ANTIBIOTIC SENSITIVITY PATTERN OF SALMONELLA SEROTYPES IN PATIENTS WITH ENTERIC FEVER IN A TEACHING HOSPITAL

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ABSTRACT

Objective: *To determine the sensitivity of salmonella serotypes to antibiotics in patients with enteric fever.*

Methodology*: This descriptive study was carried out in medical units of Lady Reading Hospital Peshawar from January 2008 to December 2011. Patients were included by consecutive sampling technique. Blood samples were collected from patients with clinically suspected enteric fever and were sent to hospital laboratory for culture and sensitivity(C/S). Salmonella colonies were identified using standard biochemical tests including fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H2S production, and fermentation of dulcitol. Serological confirmation tests included polyvalent antisera for flagellar and somatic antigens. Antibiotic susceptibility was checked by Kirby- Bauer disc diffusion method for 22 antibiotics.*

Results*: Blood cultures of 106(61%) patients out of 173 patients were positive for salmonella species. Salmonella Typhi was found in 54.7% while salmonella paratyphi A in 32.1% and B in 13.2%. The sensitivity of salmonella species for ceftriaxone and ceftazidime was found to be 100%, followed by imipenem (98.1%) and meropenem (96.2%). The sensitivity of drugs like amoxicillin, chloramphenicol and co-trimaxazole were 2.8%, 12.3% and 22.6% respectively. Regarding quinolones, most sensitive was moxifloxacin 49.1%, followed by ciprofloxacin 48.1%. Salmonella typhi was fully sensitive to meropenum, ceftriaxone and cetazidime while paratyphi A or B was fully sensitive to ceftriaxone, ceftazidime and imipenem.*

Conclusion*: The sensitivity of salmonella species was very low to the first line agents such as amoxicillin, chloramphenicol and co-trimaxazole. Salmonella species were fully sensitive to third generation cephalosporins such as ceftriaxone and ceftazidime.*

Key Words*: Enteric fever, Typhoid fever, Paratyphoid fever, Salmonella, Antibiotics sensitivity.*

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INTRODUCTION

Enteric fever remains a major public health problem in the developing world, where provision of pure water supplies and sewage control are inadequate[1]. Almost 80% of the cases and deaths due to enteric fever are in Asia while the rest occur mostly in Africa and Latin America. An estimated 22 million new cases and 200,000 deaths occur annually due to enteric fever[2].

Pakistan is considered to be one of the enteric fever endemic countries with very high disease burden. Its overall incidence is 451.7 cases per 100,000 population per year in Pakistan[3].

Enteric fever is characterized by severe systemic illness with fever and abdominal pain. It includes typhoid fever (caused by salmonella typhi) and paratyphoid fever (caused by S. Paratyphi A, B or C)[4]. The diagnosis of enteric fever is made by culture of the causative micro-organism in the setting of a compatible clinical illness. Blood cultures for Salmonella species are obtained before initiation of an-

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timicrobial therapy. The sensitivity of blood culture alone is only 50 to 70%. When patients have already received antimicrobial therapy, blood cultures may be positive in only 40% of the patients[5].

Enteric fever is known to be associated with significant morbidity and mortality due to emerging multidrug-resistant strains of salmonella[6]. The emergence of antimicrobial resistance, especially the multidrug resistance to ampicillin, chloramphenicol and co-trimoxazole, has complicated the treatment of enteric fever[7]. Multi-drugn resistant S. Typhi (MDRST) has appeared throughout the world, especially in South America, the Indian subcontinent, Africa and Southeast Asia .

Wide variation in the sensitivity pattern of various strains of salmonella makes it necessary to assess its sensitivity to antibiotics before instituting therapy. The present study was undertaken to know the antibiotic susceptibility of salmonella serotypes to the more commonly used agents like ampicillin, chloramphenicol, co-trimoxazole, ciprofloxacin and ceftriaxone. It will help in providing a suitable guideline for the treatment of enteric fever

METHODOLOGY

This was a descriptive cross sectional study carried out in medical units of Post graduate Medical Institute Lady Reading Hospital Peshawar, Khyber Pukhtoonkhwa, from January 2008 to December 2011. Patients were admitted through out-patient (OPD) and emergency departments.

After obtaining an informed consent, a total of 173 blood samples were screened for salmonella serotypes in clinically suspected enteric fever patients. Patients aged 12 years or above and of either gender were included by consecutive sampling technique.

