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Original Research Article

Emergence of Quinolone Resistance and Re-Emergence of Susceptibility to First Line Antibiotics in Salmonella Spp. Isolated in a Tertiary Care Hospital

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Abstract: Typhoid fever (enteric fever) is a global health problem causing high morbidity and mortality, especially in endemic areas such as India. It afflicts local inhabitants as well as travellers to endemic areas. It has a mortality rate of 30% if not treated properly though appropriate treatment reduces the mortality rate to as low as 0.5%. Multi drug resistant Salmonella are still common in many areas, although in some regions highly sensitive strains have re-emerged. Isolation of Salmonella spp. from blood samples was done over a period of 1 year. The isolates were confirmed as Salmonella by using a battery of biochemical reactions. Specific antisera were used for serologic characterization of Salmonella strains. Antimicrobial susceptibility testing was performed as per CLSI guidelines. Minimum inhibitory concentrations were also evaluated for ciprofloxacin by E test. Total number of Salmonella isolates from clinical samples was 78. Six isolates (7.6%) were found to be multidrug resistant. Eight isolates were sensitive to all the antimicrobials tested. Among the antibiotics used for susceptibility testing of the isolates, all the isolates were found to be susceptible to cefixime, ceftriaxone, cefotaxime and azithromycin. 92.3 % of the Salmonella spp. were susceptible to chloramphenicol, 91 % to co-trimoxazole and 88.4 % of the isolates were susceptible to ampicillin. Only 12.9% of the isolates were found to be susceptible to ciprofloxacin and 10.3% to nalidixic acid. The present study revealed the increased rate of nalidixic acid resistant Salmonella spp. associated with reduced susceptibility to fluoroquinolones in contrast to increased susceptibility of the strains to conventional first-line drugs ampicillin, chloramphenicol, and cotrimoxazole. So, the conventional first-line drugs along with the third generation cephalosporins and azithromycin can be used as empiric therapy for treatment of enteric fever in our setting.
Keywords: Salmonella typhi, Typhoid, Multidrug resistance, Re-emergence susceptibility, Quinolone resistance, Decreased ciprofloxacin susceptibility, Nalidixic acid resistance.

INTRODUCTION

Enteric fever is a global public health problem and is endemic in many developing countries, including India. It afflicts local inhabitants as well as travellers to endemic areas. It has a mortality rate of 30% if not treated properly though appropriate treatment reduces the mortality rate to as low as 0.5% [1]. Almost 80% of the cases and deaths are in Asia [2]. It is estimated that there are 22 million new cases of enteric fever annually, with 200,000 deaths [3]. Simultaneous resistance to three or more different groups of antimicrobial drugs is defined as Multi Drug Resistant Salmonella. In the past, S. Typhi infections were routinely treated with chloramphenicol, ampicillin, or trimethoprim-sulfamethoxazole [4], but increasing multidrug resistance in Salmonella enterica serotype Typhi has been reported from various parts of the world [5]. Increasing incidence of infection with Salmonellae resistant to nalidixic acid, which usually display decreased susceptibility to fluoroquinolones, has raised considerable global concern [6].

In India, Salmonella typhi drug resistance has been reported since 1960 following the first outbreak of MDR Salmonella typhi in Calicut [7]. MDR Salmonella typhi is still common in many areas, although in some regions highly sensitive strains have re-emerged [8]. In response, physicians in endemic areas shifted to fluoroquinolones, Azithromycin or third-generation cephalosporins to ensure better treatment outcomes [4]. But, recently the re-emergence of the conventional first-line drugs susceptible strains of Salmonella spp. has been reported [9, 10]. Despite the recently reported susceptibility of S. Typhi isolates to ciprofloxacin by

the disc-diffusion method, patients in many endemic areas have begun to present with clinical treatment failures leading to serious consequences. This treatment failure was observed for the first time in India in 1991 and subsequently recognized in other nearby countries [4].

The emergence of increased resistance to ciprofloxacin in Salmonella typhi would severely limit the choice of antimicrobial therapy for enteric fever. Nalidixic-acid-resistant strains exhibiting reduced susceptibility towards ciprofloxacin (MICs 0.125–1 mg/L) have become endemic in several geographical areas of the Indian subcontinent and have also been reported in US, UK and in other developed countries [11, 12]. In the present situation, when the treatment options for enteric fever are decreasing, the re-emergence of strains of the Salmonella susceptible to ampicillin, chloramphenicol, and cotrimoxazole should be evaluated to find out their therapeutic importance [9]. For timely proper management of the enteric fever the knowledge of the prevalence of the different serovars of Salmonella and their antimicrobial susceptibility patterns is of utmost importance [9]. In this study we studied the antimicrobial susceptibility patterns of Salmonella spp. isolated from the blood samples of the patients suspected of suffering from enteric fever toward different commonly used antibiotics. Reduced susceptibility of nalidixic acid-resistant strains to fluoroquinolones was also evaluated.

MATERIALS AND METHODS

Study Setting & Duration

The study was conducted in the Microbiology department of a tertiary care hospital in Nizamabad for a period of 12 months from May 2015 to April 2016. Approval of institutional ethical committee was taken for this study.

