries like India.

The antibiotic susceptibility pattern of S.typhi as depicted in Table-2 & Fig.1, showed very high susceptibility towards Imipenem (100%) followed by third generation cephalosporins [Ceftriaxone (96.67%), Ceftazidime (96.67%), Cefixime (93.33%), Cefotaxime (90%)], Monobactums-Aztreonam (90%) & fourth generation Cephalosporins-Cefepime (86.67%).

S.typhi isolates were least susceptible towards Nalidixic acid with 76.67% of test strains being resistant followed by Tetracycline with 50% resistance rate. Similar findings were reported by Hume S et al 2009,Mandal S et al 2012,Choudhary A et al 2013, Jain S et al 2013,Kumar Y et al 2013, Chane HJ et al 2014, Singhal A et al 2014,Azmat Ali et al 2017,Gurung B et al 2017 [15,18,21-27].

The susceptibility pattern of S.paratyphi A isolates as depicted in Table-2 & Fig.2 showed highest susceptibility towards Cefixime, Ceftazidime, Ceftriaxone, Amikacin & Imipenem(100%) followed by Levofloxacin, Cefotaxime, Cefepime & Aztreonam(80%). Highest resistance rates were seen against Nalidixic acid and Azithromycin.

Amongst fluoroquinolones Levofloxacin exhibited modest sensitivity against S.typhi and paratyphi(86.67% & 80% resp.) but when compared to previous studies resistance against fluoroquinolones esp. Ciprofloxacin is following an increasing trend due to the selective pressure of unrestricted rampant usage as the mainstay of typhoid therapy [3,28-32] None of the isolates came out to be positive for ESBL production as screened by combined disc diffusion test (CDST). Similar findings were reported by Bhagra S et al 2014 and Elumalai S et al 2014 [10,11].

Amongst the 35 isolates, multidrug resistance towards first line drugs was seen in 6 isolates (17 %) all being S.typhi strains. All these MDR strains were also resistant to Nalidixic acid (MDR-NAR).Similar observations were reported by Chau et al 2007,Ochiai RL et al 2008, Bhagra S et al 2014, Afroz H et al 2014, Paudel KR et al 2016, Gurung B et al 2017, Ali A et al 2017 [10,15,16, 18,19,33].

In this study multidrug resistance was not found amongst S.paratyphi strains which is consistent with the reports from Walia M et al 2005, Arjyal A et al 2011, Jain S et al 2013, Sharvani R et al 2016, Gurung B et al 2017 [3,17,18,34,35] Most of the isolates were resistant to Nalidixic acid (NARST) but many of them were susceptible to fluoroquinolones (esp.Levofloxacin). But it has been suggested that such strains(NARST) should be considered Fluoroquinolone resistant; Nalidixic acid being a surrogate marker to predict FQ failure as per CLSI guidelines. As Nalidixic acid resistance amongst Salmonella spp. is rapidly increasing in India,which may lead to dilemma in use of Fluoroquinolones considered to be one of the most effective drug in Enteric fever treatment. But the consistent use of FQ esp.Ciprofloxacin in NA resistant cases has led to a steady rise in MIC alongwith further mutations at same locus which has led to emergence of completely resistant strains.

In this situation Standard disc diffusion test could no longer be relied upon and only MIC determination by any of the available methods like E-test should be preferred for detecting

Ciprofloxacin resistance, particularly in all Nalidixic acid resistant strains [10]. In the MDR-NAR cases third generation Cephalosporins and azithromycin are potential treatment options. Azithromycin can achieve rapid remission, prevents relapse and reduces faecal carriage rates by virtue of its high intracellular concentration and long elimination half-life [3,36,37].

But in this study much higher Azithromycin resistance rate was seen for S.paratyphi A as compared to S.typhi with 60% of strains showing resistance (Arjyal A et al 2011, Jain S et al 2013) [3,34]. A low level of multidrug resistance but high level of Nalidixic acid resistance was reported in this study like many other studies conducted by Gupta V et al 2009, Nagshetty K et al 2010, Bhattacharya SS et al 2011, Shetty AK et al 2012, Rai S et al 2012, Sarika jain et al 2013, Kumar Y et al 2013, Khaparde Ashvini et al 2016, Udaykumar S et al 2016 [3,4,6,28,37-40].

