

POTENT ANTIBIOTIC AGAINST TYPHOID BACTERIA

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ABSTRACT

One hundred and fifty blood samples were collected from typhoid patients from selected hospitals in Madurai North. Among the 150 samples, analyzed 74 were recorded positive *Salmonella typhi* and *Salmonella paratyphi*. Out of this 74, about 43 were positive for *Salmonella typhi* and 31 were positive for *Salmonella paratyphi*. The eight samples were exhibited positive in 1:80 dilution were chosen for further study. These 8 samples were tested against 7 commonly employed antibiotics like Ampicillin, Chloramphenicol, Co-trimoxazole, Ciprofloxacin, ceftriaxone, Azithromycin, Ofloxacin and multi drug (Ampicillin + Co – trimoxazole + Chloramphenicol). Antibiogram shows that they are resistant and sensitive to above antibiotics. This study refers that *Salmonella typhi* isolates were highly sensitive to azithromycin (95%) and it is used as a routine and effective drug for typhoid.

Key words – Typhoid- Bacteria- sensitivity pattern- routine- antibiotics.

INTRODUCTION

Salmonellosis is an infection caused by ingesting salmonellae in food that is contaminated by feces of animals or humans directly or indirectly. Common sources of infection include poultry meat and meat products, eggs and egg products. Some of the symptoms of salmonellosis are diarrhea, vomiting fever and abdominal pain these occur 12-36 hours after eating infected food, in acute infection, blood and mucus are present in fecal specimens (Al – jurayyan *et al.*, 2004). Salmonellae can be isolated from blood, stool, urine, bone marrow, duodenal aspirates and rose spots. *Salmonellae* are Gram negative, non-lactose bacteria. All *Salmonellae* are actively motile. They are also, non-capsulated with the exception of *Salmonella typhi* belonging to the family Enterobacteriaceae (Cheesbrough, 2000). From blood, organisms can usually be detected in 75-90% of patients during the first ten days of infection, and in about 30% of patients during the third week (Cheesbrough, 2002). The risk to salmonellosis is increased due to the following factors; patients during the third week (Cheesbrough, 2002). Multi drug resistant (MDR) *Salmonella typhi*, causing enteric fever continues to be a major public health problem in tropics and subtropics of the world, affecting both local population and travelers to the endemic area. Frequent outbreaks have been reported in South Asian countries (Muvey *et al.*, 2003). *Salmonella typhi* has been found to produce a wide variety of ESBL types including TEM, SHV, PER and CTXM enzyme (Batchelor *et al.*, 2005; Paterson, 2006).

MATERIALS AND METHODS:

Collection of clinical isolates:

Clinical isolates were obtained from clinical laboratories in Madurai. The clinical samples collected were blood from hospitalized patients. In order to characterize and identify the bacteria specifically certain selective agar media were used. Single colony from the SS agar plate was streaked on *Salmonella sp* isolation agar plates. The identified from the selective agar plates were picked up, streaked on nutrient agar slants, and preserved for future identification. Thereby the two selected isolates namely, *Salmonella sp* were used for further studies. Antibiotic susceptibilities were determined on Muller Hinton Agar

(Oxoid, Basingstoke, United Kingdom) by standard disk diffusion procedures (Bauer et al., 1966) to the following antibiotics: Ampicillin, Gentamycin, Levofloxacin, Ceftazidime, Chloramphenicol, Amikacin, and Streptomycin. Serological identification of *Salmonella* species was performed by slide agglutination test with). Commercial kit was used to agglutinate and sera group salmonellae by their O antigens: A, B, C, D, and E. When positive agglutination reaction was obtained in one of the antisera, the *Salmonella* sub group was identified, and no further testing with antisera needed to be conducted. Kirby-Bauer method was carried out. The antibiotic discs (amikacin 30mcg, cephalexin 30mcg, chloramphenicol 30mcg, ciprofloxacin 5mcg, gentamicin 10mcg, kanamycin 30mcg, nalidixic acid 30mcg, neomycin 30mcg, norfloxacin 10mcg, ofloxacin 5mcg, streptomycin 10mcg and 1.25/23.75mcg) were placed on the surface of the inoculated plates in such a way that each disc was made to adhere perfectly to the surface of the agar by gently pressing. The plates were incubated at 37°C for 24 hours and after incubation, the zones of inhibition were measured with standard chart. The diameters of zones of inhibition of MDR were compared with the critical zones diameters.

RESULT AND DISCUSSION

Salmonella was detected in 16% of the collected samples under studying. NB inoculated separately with the collected samples revealed the growth of bacteria after 24 hrs of incubation at 37°C aerobically and was indicated by the presence of turbidity. All the isolates were found to be motile having swinging movement when examined using hanging drop slide under microscope. The rapid slide agglutination with poly O and the case polyvalent H antisera was conducted with all the isolated *Salmonella* serovars. In this test all culturally and biochemically positive salmonella serovars showed agglutination with poly O and in case of polyvalent H. All isolates gave positive reaction. In vitro antibiotic sensitivity pattern of isolated salmonellae was performed against seven commonly used antibiotics belonging to different groups. After incubation plates were examined and diameters of the zone of inhibition for individual antibacterial agents, moderately sensitive and resistant. Among the isolates 100% were highly sensitive to ciprofloxacin, 80% and 60% were to chloramphenicol respectively while 40% to co-trimoxazole and 20%. However 40% were moderately sensitive to co-trimoxazole while 20% chloramphenicol and ciprofloxacin. 60%, 25%, and 20% were found to be sensitive gentamicin. As regard effectively to azithromycin and 75% resistant to ofloxacin while resistant to chloramphenicol.