Study Design

Prospective, Cross sectional study

Study Population

A total of 489 patients clinically suspected of suffering from enteric fever, were included in the study. Patients who had already received antibiotics were not included in the study.

Blood samples were collected aseptically by vein puncture and inoculated immediately into brain heart infusion broth. The amount of blood collected from adults was 5 ml and that from children was 2 ml. The ratio of amount of blood to that of brain heart infusion broth was maintained to be 1:10. After incubation, at 37 °C for 24, 48 and 72 hrs subcultures were done on MacConkey agar and blood agar and were observed for bacterial growth after 24 hrs of aerobic incubation at 37 °C. Isolates were identified by biotyping (colony morphology, staining reaction and biochemical characteristics) and serotyping using specific antisera (Denka Seiken Co. Ltd, Tokyo, Japan). Samples were considered negative for Salmonella, if no growth was observed on subculture after 7 days of aerobic incubation at 37°C.

Antimicrobial susceptibility testing for Salmonella serovars was performed by Kirby Bauer disc diffusion technique following CLSI guidelines [13]. The antibiotic discs used were, ampicillin (30 µg), nalidixic acid (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), cotrimoxazole (1.25 µg), cefotaxime (30 µg), azithromycin (15 µg), and cefixime (30 µg). (Himedia Laboratories, Mumbai) The E-test (bioMerieux) was employed to determine the MIC of ciprofloxacin. MDR was defined as the simultaneous resistance of bacteria to chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole. Escherichia coli ATCC 25922 was used for quality control. Statistical analysis was performed by using SPSS 19.0.

RESULTS

Out of 489 blood samples, 78 (15.9%) samples were culture positive for Salmonella spp. Out of total 78 Salmonella spp. isolated, 61 (78.2%) were Salmonella Typhi and and 17 (21.8%) were Salmonella paratyphi A. The age of patients under the investigation ranged from 3 years to 70 years (mean 24.1±14.16). The age group 11-40 years accounted for maximum enteric fever cases. There were 41 males and 37 female patients.

Six isolates (7.6%) were found to be multidrug resistant (showing resistance to ampicillin, chloramphenicol, cotrimoxazole and nalidixic acid). Eight isolates were sensitive to all the antimicrobials tested. The highest MIC value of ciprofloxacin among the isolates was 8µg/ml. All the isolates having ciprofloxacin MIC ≥1µg/ml were resistant to nalidixic acid by disc diffusion whereas isolates having MIC ≤1µg/ml showed variable results. The difference in mean ciprofloxacin MIC in nalidixic acid resistant Typhi and Paratyphi A was statistically significant ($p<0.001$).

Among the antibiotics used for susceptibility testing of the isolates, all the isolates were found to be susceptible to cefixime, ceftriaxone, cefotaxime and azithromycin. 92.3 % of the Salmonella spp. were susceptible to chloramphenicol, 91 % to co-trimoxazole and 88.4 % of the isolates were susceptible to ampicillin. Only 12.9% of the isolates were found to be susceptible to ciprofloxacin and 10.3% to nalidixic acid.

Table 1: Antimicrobial susceptibility pattern of Salmonella isolates

Sl. no.	Antibiotic	Sensitive	Resistant
1	Ampicillin	69 (88.4%)	9 (11.6%)
2	Nalidixic acid	8 (10.3%)	70 (89.7%)
3	Ciprofloxacin	10 (12.9%)	68 (87.1%)
4	Chloramphenicol	72 (92.3%)	6 (7.7%)
5	Ceftriaxone	78 (100%)	0 (0%)
6	Cotrimoxazole	71 (91%)	7 (9%)
7	Cefotaxime	78 (100%)	0 (0%)
8	Azithromycin	78 (100%)	0 (0%)
9	Cefixime	78 (100%)	0 (0%)

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 [14]. The resistance pattern of Salmonella spp. had been varying with time and geographical locations [15].

We isolated a total of 78 Salmonella isolates from 489 samples(15.9%). Similar rate of culture was also found in other studies [16, 17]. Typhoid and paratyphoid, collectively known as 'enteric fever', remain as one of the commonest causes of the fever in most parts of developing world. As in our study higher prevalence of S. typhi (78.2%) in comparison to S. paratyphi A (21.8%) ($p < 0.05$) was also reported in another study [16].

In present study, the rate of nalidixic acid resistance, which is a phenotypic marker for reduced susceptibility to fluoroquinolones [18]; was observed high (89.7%). Similar rates of nalidixic acid resistance were also reported in other studies conducted in India [19].