This study has shown re-emergence and an appreciable increase in susceptibility of Salmonella enterica strains towards first line antibiotics attributed to a sharp decline in their usage by clinicians over last decade resulting in the withdrawal of selection pressure [4,6,38]. Loss of self-transmissible plasmids and emergence of de novo susceptible strains might be the other reasons.So,now these drugs could be reconsidered and reincorporated judiciously in Enteric fever therapy [3,17,28]. This study has shown a very high susceptibility of test strains towards third generation cephalosporins in accordance with other studies(Udaykumar S et al 2016, Khaparde A et al 2016)[6,28].So,these extended spectrum cephalosporins are often recommended as drugs of choice for treating enteric fever esp.in fluoroquinolone resistant cases.
With the high levels of resistance being reported against Fluoroquinolones, Nalidixic acid this is an alarming situation as it could seriously limit therapeutic options. Therefore appropriate judicious selection and rotation of antibiotics guided by the knowledge of their susceptibility profiles is of utmost importance[3,6,15].

Some of the important limitations of this study are that the antibiotic susceptibility pattern of Salmonella spp. was derived by Standard disc diffusion test with results not confirmed by MIC determination; and that it is a single centre retrospective study with a limited sample size.

CONCLUSION

This study indicates that first line antibiotics could now be re-incorporated in enteric fever therapy. It is recommended to determine MIC values for fluoroquinolones prior to therapy so as to avoid treatment failures. The third generation cephalosporins should be used judiciously with caution. This study emphasises the need for doing blood culture and antibiotic susceptibility testing for each and every suspected case of enteric fever. Irrational inappropriate indiscriminate usage of antibiotics must be strictly curbed through antimicrobial stewardship to prevent the emergence of multidrug resistant strains. Appropriate Surveillance strategy for regular continuous monitoring of antimicrobial susceptibility pattern with formulation of antibiotic policy at the institutional or regional level is an important prerequisite to rationalize enteric fever treatment protocols to curb the menace of rapidly emerging drug resistance amongst such pathogens.Apart from this, improving living conditions, creating public awareness regarding general hygiene, infection control practices and appropriate usage of typhoid vaccines will help in controlling typhoid in a community.

Conflicts of interest: None

Table-1: Agewise distribution of culture confirmed Enteric Fever cases

S.no	Age group (in yrs.)	Enteric fever cases
1.	Upto 10 yrs.	4
2.	11-20 yrs.	7
3.	21-30 yrs.	11
4.	31-40 yrs.	5
5.	41-50 yrs.	4
6.	51-60 yrs.	2
7.	> 60 yrs.	2
Total		35

Table-2: Antibiotic susceptibility profile of Enteric fever pathogens

S.No.	Antibiotics	S.typhi(30)	S.paratyphi A(5)
1.	Ampicillin	22(73.33)	3(60)
2.	Azithromycin	23(76.67)	2 (40)
3.	Ciprofloxacin	21(70)	3 (60)
4.	Levofloxacin	26(86.67)	4 (80)
5.	Ceftriaxone	29(96.67)	5 (100)
6.	Cefixime	28(93.33)	5(100)
7.	Cefotaxime	27(90)	4 (80)
8.	Ceftazidime	29(96.67)	5 (100)
9.	Cefepime	27(90)	4 (80)
10.	Chloramphenicol	24(80)	4(80)
11.	Cotrimoxazole	21(70)	4 (80)
12.	Nalidixic acid	7(23.33)	1 (20)
13.	Tetracycline	15(50)	3 (60)
14.	Amikacin	26(86.67)	5(100)
15.	Imipenem	30(100)	5(100)
16.	Aztreonam	28(93.33)	4 (80)

