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RESEARCH ARTICLE

QUINOLONE RESISTANCE AMONG ENTERIC FEVER ISOLATES IN A TERTIARY CARE HOSPITAL

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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.

Results: 8.77% of samples received for blood culture yielded the growth of enteric fever Salmonellae, 31 S.Paratyphi A and 25 S.Typhi were isolated. S.Paratyphi A isolates are on the rise. 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.

Conclusion: Enteric fever continues to be a major public health problem around Mysore. 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. Nalidixic acid is not a good screening test for detection of quinolone resistance.

INTRODUCTION

Enteric fever continues to be a major public health problem, especially in the developing countries of the tropics (Gautam *et al.,* 2002). It is an important cause of morbidity and mortality with an estimated 33 million cases world wide (Kumar *et al*., 2008) and an estimated 600,000 deaths annually (Gautam *et al.,* 2002). Persistence of enteric fever directly indicates failure of public health measures. Enteric fever is endemic in all parts of India. *Salmonella enterica subspecies enterica and serovar Typhi* (*S.Typhi*) and *Salmonella enterica subspecies enterica serovar Para Typhi A* (*S.Paratyphi A)* are the predominant types of *Salmonella* responsible for enteric fever in India (Gautam *et al.,* 2002). 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 % (Manchanda *et al*., 2006). In Mysore and surrounding areas, an outbreak of enteric fever was observed in 2007, which is still persisting in the community. Majority of these isolates were found to be Nalidixic acid resistant strains (NAR). In this study we made an attempt to detect the occurrence of enteric fever in patients attending JSS Hospital, Mysore from Jan 2008 to Dec 2008 and also to determine the seasonal variation in the occurrence of enteric fever.

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Aims and Objectives

- To detect the occurrence of enteric fever.
- To determine the seasonal variation in the incidence of enteric fever.
- Antimicrobial susceptibility of Enteric fever Salmonellae.

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.
- Culture & Identification of Salmonella.
- Processing of blood culture:

Blood samples received from suspected cases of enteric fever were processed by the conventional method. The bottles were incubated at 37^0C and examined every alternate day for signs of growth. The bottles which showed signs of growth were sub

cultured on Blood agar and Mac conkey agar taking precautions to prevent contamination. The plates were incubated aerobically at 37^0C for 24 hours. The presence of greyish opaque colonies on Blood agar and non-lactose fermenting colonies on Mac conkey were further subjected for a battery of identification tests to identify the growth as Salmonella Typhi or Salmonella Paratyphi A. The *Salmonella* isolate was further confirmed by slide agglutination using factor sera. The serotype was confirmed by using *Salmonella* factor sera O-9, O-2, and O-4.

Antibiotic susceptibility testing

The *Salmonella* isolates were subjected for antibiotic susceptibility testing 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). Nalidixic acid disc (30µg), was used as a screening tool to determine quinolone resistance among enteric fever Salmonellae, in addition Ciprofloxacin disc (5µg) was also used to look for any discrepancy. 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.

Quinolone susceptibility testing

- 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.

RESULTS

Out of the 3448 blood samples collected and cultures, 638 (18.5%) yielded bacterial growth. 56 (8.77%) of these were enteric fever Salmonellae. Among these 42 (75%) were isolated from males and 24 (26%) from females. 51 of the Salmonella isolates were isolated from patients within the first 3 decades, 10,22,19 cases in the 1st 2nd& 3rd decades respectively. Out of the 56 isolates of Salmonellae, 31 were S.Paratyphi A and 25 were S.Typhi strains. In males there were equal distribution of the two species, but in females 19 were S.Paratyphi A and 5 were S.Typhi. In this study the mean time for positive blood culture was 3-5 days. 6 cases yielded growth on 6th day, 2 each yielded growth on 7th& 8th day. During this study 23 cases were isolated during the months of July-September. Least (one case) was in January.