Patients with fever due to non-typhoidal illnesses (i.e. pneumonia, malaria, urinary tract infection) were excluded from the study clinically and on the basis of appropriate investigations.

The diagnosis of Enteric fever was based on patients having fever for five days or more prior to admission plus documentation of fever (≥ 1000F) in the hospital with headache, constipation / diarrhea, abdominal pain, nausea, rose spots, splenomegaly, hepatomegaly, signs of toxemia, leukopenia / leukocytosis; and positive blood culture for S. Typhi / S. Paratyphi A, B, C.

Patients were assessed through a detailed history and physical examination. Chest x-ray, urine detailed report and blood malarial parasites were checked to exclude other non-typhoidal illnesses.

Blood samples from patients with clinically suspected enteric fever were collected and were sent to hospital laboratory for culture and sensitivity(C/S) to determine the pattern of sensitivity of salmonella serotypes. All blood cultures were inoculated in thioglycolate broth and incubated at 37° for 7 days. Salmonella colonies were identified using standard biochemical tests including fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H2S production, and fermentation of dulcitol. Serological confirmation tests included polyvalent antisera for flagellar (H) and somatic (O) antigens. Antibiotic susceptibility was checked by Kirby- Bauer disc diffusion method for 22 antibiotics.

All information was recorded on a standard proforma. Computer software SPSS (windows version 15.0) was used for data entry, storage, processing and analysis. Mean + standard deviation (SD) was calculated for quantitative variables like age. Frequencies and percentages were obtained for qualitative variables like gender and antibiotic sensitivities. Data are presented in the form of tables.

RESULTS

Blood cultures of 106 (61%) patients out of 173 patients with clinically suspected enteric fever were positive for salmonella serotypes. Salmonella typhi were 54.7% (n=58) while salmonella paratyphi A 32.1% (n=34) and salmonella paratyphi B were 13.2% (n=14).

Salmonella infection was more common in males 62 (58.5%) than females 44 (41.5%), having male to female ratio of 1.4:1. Gender distribution of salmonella serotypes is shown in table 1.

Age of the patients ranged from 12-50 years. The mean age of study groups was 28.61±9.464 years.

The sensitivity of drugs like ampicillin, chloramphenicol and co-trimaxazole were 2.8%, 12.3% and 22.6% respectively. Regarding quinolones the most

Table 1: Gender distribution of patients with enteric fever

Gender	Salmonella Serotypes			Total
	Salmonella Typhi	Salmonella Paratyphi A	Salmonella Paratyphi B	
Male	41 (66.1%)	17 (27.4%)	4 (6.5%)	62 (100%)
Female	17 (38.6%)	7 (38.6%)	10 (22.7%)	44 (100%)
Total	58 (54.7%)	34 (32.1%)	14 (13.2%)	106 (100%)

Table 2: Antimicrobial sensitivity pattern of salmonella in in-patients with enteric fever

Antibiotics	Sensitivity	Resistance
Amoxicillian	03 (2.8%)	103 (97.2 %)
Chloramphenicol	13 (12.3 %)	93 (87.7 %)
Cotrimexazole	24 (22.6 %)	82 (77.4 %)
Coamoxiclav	99 (93.4%)	07 (6.6%)
Gentamicin	0 (0%)	106 (100%)
Amikacin	88 (83.0 %)	18 (17.0 %)
Imipenem	104 (98.1 %)	02 (1.9 %)
Meropenem	102 (96.2 %)	04 (3.8%)
Pipercillin	90 (84.9%)	16 (15.1 %)
Cephradine	06 (5.7 %)	100 (94.3 %)
Ceftriaxone	106 (100%)	0 (0%)
Cefpodoxime	45 (42.5 %)	61 (57.5 %)
Cefoperazone	95 (89.6 %)	11 (10.4 %)
Cefuroxime	03 (2.8%)	103 (97.2 %)
Cefotaxime	98 (92.5 %)	08 (7.5 %)
Ceftazidime	106 (100%)	0 (0%)
Ciprofloxacin	51 (48.1 %)	55 (51.9 %)
Ofloxacin	44 (41.5 %)	62 (58.5 %)
Moxifloxacin	52 (49.1 %)	54 (50.9 %)
Levofloxacin	46 (43.4 %)	60 (56.6 %)
Sparfloxacin	47 (44.3 %)	59 (55.7 %)
Enoxacin	13 (12.3 %)	93 (87.7 %)

sensitive was moxifloxacin (49.1%), followed by ciprofloxacin (48.1%), sparfloxacin (44.3%), levofloxacin (43.4%), ofloxacin (41.5%) and enaxocin (12.3%).