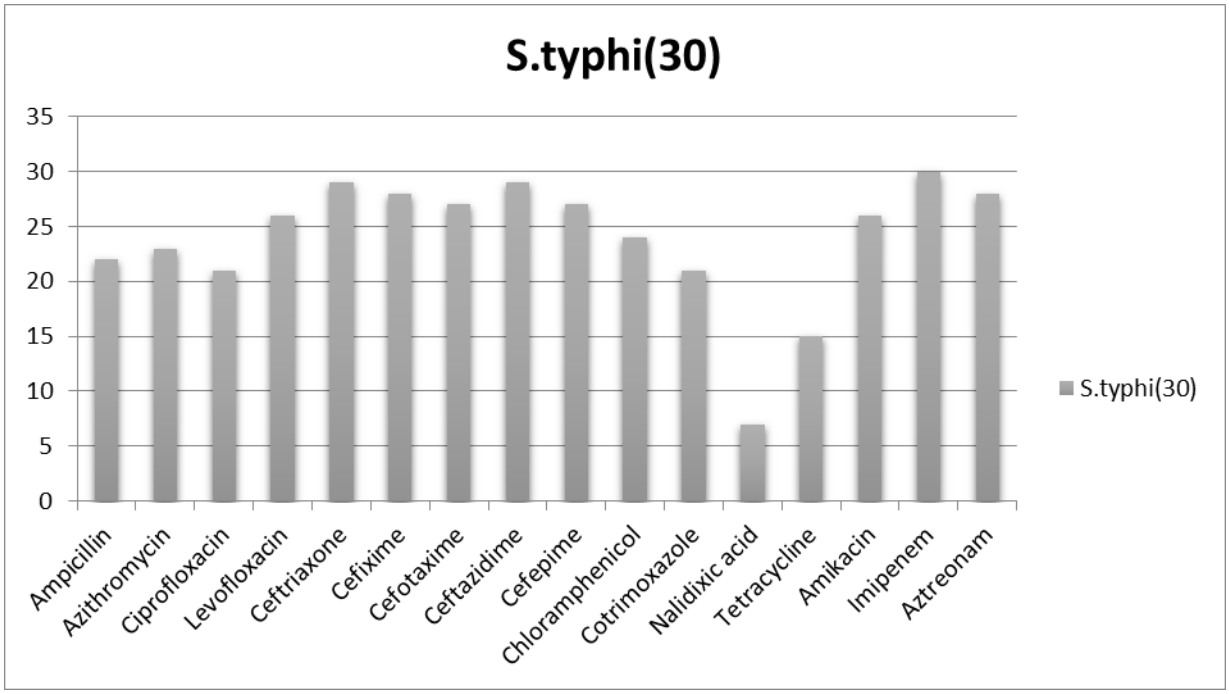

Fig.1: Antibiotic Susceptibility pattern of S.typhi strains

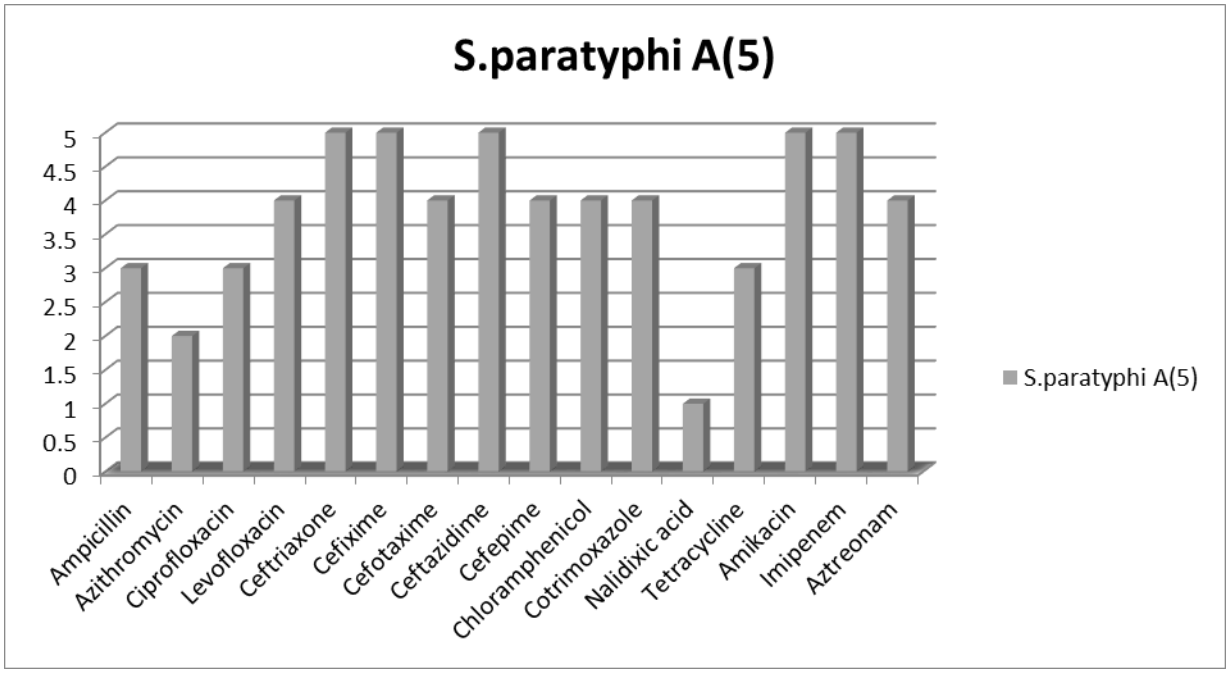

Fig.2: Antibiotic Susceptibility pattern of S.paratyphi A strains

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