

Detection of quinolone resistance among enteric fever Salmonellae

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Abstract

Introduction: Enteric fever is an important public health problem in developing countries like India. Antibiotics are still the main stay in the treatment of enteric fever, but enteric fever Salmonellae have developed drug resistance and this has further hampered the process of reducing the mortality and morbidity associated with this disease. **Materials and Methods:** Phenotypically and serologically confirmed enteric fever Salmonellae were subjected for antimicrobial susceptibility testing by Kirby Bauer disc diffusion testing and MIC of the Salmonellae were detected using Agar dilution, E test and Hi comb. Results: 8.46% of samples received for blood culture yielded the growth of enteric fever Salmonellae, 29 S. Paratyphi A and 25 S. Typhi were isolated. Male to female ratio was 3:1, more commonly isolated in the first 3 decades. Seasonal variation was observed with majority of them isolated between July to September. Mean time for positive blood culture was 3-5 days. 83.3% of the isolates were resistant to Nalidixic acid. MIC values of all isolates were in susceptible range but with increasing values. Conclusion: Enteric fever continues to be a major public health problem around Mysore. Paratyphoid fevers which were rarely detected previously have now emerged on par to Typhoid fever. During monsoon months the number of Enteric fever cases increases. Young adults are more commonly susceptible. Ciprofloxacin resistance is not very common in this region.

Keywords: Enteric fever, E test, NARST, Salmonella Typhi.

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INTRODUCTION

Enteric fever continues to be a major public health problem, especially in the developing countries of the tropics¹. It is an important cause of morbidity and mortality with an estimated 33 million cases worldwide² and an estimated 600,000 deaths annually¹. Persistence of enteric fever directly indicates failure of public health measures. Enteric fever is endemic in all parts of India. Salmonella enterica subspecies enterica serovar Typhi (S.

Typhi) and Salmonella enterica subspecies enterica serovar Paratyphi A (S. Paratyphi A) are the predominant types of Salmonella responsible for enteric fever in India¹. Failure to implement or delay in starting effective treatment is associated with high mortality (20%). Timely and effective treatment reduces the mortality rate to as low as 1 %³. Chloramphenicol was first introduced in 1948 as effective antibiotic in the treatment of enteric fever and was the undisputed drug of choice until the mid-1970⁴. Chloramphenicol resistant Salmonella species were detected all over India. Hence, Ampicillin and Trimethoprim/Sulfamethoxazole (TMP-SMZ) were used for treatment. In 1987 Salmonella not responding to Ampicillin, TMP-SMZ and Chloramphenicol was observed first in South East Asia and these strains were labelled as Multi drug resistant strains (MDR)⁵ and it was discovered that this property was plasmid mediated^{6,7,8}. Due to the emergence of MDR strains in 1990, Quinolones were suggested as an alternative drug due to its quicker defervescence (3-5 days) and early abatement of symptoms⁵. A screening

method to detect invitro decreased susceptibility to Quinolones was introduced by using Nalidixic acid (30µg) disc in the routine Antibiotic susceptibility testing (AST) testing for enteric fever Salmonellae⁹ But, Salmonella has shown decreased susceptibility to Ciprofloxacin from 1993 onwards and genetic analysis has shown that mutation in the gene Gyr A is responsible for this¹⁰. Off late it has been noticed that enteric fever patients treated with Ciprofloxacin have showed delayed defervescence. MIC determination of Ciprofloxacin against Salmonella isolates has showed increasing MIC levels.^{11,12,13} Hence, an attempt was made to detect the occurrence of enteric fever in patients attending JSS Hospital, Mysore and find out the MIC of Ciprofloxacin among isolates obtained from these cases, and compare the results of disc diffusion with the MIC results.

