

Antimicrobial Sensitivity Pattern of Salmonella Enterica Serovar Typhi and Paratyphi a Isolated from Blood Culture in a Tertiary Care Hospital, Bettiah, Bihar, India

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ABSTRACT

BACKGROUND

Typhoid fever continues to remain a major public health problem, especially in Bihar, due to poor sanitation and personal hygiene. Typhoid fever continues to remain a health problem as the causative organism Salmonella Typhi has developed resistance to many of the antibiotics used. This study was done to evaluate status in antimicrobial susceptibility patterns of Salmonella enterica serovar Typhi (S. Typhi) and S. Paratyphi obtained from blood culture in a tertiary care hospital in Bettiah, Bihar, India.

METHODS

Blood samples were obtained from patients, suspected with enteric fever. Blood isolates of Salmonella species over a one year period between July 2018 and August 2019 were studied. 120 strains of S. Typhi were isolated. Sensitivity to ampicillin, chloramphenicol, gentamicin, ciprofloxacin and ceftriaxone were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method.

RESULTS

Of the total 120 isolates studied, 68 (56.67%) were S. Typhi and 52 (43.33%) were S. Paratyphi A. Of these isolates, 104 (86.33%) were sensitive to ciprofloxacin (MIC<0.25 mg/ml), 110 (91.67%) were nalidixic acid resistant. Of the 110 nalidixic acid resistant isolates, 104 (86.33%) were susceptible to ciprofloxacin (MIC <0.25 mg/ml). All 120 isolates were sensitive to co-trimoxazole and ceftriaxone, 104 isolated (86.66%) were sensitive to amoxicillin and 110 (91.67%) were sensitive to chloramphenicol.

CONCLUSIONS

Nalidixic acid resistance screening is not a consistent surrogate indicator of ciprofloxacin resistance. Ciprofloxacin MIC should to be routinely done. The isolates showed a high degree of susceptibility to ampicillin, co-trimoxazole and chloramphenicol. Thus, antibiotics like amoxicillin, co-trimoxazole, and third generation Cephalosporins (Cefotaxime) may once again be useful for the management of enteric fever in tertiary care hospitals in Bettiah, Bihar, India.

KEYWORDS

Multidrug Resistance, Typhoid, Salmonella Typhi, Salmonella Paratyphi A

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BACKGROUND

Typhoid fever is a common infection caused by the gram negative bacterium, *Salmonella enterica* serovar typhi.¹ It remains a great public health problem in developing countries mainly in the tropics and subtropics having substandard personal hygiene and poor sanitation.² It is endemic in many parts of the world as well as India and Bangladesh.^{3,4} At least 21.7 million new cases emerge each year of which 90% occur in South East Asia, resulting in about 216000 deaths. The annual attack rate ranges from 358 to 1100 per 100,000 populations.^{5,6,7,8} Multidrug-resistant *S. Typhi* (MDRST) is epidemiologically defined as strains resistant to any two antimicrobials in vitro even if the antimicrobials tested are known to be clinically ineffective.⁹ A more useful definition of MDRST is reserved for strains resistant to all three first-line antityphoidal antimicrobial agents, namely ampicillin, chloramphenicol, and trimethoprim-sulphamethoxazole.⁹ Typhoid fever, caused by MDRST, has become a significant cause of morbidity and mortality over recent years. With the emergence of MDRST, fluoroquinolones have gained importance for the treatment of enteric fever in recent years. A considerable variation has been noted in the antimicrobial susceptibility patterns among isolates of *S. Typhi* as suggested in various studies conducted in different geographical locations.¹⁰ Knowledge of the prevalence of *S. Typhi* and their antimicrobial susceptibility patterns is of utmost importance in the institution of appropriate antimicrobial therapy.

