ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA ENTERICA SEROVARS IN A TERTIARY CARE HOSPITAL IN KOSHI REGION (NORTHERN BIHAR), INDIA

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ABSTRACT

BACKGROUND

Typhoid fever continues to remain a major public health problem, especially in regions such as Koshi Region due to poor sanitation and personal hygiene. Koshi Region is often prone to enteric fever outbreaks and is an endemic region of typhoid fever. *Salmonella enterica* serovars Typhi and Paratyphi are predominantly known to cause enteric fever. Multidrug resistance in *S. Typhi* and *S. Paratyphi* has emerged as a cause of concern. 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 our tertiary care hospital in Northern Bihar, India.

MATERIALS AND METHODS

A total of 69 isolates of *Salmonella Typhi* and *Salmonella Paratyphi A* collected from different clinical isolates were tested for antimicrobial susceptibility according to the CLSI guidelines. Blood isolates of *Salmonella* species over a two year period between December 2013 and December 2015 were studied. A total of 69 isolates of *Salmonella* species were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method.

RESULTS

Bacterial cultures included 69 *S. Typhi* isolates from blood cultures of patients from suspected typhoid fever who attended the outpatient clinics or were admitted in the hospitals during December 2013-December 2015. 39 (56.52%) were *S. Typhi* and 30 (43.47%) were *S. Paratyphi A*. Of these isolates, 60 (86.95%) were sensitive to ciprofloxacin (MIC<0.25 mg/mL), 63 (91.30%) were nalidixic acid resistant. Of the 63 nalidixic acid resistant isolates, 60 (95.23%) were susceptible to ciprofloxacin (MIC <0.25 mg/mL). All 69 isolates were sensitive to co-trimoxazole, gentamycin and ceftriaxone, 60 isolated (86.95%) were sensitive to amoxicillin and 63 (91.30%) were sensitive to chloramphenicol.

CONCLUSION

Nalidixic acid resistance screening is not a reliable surrogate indicator of ciprofloxacin resistance. Ciprofloxacin MIC should 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 our tertiary care hospital in Northern Bihar, India.

KEYWORDS

Typhoid, Multidrug Resistance, *Salmonella Typhi* and *Salmonella Paratyphi A*.

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BACKGROUND

Enteric fever is a global public health problem. Almost 80% of the cases and deaths are in Asia and the rest occur mostly in Africa and Latin America.^[1] It is estimated that there are 22

million new cases of enteric fever annually, with 200,000 deaths.^[2] Regions with the highest incidence of enteric fever (>100 cases per 100,000 persons per year) are South Central Asia and Southeast Asia. Regions of moderate incidence (10-100 cases per 100,000 persons per year) include the rest of Asia, Africa, Latin America and the Caribbean and Oceania, except for Australia and New Zealand. In Delhi, India, the incidence of enteric fever is 9.8 cases per 1,000 person-years.^[3] *Salmonella enterica* serovar Typhi and Paratyphi A are the predominant types of aetiological agents responsible for enteric fever in India, particularly during summer.^[4] The emergence of antimicrobial resistance, especially the multidrug resistance to ampicillin, chloramphenicol and co-

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trimoxazole, has further complicated the treatment and management of enteric fever.^[4,5]

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.^[6] Since then MDRST has appeared throughout the world, especially in South America, the Indian subcontinent, Africa and Southeast Asia.^[5] 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) and Calcutta (2000).^[7-10] However, resurgence of resistant strains in Ludhiana in 2002 is of concern.^[11] A study of imported strains that was based in the United States^[12] noted an increase in the number of MDR and nalidixic acid resistant *S. Typhi* globally (NARST), although all isolates remained sensitive to ciprofloxacin and ceftriaxone. In Bangladesh there has been a reported decrease in MDR isolates with no corresponding increase in sensitive strains.^[13] For ciprofloxacin there has been an increase in MIC strains imported into the United Kingdom,^[14] Bangladesh,^[15] and India.^[16,17,18]

Multidrug resistant (MDR) strains (resistant to chloramphenicol, ampicillin and co-trimoxazole) of *Salmonella enterica* have emerged worldwide in the last two decades. Isolates of *S. enterica* with reduced susceptibility to fluoroquinolones have now appeared in the Indian subcontinent and other regions.^[19,20] However, in India the degree of resistance to commonly used antibiotics such as chloramphenicol, ampicillin and co-trimoxazole in the era of quinolone resistance is not clear.^[21,22] 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 2013-2015 in a tertiary care hospital in Northern Bihar, India.

MATERIALS AND METHODS

Study Design

All *S. enterica* isolates obtained from blood cultures of clinically suspected cases of enteric fever seen in Lord Buddha Hospital (Central Hospital) and Associated Hospital, Lord Buddha Koshi Medical College, a tertiary care center in Koshi Region (Northern Bihar), from December 2013 to December 2015 were included in the study. The study protocol was approved by the hospital ethics committee.

Criteria for selection of patients-

Patients included 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 diarrhoea). Members of both sexes representing all ages will included.