MATERIALS AND METHODS

Patients attending JSS Hospital who were clinically suspected as Enteric fever during the study period. Sample: 5- 10 ml of venous blood from these patients for blood culture. Inclusion criteria: Phenotypically and serologically confirmed Enteric fever Salmonellae isolated from the blood of patients over one year of age Exclusion criteria: Non Salmonella species isolated from blood cultures and patients below one year of age. Processing of blood cultures and Culture and Identification of Salmonella was done according to the standard protocols. Species identification was confirmed by agglutination using factor sera (Denka Seiken). Antibiotic susceptibility testing for Salmonella isolates was done by Kirby Bauer disc diffusion technique against Ampicillin(10µg) Amoxycillin Clavulanicacid (20/10µg), Trimethoprim / Sulfamethoxazole (1.25/23.75µg), Tetracycline (30µg), Gentamicin (10µg), Ceftriaxone (30 µg), Chloramphenicol (30µg), Nalidixic acid (30µg), Ciprofloxacin (5µg). All discs were procured commercially (Hi-media laboratories private limited). The diameter of the zone of inhibition was measured and interpreted according to the guidelines of Clinical Laboratory Standards institute (CLSI). ATCC E. coli 25922 was used as a control for confirming the efficacy of the above said discs. Nalidixic acid disc (30µg) helped in detecting the possible decreased susceptibility to fluoroquinolone. In addition Ciprofloxacin (5µg) discs used in the present study helped to detect the invitro susceptibility pattern to Quinolones. MIC of Ciprofloxacin against Salmonella isolates was done by Agar dilution, Hi comb (Himedia) and E test (AB Biodisk, Solna, Sweden). All the statistical methods were carried out through the SPSS for Windows (version 16.0).

RESULTS

Out of the 3446 blood samples collected and cultured, 638 (18.5%) yielded bacterial growth. 54 (8.46%) of these were enteric fever Salmonellae. Among these 40(74%) were isolated from males and 24 (26%) from females with a male to female ratio of 1.6:1.. 51 of the Salmonella isolates were isolated from patients within the first 3 decades, 10, 22,19 cases in the 1st 2nd and 3rd decades respectively. Out of the 54 isolates of Salmonellae, 29 were *S. Paratyphi A* and 25 were *S. Typhi* strains. In males there were equal distribution of the two species, but in females 9 were *S. Paratyphi A* and 5 were *S. Typhi*. In this study the mean time for positive blood culture was 3-5 days. During this study 23 cases were isolated during the months of July- September. Least (one case) was in January. All 54 isolates were susceptible to Chloramphenicol, TMP-SMZ and Ceftriaxone. 20 *S. Typhi* and 25 *S. Paratyphi A* was found to be resistant to Nalidixic acid. This included *S. Typhi*² and *S. Paratyphi A*¹ which were resistant to Ampicillin, Amoxycyclavulnic acid and Tetracycline. Among the 20 *S. Typhi* isolates detected as NARST, only 2 of them showed resistance to Ciprofloxacin by Disc diffusion method and among the 25 *S. Paratyphi A* only 2 were resistant to Ciprofloxacin by the same method. All 54 isolates were susceptible to Chloramphenicol, TMP-SMZ and Ceftriaxone. Only one *S. Paratyphi A* was found to be resistant to both Ampicillin and Amoxycyclavulnic acid, this isolate was also resistant to Tetracycline and Gentamicin. 2 *S. Typhi* were resistant to Ampicillin and Tetracycline. E test was performed on 13 *S. Typhi* isolates among which 7 isolates showed an MIC of 0.38, 2 isolates showed an MIC of 0.25 and 1 isolate each showed an MIC of 0.16, 0.23, 0.125 and 0.19. Among the 10 isolates of *S. Paratyphi A* tested by E test 4 isolates had an MIC of 0.38, 2 isolates had an MIC of 0.50, another 2 isolates had an MIC of 0.19 and two isolates had an MIC of 0.25. Though all the isolates had the MIC values in the susceptible range according to CLSI guidelines, 17 isolates had an MIC in the zone of reduced susceptibility to Ciprofloxacin (0.25-1µg/ml) All 54 isolates were tested by Agar dilution method and detected MIC of Ciprofloxacin. 33 showed MIC of less than 0.25µg/ml, 17 showed MIC of 0.25µg/ml, 4 isolates had an MIC of 0.5µg/ml, 2 had MIC of 1µg/ml. During the present study MIC level, for Ciprofloxacin was also detected by Hi-comb strips. 40 isolates were tested, 9 of these were tested by both E test and agar dilution test. Remaining 31 isolates were tested only by agar dilution and Hi-Comb. Among the 9 isolates tested by all 3 methods, 4 were *S. Paratyphi A* and 5 were *S. Typhi*. Of the 4 *S. Paratyphi A*, Hi-Comb showed an MIC of 0.5µg/ml in one isolate. Among 5 *S. Typhi* isolates 4 had

similar results (0.25µg/ml) but one strain showed an increase MIC of 0.5 µg/ml by Hi-Comb test. Hi-Comb showed higher MIC values for 2 isolates. Of the remaining 31 tested by Hi-Comb and agar dilution 1 *S. Paratyphi A* and 1 *S. Typhi* showed MIC of 1µg/ml, the other 29 had a low MIC by both the methods.