The increasing emergence of nalidixic resistant S. typhi may probably be attributed to the use of quinolone antibiotics in animal feeds in the country. Several workers have reported from elsewhere that the use of quinolones in food animals have led to the rapid emergence of resistant Salmonella infections to humans [20]. Studies have also revealed that antimicrobial agents used in agriculture and closely related agents used in human medicine have been exerting selective pressure on their target bacteria [21]

NA-resistant Salmonella isolates were found to have almost tenfold higher MIC to ciprofloxacin. This increase in high-level ciprofloxacin resistance probably reflects the overuse or irrational use of ciprofloxacin in the treatment of typhoid as well as in other unrelated infections. Incomplete treatment may be another factor contributing to development of resistance. There are several reports of therapeutic failure of fluoroquinolones in patients with enteric fever [22,23]. Although reported as susceptible by disc diffusion assay using recommended breakpoints to fluoroquinolones, these isolates have smaller zones of inhibition to fluoroquinolones by Kirby- Bauer disc diffusion method and MIC is almost tenfold higher than fully susceptible strains [23]. Renuka *et al.* reported isolation of Salmonella enterica serotype Typhi strains showing high level resistance to ciprofloxacin [24].

The selective pressure on the bacterial population from the uncontrolled use of quinolones has led to the emergence of a resistance to this group of antimicrobials. One mutation in the gyr A gene mediates full resistance to narrow-spectrum quinolones, such as nalidixic acid, and decreased susceptibility to fluoroquinolones. A second mutation in either the gyr A or the gyr B genes mediates full resistance to fluoroquinolones [25].

In contrast to nalidixic acid resistance, in the present study, re-emergence of susceptibility (92.3% susceptibility to chloramphenicol, 91% to co-trimoxazole and 88.4% susceptibility to ampicillin) to conventional first line drugs used for treatment of enteric fever was observed. Similar types of findings were also observed by Garg *et al*. [19] and Acharya *et al* [26] The increased use of the fluoroquinolones and the discontinuation in the use of the conventional first line antibiotics (ampicillin, chloramphenicol, and co-trimoxazole) for treatment of the enteric fever for long periods of time may be the reason behind the reduced susceptibility of Salmonella strains to fluoroquinolones and re-emergence of first line antibiotics susceptible S. Typhi and S. Paratyphi A isolates. Further, the loss of the plasmids responsible for resistance to first line drugs may be the reason for the re-emergence of the susceptible strains [9].

Reports from African countries such as Senegal and Egypt also indicate significant shift in the antibiotics susceptibility to the first line antibiotics. Similar observations of increased in susceptibility of S. typhi to the first line antibiotics particularly chloramphenicol had since been made in India and Bangladesh and were attributed to the restricted use of chloramphenicol for few years [21, 23]. Discontinuation of Chloramphenicol therapy relieved the selection pressure paving the way for re-emergence of S. enterica serovar Typhi isolates sensitive to Chloramphenicol.

The, high level of Chloramphenicol MICs, in resistant isolates, was due to acquisition of R-plasmid under selective pressure. Loss of R-plasmid would lead emergence of Chloramphenicol-sensitive strains showing very low MICs. High degree of Chloramphenicol susceptibility to S. enterica serovar Typhi isolates has also been reported very recently from many other parts of India. Over period of years several Indian studies have documented a 90-95% re-emergence of Chloramphenicol susceptibility [25, 26].

In contrast to our study, 100 % susceptibility of Salmonella typhi and 96.7 % susceptibility of Salmonella paratyphi A to ciprofloxacin were observed in a study by Chand *et al* [9]. The increased haphazard use of ciprofloxacin as empirical therapy for treatment of enteric fever in recent years may have contributed to this discrepancy.

Among cephalosporins and macrolides all the isolates were susceptible to cefotaxime, cefixime, ceftriaxone and azithromycin. But among the cephalosporins, cefixime may attract more attention as drug of choice as it can be used orally. Only six isolates (7.6%) were found to be multidrug resistant in present study and the finding correlated with the previous study that reported the decreasing trend in multidrug resistant isolates [9]. Due to irrational use of antibiotics, the rate of drug resistance among bacteria is increasing and the situation is worse in developing countries [11, 12]. So antibiotics should be used only on the basis of culture and sensitivity report. Our findings will be helpful for the clinician to choose the appropriate empirical therapy for the treatment of enteric fever. Further it will also be helpful to make policy for empirical therapy for treatment of enteric fever.

CONCLUSION

The present study revealed the increased rate of nalidixic acid resistant Salmonella spp. associated with reduced susceptibility to fluoroquinolones in contrast to increased susceptibility of the strains to conventional first-line drugs ampicillin, chloramphenicol, and cotrimoxazole. Ciprofloxacin can no longer be considered the drug of choice in treating Salmonella infections due to its high-level resistance So, the conventional first-line drugs along with the third generation cephalosporins and azithromycin can be used as empiric therapy for treatment of enteric fever in our setting. Considering the rapid emergence of high level Ciprofloxacin resistance, is it time to re-think about Ciprofloxacin breakpoints or an alternate therapy for enteric fever. Examining the antibiotic susceptibility patterns of pathogens is important toward tailoring treatment to the ever changing resistance patterns and distribution of these organisms. Multicenter studies covering wide geographical area and large population are required to generate more significant data regarding the susceptibility of the Salmonella spp. toward ampicillin, co-trimoxazole and chloramphenicol and to determine the possibility of using these drugs for empirical therapy for treatment of enteric fever.

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