In India, antibiotic resistance among *S. Typhi* has been reported since 1960, and the first outbreak of multidrug resistant *S. Typhi* (MDRST) was reported in Calicut.¹¹ Since then MDRST has appeared during the world, especially in South America, the Indian subcontinent, Africa and Southeast Asia.¹² The incidence of multidrug resistant (MDR) *S. Typhi* has been reported to be as high as 60% but then declined in Pune (1999), Nagpur (2001), Delhi (2004), Calcutta (2000) and Northern Bihar (2017).¹³⁻¹⁷ However, reappearance of resistant strains in Ludhiana in 2002 is of concern.¹⁸ A study of imported strains that was based in the United States¹⁹ noted an increase in the number of MDR and nalidixic acid resistant *S. Typhi* globally (NARST), while all isolates remained sensitive to ciprofloxacin and ceftriaxone. In Bangladesh there has been a reported decrease in MDR isolates with no consequent increase in sensitive strains.²⁰ For ciprofloxacin there has been an increase in MIC strains imported into the United Kingdom,²¹ Bangladesh,²² and India.^{23,24,25}

Multidrug resistant (MDR) strains (resistant to chloramphenicol, ampicillin and co-trimoxazole) of *Salmonella enterica* have emerged worldwide in the last two decades.²⁶ Isolates of *S. enterica* with reduced susceptibility to fluoroquinolones have now appeared in the Indian subcontinent and other regions.^{27,28} However, in India the degree of resistance to frequently used antibiotics such as chloramphenicol, ampicillin and co-trimoxazole in the era of quinolone resistance is not clear.^{26,29,30} The present study was undertaken to document the change in the antibiotic

susceptibility of *S. enterica* serovar Typhi and *S. Paratyphi* isolates obtained from blood culture during 2018-2019 in a tertiary care hospital in Bettiah, Bihar, India.

METHODS**Study Design**

One hundred twenty (120) clinically suspected cases of typhoid fever of different age and sex attended at outpatient department of Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital from July 2018 to August 2019 were included in the study. The study protocol was approved by the hospital ethics committee.

Patients

Patients integrated in this study had fever for less than 3 days, had no anti-typhoid treatment, or any condition suggestive of typhoid (soft enlarged spleen, headache, and abdominal discomfort with diarrhea). Members of both sexes instead of all ages will include. Bacterial Culture included 120 *S. Typhi* isolates from blood cultures of patients suffering from suspected typhoid fever who attended the outpatient clinics or were admitted in the hospitals of the during July 2018 to August 2019 in a tertiary care hospital in (West Champaran) Bihar India. A total of 1500 blood samples Bile salt broth (broth culture)³¹ and streptokinase broth (clot culture)³² blood samples were used for enrichment as well as Selenite F-broth for environmental samples. The enriched samples subsequent to visible turbidity were streaked on MacConkey, XLD and Wilson Blair media. All isolate will identified by biochemical test.

Antimicrobial Susceptibility Testing

All the isolates of *Salmonella Typhi* were tested for their antimicrobial susceptibility pattern on Mueller Hinton agar media by disc diffusion method against Ampicillin (10 µgm), Ciprofloxacin (5 µgm), Cotrimoxazole (25 µgm), Ceftriaxone (30 µgm), Azithromycin (15 µgm), Chloramphenicol (30 µgm), Ceftazidime (30 µgm) and Nalidixic acid (30 µgm). The disk strength and zone-size analysis was in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby-Bauer disc diffusion method.³³ Interpretation of zone size Inhibition zones produced by each drug was measured into two susceptibility categories to be exact sensitive (S) and Resistant (R) Strains produce zone size between sensitive and resistant are regarded as intermediate sensitive which is not measured in this study. MICs of isolates resistant to chloramphenicol, ampicillin and nalidixic acid were determined by agar dilution test via purified antibiotic powders (HiMedia Laboratories, Mumbai). MIC of ciprofloxacin were unwavering for only 20 randomly selected nalidixic acid resistant isolates. Isolates resistant to ampicillin, chloramphenicol and cotrimoxazole were termed MDR.

RESULTS

Antibiotics Screened	No. of Isolates	Sensitive Isolates
Nalidixic acid (30 µg)	120	10 (08.33%)
Amoxicillin (10 µg)	120	104 (86.66%)
Chloramphenicol (30 µg)	120	110 (91.67%)
Ciprofloxacin (5 µg)	120	104 (86.33%)
Co-trimoxazole (1.25/23.75 µg)	120	120 (100%)
Ceftriaxone (30 µg)	120	120 (100%)
MDR*	120	110 (91.67)

Table 1. Sensitivity Rates to Various Antibiotics in Tertiary Care Hospital in Northern Bihar, India

*Defined as resistance to amoxicillin, chloramphenicol and co-trimoxazole

Of the total 120 isolates studied, 68 (56.67%) were *S. Typhi* and 52 (43.33%) were *S. Paratyphi A*. Of these isolates, 104 (86.33%) were sensitive to ciprofloxacin (MIC < 0.25 mg/ml), 110 (91.67%) were nalidixic acid resistant. Of the 110 nalidixic acid resistant isolates, 104 (86.33%) were susceptible to ciprofloxacin (MIC < 0.25 mg/ml). All 120 isolates were sensitive to co-trimoxazole and ceftriaxone, 104 isolated (86.66%) were sensitive to amoxicillin and 110 (91.67%) were sensitive to chloramphenicol (Table 1).