DISCUSSION

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately, the emergence of antibiotic resistant bacteria in general and also among enteric fever *Salmonellae* is threatening the effectiveness of the antimicrobial agents in the management of infectious diseases including enteric fever. Ampicillin, TMP-SMZ was used as substitutes for treatment of Chloramphenicol resistant enteric fever cases. Soon the *Salmonellae* developed resistance to these drugs also, resulting in the emergence of Multidrug resistant enteric fever *Salmonellae*. This problem spread all over the world very rapidly leading to shelving of these three magic bullets in the management of enteric fever. 54 strains of *Salmonellae* were isolated from blood culture. The male to female ratio was 3:1. More cases are reported among males compared to females probably as a result of increased exposure to infection¹⁴. In the present study adolescents and young adults were the major sufferers (1-30 yrs) this is the same scenario in majority of places, and the only explanation for this increased rate in younger generation could be their eating habits in public places. Saha *et al*¹⁵ and Kumar *et al*² (134 /174) also found similar high rate of enteric fever in young adults, Mukherjee *et al*¹⁶ observed that the median age of enteric fever is 19 yrs. Seasonal variation worldwide is noticed in enteric fever, in some countries very high rate is found in summer months, overall it is commonly seen during the monsoon months, in the present study also more number of cases were detected in July- Aug period. Open field defecating habits common in South Asian countries results in contamination of soil. This contaminated soil gets washed during the monsoon which may lead to floods. During this period, due to poor living conditions, the economically weaker section of the population is at increased risk of contracting the enteric fever^{2,17}. 54 Enteric fever *Salmonellae* were isolated in the study, (8.46%). 12.11 % rate was reported at Pondicherry¹⁷, 7% enteric fever rate was noticed at New Delhi². 29 *S. enterica subspecies enterica serovar Paratyphi A* (53.7%) and 25 strains of *S. enterica subspecies enterica serovar Typhi* (46.3%) were isolated in the present study. *Salmonella enterica serotype Paratyphi A* causes a milder form of enteric fever, but there has been a gradual increased report of Paratyphoid fevers in India. The rate has

increased to 44.9% in Delhi, 59% in Calcutta, 46.15% in Nagpur and 53.3% in Sevagram¹⁸. In addition a changing antibiotic susceptibility of *Salmonella Paratyphi A* also has been observed in different parts of India. The exact factor responsible for this changing trend is not known; it may be due to changing ecology, environmental changes, increased suspicion of enteric fever in PUO cases and improved Microbiology diagnostic laboratory services. The finding in the present study endorses the observations made by other workers that Paratyphoid fever is an Emerging illness and the Clinical Microbiologist must look out for isolation of *S. Paratyphi A* in Blood and faeces cultures. *S. enterica subspecies enterica serovar Typhi* (46.3%) continues to be the main culprit in enteric fever. It appears that *S. Typhi* strains have been marginally overtaken by *S. Paratyphi A* as the major pathogen. Still Typhoid fever is more harmful and if either neglected or irregularly treated it can result in serious complications including fatality which may be up to 20%³. Rate of isolation of *S. Typhi* was 21.1% at Kolkata¹⁵. The average period of incubation required for growth of *Salmonella* to produce positive results was 4-5 days in the present study which is in concordance with the study of Shaw B¹⁹. The antibiotic susceptibility of both *Salmonella Typhi* and *Paratyphi A* showed a mixed pattern. Majority of Isolates were found to be susceptible to Ampicillin, Trimethoprim/Sulphamethoxazole, Chloramphenicol, Ceftriaxone. 100% susceptibility was seen for Chloramphenicol, TMP-SXZ and Ceftriaxone. Only 3 out of 54 isolates were resistant to Ampicillin (5.6%). By disc diffusion method it was found that 4 isolates were resistant to Ciprofloxacin, 2 of these were *S. Typhi* and the other 2 were *S. Paratyphi A*. In Contrast Nalidixic acid resistance was seen in 45 /54 isolates (83.3%). A very high rate of resistance to Ampicillin, TMP-XZ and Chloramphenicol has been reported recently at Pondicherry and other places^{10,19,20,21}. Multidrug resistance (resistance to Ampicillin, Chloramphenicol and TMP-SMZ) in the present study was 7.4%. Das U²² *et al* have reported MDR among 7.9% of isolates in their study and Capoor²³ *et al* reported 7% isolates showing multidrug resistance. In the study in vitro susceptibility to Ciprofloxacin was seen in 92.6%, this finding was in sharp contrast to the Nalidixic acid susceptibility pattern seen in Disc diffusion method. Nalidixic acid resistance was seen in 83.3%. 71.4% resistance to Nalidixic acid is reported from Lucknow²⁴. Nalidixic acid resistance by disc diffusion method is used as an indirect marker for finding decreased susceptibility to Ciprofloxacin. There are several discordant findings regarding Nalidixic acid resistance being used as a marker for Quinolone resistance. In the present study it was found that 83.3 % isolates were Nalidixic acid (NA)