The sensitivity of salmonella serotypes to ceftriaxone and ceftazidime was found to be 100%, followed by imipenem (98.1%), meropenem (96.2%), coamoxiclav (93.4%), cefotaxime (92.5%) and pipercillin (84.9%) [Table 2].

Salmonella typhi was fully sensitive to meropenum, ceftriaxone and cetazidime while paratyphi A or B was fully sensitive to ceftriaxone, ceftazidime and imipenem (Table 3).

DISCUSSION

Enteric fever continues to be a public health problem due to improper sanitation, inadequate human waste treatment[9] and presence of a large number of carriers in the society[10]. Inappropriate use of antimicrobial agents11 has led to widespread resistance among bacterial pathogens including salmonella serotypes[12-14].

In our study the frequency of salmonella typhi was 54.7% while that of salmonella paratyphi A was 32.1% and salmonella paratyphi B were 13.2%. The findings of our study are similar to other studies,[5,15-17] showing that there has been a shift in the isolation rate of S. typhi to S. paratyphi A. Isolation rate of S. typhi has decreased to a greater extent whereas the incidence of S. paratyphi A has drastically increased. The reason might be due to widespread use of vaccines which are effective only against S. typhi[1,18].

The sensitivity to first line drugs like ampicillin, chloramphenicol and co-trimaxazole were 2.8%, 12.3% and 22.6% respectively in the present study. Local data suggests that resistance to first line agents among salmonella serotypes varies from 39 to 46%[19]. In the years 1982-89 the rate of MDRST in India was below 15%, but it increased to 50% in 1990, to more than 70% in 1992 and then to 90% in Bangalore in 1994[8,20,21].

Resistance to the conventionally used first line antibiotics is termed multi-drug resistant (MDR)

Table 3: Antimicrobial sensitivity pattern of Salmonella serotypes

Antibiotics	Salmonella Typhi (n=58)		Salmonella Para-typhi A (n=34)		Salmonella Para-typhi B (n=14)	
	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance
Amoxicillian	3(5.2%)	55(94.8%)	0(0%)	34(100%)	0(0%)	14(100%)
Chloramphenicol	0(0%)	58(100%)	9(26.5%)	25(73.5%)	4(28.6%)	10(73.4%)
Co-trimexazole	7(12.1%)	51(87.9%)	13(38.2%)	21(61.8%)	4 (28.6%)	10(71.4%)
Co-amoxiclav	54(93.1%)	4 (6.9%)	31(91.2%)	3(8.8%)	13(92.9%)	1(7.1%)
Gentamicin	58(100%)	0(0%)	34(100%)	0(0%)	14(100%)	0(0%)
Amikacin	46(79.3%)	12(20.7%)	28 (82.4%)	6 (17.6%)	14(100%)	0(0%)
Imipenem	56(96.6%)	2(3.4%)	34(100%)	0(0%)	14(100%)	0(0%)
Meropenem	58(100%)	0(0%)	31(91.2%)	3(8.8%)	13(92.9%)	1 (7.1%)
pipercillin	54(93.1%)	4(6.9%)	27(79.4%)	7(20.6%)	9(64.3%)	5 (35.7%)
Cephradine	3(5.2%)	55(94.8%)	3(8.8%)	31(91.2%)	0(0%)	14(100%)
Ceftriaxone	58(100%)	0(0%)	34(100%)	0(0%)	14(100%)	0(0%)
Cefpodoxime	30(51.7%)	28(48.3%)	12(35.3%)	22(64.7%)	3 (21.4%)	11(78.6%)
Cefoperazone	52(89.7%)	6(10.3%)	32 (94.1%)	2(5.9%)	11 (78.6%)	3(21.4%)
Cefuroxime	3(5.2%)	55(94.8%)	0(0%)	34(100%)	0(0%)	14(100%)
Cefotaxime	57(98.3%)	1(1.7%)	30 (88.2%)	4(11.8%)	11(78.6%)	3(21.4%)
Ceftazidime	58(100%)	0(0%)	34 (100%)	0(0%)	14 (100%)	0(0%)
Ciprofloxacin	6(10.3%)	52(89.7%)	33(97.1%)	1(2.9%)	12(85.7%)	2(14.3%)
Ofloxacin	4(6.9%)	54(93.1%)	27(79.4%)	7(20.6%)	13 (92.9%)	1(7.1%)
Moxifloxacin	12(20.7%)	46(79.3%)	27(79.4%)	7(20.6%)	13(92.9%)	1(7.1%)
Levofloxacin	4(6.9%)	54(93.1%)	30(88.2%)	4(11.8%)	13(92.9%)	1(7.1%)
Sparfloxacin	4(6.9%)	54(93.1%)	30 (88.2%)	4(11.8%)	13(92.9%)	1(7.1%)
Enoxacin	7(12.1%)	51(87.9%)	3(8.8%)	31(91.2%)	3 (21.4%)	11(78.6%)