Of the total 150 blood culture samples, from the suspected patient of enteric fever, 78 (2.0%) sample showed positive growth for *Salmonella species*. Out of total positive cases 47 (60.3%) were *S.typhi* and 31 (39.7%) were *S.para typhi A*. Isolates were from all age group, the median age being 25 years. The male to female ratio was 1.5:1. *S. typhi* was most susceptible towards Ciprofloxacin 47 (100%), followed by Gentamicin 46 (97.9%), Ofloxacin 45 (95.7%), Ceftriaxone 45 (95.7%) and Chloramphenicol 44 (93.6%). In case of *S. paratyphi A* most of the tested antibiotics showed high percentage of susceptibility and the least susceptible antibiotic was Ampicillin 8 (25.8%).

Out of the 47 *S.typhi* isolates; three isolates were multi drug resistance, showing resistance simultaneous to Ampicillin, Chloramphenicol, and Co-trimoxazole which are first line anti typhoidal drugs (Table 2). Most of the positive cases lie in the age group 21-40 years. Median age being the 25 years (Table 3). Enteric fever is a major health problem in developing countries. Isolation of *Salmonella species* occurs throughout the year. In case of *S.paratyphi A* all isolates were susceptible to chloramphenicol. No multidrug *S.paratyphi A* was isolated. Co-trimoxazole is the second cost effective drug used to treat typhi and paratyphi infection. Present study showed that 43 (91.5%) *S.typhi* were

susceptible to Co-trimoxazole and 4 (8.5%) were resistance. As 5% of the isolates were resistance to Co-trimoxazole. Similarly, in case of *S.paratyphi A* all the isolates were susceptible to Co-trimoxazole

Among multidrug resistance *S.typhi* all, the isolates were resistance to Co-trimoxazole, this finding was in agreement with the whom among multidrug resistance *S.typhi*, 97% isolates were resistance to Co-trimoxazole. In this study Ceftriaxone was 100% susceptible to *S.paratyphi, A* which was, showed 100% susceptibility to this drug. In case of *S.typhi 2* (4.3%) showed resistance to Ceftriaxone this finding was similar to the finding reported. Fluoroquinolones particularly ciprofloxacin was the most frequently used antibiotics in *typhi* and *paratyphi* case but none of the isolates were resistance to this antibiotics. Paterson. D 2006 Islam also reported this high percentage of resistance to chloramphenicol. This resistance is due to the frequent used of this drug for typhoid fever universally over a long span of time. In the current study, we observed high percentage of resistance to chloramphenicol. These results were expected because of the high prevalence of multi-drug resistance to chloramphenicol, ampicilline, co-trimoxazole and ciprofloxacin. The study showed that out of 45 *S.typhi* isolates, 43 are susceptible to azithromycin. Resistance was not expected because this drug has not been used extensively in our country. When 45 isolates of *S.typhi* were tested for azithromycin MICs, all isolates except 2 susceptible, with MICs of 4-8mg/l ,this difference between results of susceptibility testing by disk zone diameter and MICs suggests that there was less azithromycin resistance in our isolates of *S.typhi* than was reported initially from results of disk diffusion. The study recorded a high activity of azithromycin towards *Salmonella typhi* isolates. Nearly more than 95% Susceptibility was reported. Batchelor.M 2005 reported similar findings.

Table1 Sensitivity pattern for following isolates.

| Sensitivity Pattern | ER | AX | CP | CK | CT | OF | CL | GE |
|----------------------|-----|----|----|----|----|----|-----|----|
| Resistance | 100 | 75 | 0 | 20 | 0 | 0 | 0 | 20 |
| Less Resistance | 0 | 25 | 60 | 0 | 20 | 0 | 0 | 0 |
| Moderately Sensitive | 0 | 25 | 60 | 0 | 20 | 0 | 0 | 0 |
| Highly Sensitive | 0 | 0 | 20 | 60 | 40 | 80 | 100 | 40 |

Table2: Antibiotic susceptibility patterns of isolated Salmonella species against tested antibiotics at 37⁰ c

| ANTIBIOTICS | SUSCEPTIBILITY | |
|------------------------|----------------|---------------------|
| | <i>S.typhi</i> | <i>S.para typhi</i> |
| Amphicilline | 33(70.2) | 8(25.80) |
| Chloramphenicol | 44(93.6) | 31(100) |
| Co-trimoazole | 43(91.5) | 31(100) |
| Ciproflaxin | 47(100) | 31(100) |
| Ceftriaxacin | 45(95.7) | 31(100) |
| Centamicin | 46(97.9) | 30(96.8) |
| Ofloxacin | 3(6.38) | 31(100) |
| MDR (amp+co+tri+chlor) | 3(6.38) | - |

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