resistant and 92.6% isolates showed Susceptibility to Ciprofloxacin (5µg) disc. This discrepancy may be solved by using Ciprofloxacin instead of Nalidixic acid as the marker of Quinolone resistance. In the present study further analysis of the isolates was done by detecting MIC value for Ciprofloxacin using 3 different Methods. Among the 23 isolates (13 *S.Typhi* and 10 *S.Paratyphi A*) out of 50 susceptible to Ciprofloxacin by Kirby Bauer method, 7 *S.Typhi* and 4 *S.Paratyphi A* had MIC level of 0.38 µg per ml, 2 *S.Paratyphi A* isolates had an increased MIC level of 0.5 µg per ml. An MIC level of 0.25 and above is considered as showing reduced susceptibility. All 14 isolates had a zone diameter of more than 23 mm in the disc diffusion method and were found NA resistant. This endorses that it is mandatory to find MIC values of all *Salmonella* isolates to ciprofloxacin in the routine diagnostic labs. Using only disc diffusion method of AST increases the damage to the suffering. It was found in the study that agar dilution if carried out by academically active clinical Microbiologist is almost on par to E test in performance. The disparity between these two methods observed in the study was that some of the isolates showed MIC level of 0.38 by E test and 0.25 by Agar dilution. One isolate of *S.Typhi* and one *S.Paratyphi A* had an MIC level of 1 µg/ml by Agar dilution and Hi Comb method. Both these isolates were resistant to Ciprofloxacin by Disc diffusion method also. Hi Comb showed increased MIC level of 0.5 µg per ml in 1 *S.Typhi* and one *S.Paratyphi A* isolates, the findings were almost identical in agar dilution and Hi Comb method. For laboratories with limited financial resources Agar dilution method is ideal and for economically sound hospitals and laboratories E test and Hi Comb strips can be used to report the MIC level of Ciprofloxacin.

CONCLUSION

Salmonella enterica subspecies enterica serotype Typhi was isolated in 25 out of 54 positive cases (46.3%) and *Salmonella enterica subspecies enterica serovar Paratyphi A* was isolated in 29 cases (53.7%). The rate of these two most commonly prevalent enteric fever salmonellae was almost same. During the previous 5 years a significant observation made in our hospital is the gradual increase in the number of Paratyphi A isolates in enteric fever cases. Changing Ecology has probably led to increase in the rate of Paratyphoid fevers and this may also be attributed to increased administration of Typhoid specific vaccination by the practitioners. In this region of Karnataka Enteric fever rate is high during the Monsoon season and is almost negligible during the winter months. This could be due to the rain water carrying the contaminated soil and draining it in to the water bodies in the rural areas. Many of the rural areas in our country

even today depend on these water bodies as the source of both drinking water and water for household use. This is the epidemiological observation made during the course of the present study. Majority of the isolates were found to be susceptible to the commonly used economical and safe drugs such as TMP-SMZ and Ampicillin. The isolates were also susceptible to another old antibiotic – Chloramphenicol. Majority (83.3%) of the isolates were found to be Nalidixic acid resistant by disc diffusion method. As per the CLSI guidelines Nalidixic acid 30microgram disc is employed as a screening method to detect Decreased susceptibility or Resistance to Quinolones. *Gyr* mutation in salmonellae leads to Quinolone resistance development. The mode of action of Nalidixic acid and the Quinolones is identical. But discordant findings were observed during the study and similar discordant observations have been made by other workers regarding this guideline. 92.6% isolates were susceptible to Ciprofloxacin all these were Nalidixic acid resistant. But by E Test, Agar Dilution method and by Hi Comb method only one *S.Typhi* and one *Paratyphi A* isolates showed an MIC of 1 microgram /ml. From the study we would like to conclude that Enteric fever continues to be a major public health problem around Mysore. Paratyphoid fevers which were rarely detected previously have now emerged on par to Typhoid fever. During monsoon months the number of Enteric fever cases increases. Young adults are more commonly susceptible. The Enteric fever Salmonellae in this region have regained susceptibility to Ampicillin, Trimethoprim/Sulfmethoxazole and Chloramphenicol indicating the disappearance of MDR cases. Ciprofloxacin resistance is not very common in this region.

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