typhoid fever and is a major therapeutic concern for physicians in developing countries[18,22,23]. Contributory factors may be drug overuse, misuse and inappropriate prescribing practices by physicians11 along with intrinsic microbiological plasmid-mediated factors. A recent observation of plasmid-mediated quinolone resistance[24] in Enterobacteriaceae is of great concern since this resistance gene could be disseminated rapidly across bacterial populations by conjugation.

Drug resistance to chloramphenicol in S. typhi first emerged in the United Kingdom (UK) in the 1950s and subsequently in Greece and Israel followed by the epidemics of MDR Salmonella in Mexico, India and other regions8. A high (52–82%) prevalence of MDR S. typhi has also been reported in Kenya and Ghana[25].

The resistance to chloramphenicol increased steadily in India from 1960 onwards but showed a downward trend from 15.8% to 7.8% from 1998 to 2001. This indicates a re-emergence of chloramphenicol sensitivity in S. typhi[26]. Jevanand et al[27] found that 29.6% of the strains were sensitive to chloramphenicol by the disc diffusion method while 100% sensitivity was observed with MIC methodology.

In a search for improved treatments for enteric fever and MDRST in particular, attention has been focused on fluoroquinolone compounds and broad-spectrum cephalosporins because of their excellent properties[9].

In the present study the sensitivities of salmonella serotypes were moxifloxacin 49.1% followed by ciprofloxacin 48.1%, sparfloxacin 44.3%, levofloxacin43.4% and ofloxacin 41.5%.

Fluoroquinolones when first introduced in early 1990's were very effective but the past decade has seen a progressive increase in the MICs of ciprofloxacin and high incidence of clinical failure to quino-

lones[28,29]. With an increase in MIC of ciprofloxacin, effective use may require parenteral or higher dosages to achieve serum levels required for effective therapy; however, the latter could have unwanted health consequences.

The development of resistance is due to the overuse of Ciprofloxacin in the treatment of enteric fever. Incomplete treatment may also be a factor contributing to the development of resistance. Similarly in other studies the isolates were found to have decreased sensitivity to ciprofloxacin[30,31]. The results are similar to what our study presents.

This rise in fluoroquinolone resistance has serious implications for empiric treatment of enteric fever in the community[9,17]. Fluoroquinolone-resistant strains require treatment with an alternative agent, e.g. Ceftriaxone, resistance to which remains low at 0.1-0.5%[17,31,32].

In our study the sensitivities found to third generation cephalosporins were ceftriaxone (100%), ceftazidime (100%), cefotaxime (92.5%) and Cefoperazone (89.6%). This underlies the importance of these drugs for treating MDR and Ciprofloxacin resistant Enteric fever cases. The profile of resistance pattern is comparable to other studies[18,33-36].

CONCLUSION

The sensitivity of salmonella species was very low to the first line agents such as amoxicillin, chloramphenicol and co-trimaxazole. Salmonella species were fully sensitive to third generation cephalosporins such as ceftriaxone and ceftazidime.

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CONTRIBUTORS

RM conceived the idea, planned and wrote the manuscript of the study. ZA, KM, Z and ARA designed the study and approved the final draft of manuscript. FB helped in the data aquisition and gave input in the manuscript. All the authors contributed significantly to the research that resulted in the submitted manuscript.