Bacterial Cultures included 69 *S. Typhi* isolates from blood cultures of patients suffering from suspected typhoid fever who attended the outpatient clinics or were admitted in the hospitals during December 2013-December 2015 in a tertiary care hospital in Koshi Region (Northern Bihar), India. A total of 1,000 blood samples of Bile salt broth (broth culture),^[23] and streptokinase broth (clot culture)^[24] blood samples were used for enrichment as well as Selenite F-broth for environmental samples. The enriched samples after visible turbidity were streaked on MacConkey, XLD and

Wilson Blair media. All isolates were identified by biochemical test.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility patterns were determined using commercial antimicrobial disks (HiMedia, Mumbai): Chloramphenicol (30 µg), nalidixic acid (30 µg), ampicillin (10 µg), azithromycin (15 µg), co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), and ceftriaxone (30 µg). Antimicrobial susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby-Bauer disc diffusion method.^[25]

MICs of isolates resistant to chloramphenicol, ampicillin and nalidixic acid were determined by agar dilution test using purified antibiotic powders (HiMedia Laboratories, Mumbai). MIC of ciprofloxacin were determined for only 20 randomly selected nalidixic acid resistant isolates. Isolates resistant to ampicillin, chloramphenicol and cotrimoxazole were termed MDR.

RESULTS

Of the total 69 isolates studied, 39 (56.52%) were *S. Typhi* and 30 (43.47%) were *S. Paratyphi A*. Of these isolates, 60 (86.95%) were sensitive to ciprofloxacin (MIC<0.25 mg/mL), 63 (91.30%) were nalidixic acid resistant. Of the 63 nalidixic acid resistant isolates, 60 (95.23%) were susceptible to ciprofloxacin (MIC <0.25 mg/mL). All 69 isolates were sensitive to co-trimoxazole, gentamycin and ceftriaxone, 60 isolated (86.95%) were sensitive to amoxicillin and 63 (91.30%) were sensitive to chloramphenicol (see Table below).

Enteric fever is a major public health problem in India. Various studies document *S. Typhi* as the commonest serovar isolated over the years,^[26] and our study also showed 56.52 percent isolates of serovar *Typhi* while 43.47 percent were serovar *Paratyphi A*.^[27]

Antibiotics Screened	No. of Isolates	Sensitive Isolates
Nalidixic Acid (30 µg)	69	6 (08.69%)
Amoxicillin (10 µg)	69	60 (86.95%)
Chloramphenicol (30 µg)	69	63 (91.30%)
Co-trimoxazole (1.25/23.75 µg)	69	69 (100%)
Ciprofloxacin (5 µg),	69	60 (86.95%)
Ceftriaxone (30 µg)	69	69 (100%)
MDR*	69	63 (91.30)
<i>Sensitivity Rates to Various Antibiotics in A Tertiary Care Hospital in Northern Bihar, India</i>		

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

DISCUSSION

Due to a combination of factors including poor sanitation and health care infrastructure, 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 Northern Bihar, India. Enteric fever is a major infectious disease occurring at high fluctuating incidences in tertiary care center in Koshi Region (Northern Bihar), Lord Buddha Koshi Medical College (Northern Bihar).

In the last decade, there have been some reports of ciprofloxacin resistance in *Salmonella*.^[28] 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.^[29] In our study, 13.04 percent of isolates displayed reduced susceptibility to ciprofloxacin (MIC >0.5 µg/mL). However, as many as 91.30 percent 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 remains the standard against which newer antimicrobials are compared. Treatment with chloramphenicol reduces mortality due to typhoid fever from about 20 to 1 percent and the duration of fever from 14-28 days to 3-5 days.^[30] 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.^[31] Ampicillin and co-trimoxazole could be effective alternative drugs. In our study, *Salmonella* spp. remained sensitive to chloramphenicol, amoxicillin, ceftriaxone and co-trimoxazole (91.30, 86.96, 100 & 100%, respectively) over the two-year study period as reported earlier.^[32,33] These drugs may be preferred for treatment of enteric fever in Northern 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 8.69% for 2013-2015. This finding is in accordance with recent reports from some regions where the incidence of MDR *S. Typhi* isolates appeared to have decreased.^[32-34] The low frequency of MDR *S. Typhi* isolated is remarkable, since these drugs could once again be used for the treatment of enteric fever.^[10,34,35]

Azithromycin has done well in previous clinical studies for typhoid; however, there have been sporadic reports of azithromycin resistance.^[36] All isolates in our study were sensitive to ceftriaxone in contrast to some studies that reported resistance to ceftriaxone.^[37,38]

A limitation of our study was that 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.^[39]

CONCLUSION

The main approaches for combatting typhoid fever today is the control of the dissemination of causative agent. To this end, investigation of *S. typhi* is of major importance.

Nalidixic acid resistance screening is not a reliable surrogate indicator of ciprofloxacin resistance. Ciprofloxacin MIC should be routinely 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 useful for the management of enteric fever in our tertiary care hospital in Northern Bihar, India. However, chloramphenicol, co-trimoxazole and amoxicillin have re-emerged as valuable oral options and ceftriaxone remains a viable parenteral option for treatment of typhoid in our tertiary care hospital in Northern Bihar, India.

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