20 S.Typhi and 25 S.Paratyphi A were resistant to Nalidixic acid by Kirby Bauer disc diffusion method. Among the 20 *S.Typhi* isolates detected as Nalidixic acid resistant Salmonella Typhi (NARST), only 2 of them showed resistance to Ciprofloxacin by Disc diffusion method. Among the 25 *S.Paratyphi* A detected as Nalidixic acid resistant S.*Paratyphi* A by disc diffusion method only 2 were resistant to Ciprofloxacin by the same method. All 56 isolates were susceptible to Chloramphenicol, TMP-SMZ and Ceftriaxone. Only one S.*Paratyphi* A was found to be resistant to both Ampicillin & Amoxyclavulinic acid, this isolate was also resistant to Tetracycline and Gentamicin. 2 S.*Typhi* were resistant to Ampicillin and Tetracycline.

DISCUSSION

Enteric fever continues to be a major public health problem, especially in the developing countries of the tropics (Gautam *et al.,* 2002). Enteric fever is endemic in all parts of India (Gautam *et al.,* 2002). 56 Salmonellae were isolated in this study (8.77%). In a study done by Harish *et al.* in 2006 at Pondicherry, the isolation rate was 12.11% (Harish *et al.,* 2006). Kumar *et al.* in 2008 reported an incidence of 7% from New Delhi, in a study done by Gupta V (Gupta *et al*., 2009) et al in 2009 reported an increased incidence of enteric fever due to S.Paratyphi A in and around Chandigarh which is similar to this study The findings of the present study endorse the observations made by other workers that Paratyphoid fever is an emerging illness. (Saha *et al.,* 2003) reported an incidence rate of 21.1% at Kolkata where as in other countries the rates were as follows Rhodesia (72%), S. Africa (73%) as per reported by (Watson, 1978). 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 infection (Park, 2002).

In the present study adolescents and young adults were the major sufferers (1-30 yrs) 51/54, and this is the same scenario in majority of places, and the only explanation for this increased rate in younger generation would be their eating habits in public places. (Gupta *et al*., 2009; Saha *et al*., 2003 and Kumar *et al*., 2008) also found similar high incidence in young adults which is in correlation to the study done by (Mukherjee *et al.,* 1991) in which they observed the median range of enteric fever as 19 years. Most of the studies support the findings of this study that enteric fever is commonly see during the monsoon months (Kumar *et al*., 2008 and Harish *et al.,* 2006). 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-1970's (Lakshmi *et al.,* 2006). First epidemic of Chloramphenicol resistant strain occurred in Mexico in 1972 (Butt *et al.,* 2003) and at the same time another outbreak was reported in India from Kerala (Paniker and Vimala, 1972). Subsequently Chloramphenicol resistant *Salmonella* species were detected all over India.

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*. 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 56 isolates were resistant to Ampicillin (5.3%). 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 /56 isolates (80.3%). A very high rate of resistance to Ampicillin, TMP-SXZ and Chloramphenicol has been reported recently at Pondicherry and other places (Wain *et al.,* 1997; Chowta and Chowta, 2005; Chomal and Deodhar, 2000 and Madhulika *et al.,* 2004). Multidrug resistance (resistance to Ampicillin, Chloramphenicol and TMP-SMZ) in the present study was 7.4%. (Das *et al*., 2006) have reported MDR among 7.9% of isolates in their study and (Capoor *et al.,* 2007) reported 7% isolates showing multidrug resistance. This changing pattern of antibiotic susceptibility is reported from many places across the globe. Complete disappearance of resistant mutants from the prevailing strains is the only explanation for this remarkable turnaround in the behaviour of Enteric fever *Salmonellae*. Future usage and retaining the susceptibility to these safe antibiotics is in the hands of the practicing clinicians.

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 Lucknow (Capoor *et al.,* 2007). Nalidixic acid resistance by disc diffusion method is used as an indirect marker for finding decreased susceptibility to Ciprofloxacin. But further analysis by detecting MIC to ciprofloxacin is the reliable indicator to detect Quinolone resistant strains or strains showing 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.

Conclusion

Enteric fever is a disease of the developing countries, low socioeconomic status, unhygienic habits, carrier state are the risk factors for the persistence of a comparatively high rate of enteric fever. S.Paratyphi A has evolved as the main culprit in enteric fever replacing S.Typhi. This makes the isolation and identification of Salmonella species mandatory in routine diagnostic. Typhoid fever, if neglected or irregularly treated can lead to serious complication including fatality 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.

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. G*yr* A 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. 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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