DISCUSSION

Typhoid fever remains a major public health problem in most resource-poor countries such as India. This is the first report of *S. Typhi* antimicrobial susceptibility from in Bettiah, Bihar, India. Enteric fever is a major infectious disease happening at high fluctuating incidences in a tertiary care center in Bettiah, Bihar, India.

Enteric fever is a major public health problem in India. Various studies document *S. Typhi* as the commonest serovar isolated over the years,³⁴ and our study also showed 56.67 per cent isolates of serovar *Typhi* while 43.33 per cent were serovar *Paratyphi A*.³⁵ In the last decade, there have been some reports of ciprofloxacin resistance in *Salmonella*.³⁶ It is believed that nalidixic acid resistance is a surrogate marker for ciprofloxacin resistance, as clinical failures have been documented in cases where ciprofloxacin has been used (based on susceptibility) for nalidixic acid resistant strains.³⁷ In our study, 13.67 per cent of isolates displayed reduced susceptibility to ciprofloxacin (MIC > 0.5 µg/ml). However, as many as 91.67 per cent of nalidixic acid resistant isolates were ciprofloxacin sensitive by MIC testing. Kirby-Bauer disc diffusion assay using currently recommended breakpoints to ciprofloxacin may not be a reliable method; E-test should be the preferred method of choice to determine ciprofloxacin MIC.

Since its introduction in 1948, chloramphenicol has been the treatment of choice for typhoid fever and the treatment with chloramphenicol reduces death due to typhoid fever from about 20 to 1 per cent and the duration of fever from 14-28 days to 3-5 days.³⁸ However, chloramphenicol therapy has been associated with the emergence of resistance to chloramphenicol, a high relapse rate, bone marrow toxicity and high mortality rates in a recent study reported from the

developing world.³⁹ Ampicillin and co-trimoxazole could be effective alternative drugs. In our study *Salmonella sp.* remained sensitive to chloramphenicol, amoxicillin, Ceftriaxone and co-trimoxazole (91.67, 86.66, 100 & 100%, respectively) over the two-year study period as reported earlier.^{40,41} These drugs may be preferred for treatment of enteric fever in Bettiah, Bihar, India.

The data presented in our study highlights that MDR, although small, exists in this region. The presence of MDR (i.e. resistance to ampicillin, chloramphenicol and co-trimoxazole) was 9.33% for the year 2018-2019. This finding is in accordance with recent reports from some regions where the incidence of MDR *S. Typhi* isolates appeared to have decreased.⁴⁰⁻⁴⁴ The low frequency of MDR *S. Typhi* isolated is remarkable, since these drugs could once again be used for the treatment of enteric fever.^{44,45}

Azithromycin has performed well in previously clinical studies for typhoid; however, there have been sporadic reports of azithromycin resistance.⁴⁶ All isolates in our study were sensitive to ceftriaxone in contrast to some studies that reported resistance to ceftriaxone.^{47,48}

Limitations

Clinical outcomes were not analysed. Quinolones may remain effective despite in vitro resistance and ceftriaxone may be associated with prolonged time to fever resolution despite in vitro sensitivity.⁴⁹

CONCLUSIONS

Nalidixic acid resistance is not a reliable surrogate indicator of ciprofloxacin resistance. Ciprofloxacin MIC should be regularly done. Azithromycin resistance appears to be emerging. However, isolates showed a high degree of susceptibility to ampicillin, co-trimoxazole and chloramphenicol. Thus, antibiotics like amoxicillin, co-trimoxazole, and third generation Cephalosporins (Cefotaxime) may once again be helpful for the management of enteric fever in tertiary care hospitals in Bettiah, Bihar, India. However, chloramphenicol, co-trimoxazole and amoxicillin have re-emerged as precious oral options and ceftriaxone remains a viable parenteral option for management of typhoid in tertiary care hospitals in Bettiah, Bihar, India.

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