ORIGINAL ARTICLE

Enteric Fever in Karachi: Current Antibiotic Susceptibility of *Salmonellae* Isolates

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ABSTRACT

Objective: To determine the current sensitivity and resistance profile of *Salmonellae (S.)* isolates in a laboratory setting.

Study Design: An observational study.

Place and Duration of Study: Dr. Essa`s Laboratory and Diagnostic Centre, Karachi, Pakistan, from November 2008 - October 2010.

Methodology: Isolates from blood culture specimens of 481 bacteraemic patients were identified using conventional biochemical tests. *Salmonellae* was confirmed with specific antisera and their antibiograms determined by Kirby-Bauer Disc Diffusion method using 12 relevant antibiotics. Inclusions of the study were bacteraemia documented in all blood samples positive for *S. typhi*, *S. paratyphi-A* and *B*. Exclusions were all samples other than blood and blood samples negative for *S. typhi* and *S. paratyphi-A* and *B* during the same period. Multidrug resistance (MDR) of isolates was defined as the isolates showing resistance to all conventional anti-typhoid medicines i.e., Chloramphenicol, Ampicillin and Co-trimoxazole.

Results: Specimens (n=217) yielded 131 *Salmonellae typhi* (60.36%), 71 *S. paratyphi-A* (32.71%), and 15 *S. paratyphi-B* (6.9%); these were sensitive to the Quinolones [Enoxacin: 94.96% (n=91), Ciprofloxacin, 96.47% (n=182), Ofloxacin: 95.74% (n=203)], and Cephalosporins [Cefixime: 96.62% (n=202), Cefotaxime: 99.17% (n=206), Ceftriaxone: 98.79% (n=208)]. Resistance to Amoxicillin was 96.48% (n=128) and 29.91% (n=78) to Co-trimoxazole. About 62.64% (n=136) of the isolates were MDR strains.

Conclusion: Ciprofloxacin is currently a suitable empirical choice in presumed enteric fever cases, but culture and sensitivity analysis should be encouraged and results incorporated in prescription strategy. Increasing frequency of *S. paratyphi-A* isolates possibly suggests incomplete coverage employing monovalent vaccine.

Key words: *Typhoid. Salmonellae. Multidrug resistance. Fluoroquinolones.*

INTRODUCTION

Typhoid fever is a major public health concern, especially in developing countries,[1-3] with an estimated annual infection rate of approximately 21 million illnesses and 200,000 deaths worldwide.[3] In our region, *Salmonellae enterica Serovar typhi (S. typhi)* has emerged as the most common etiological agent of Enteric fever,[1,4] followed by *S. paratyphi-A*,[1] whose prevalence has reportedly increased from 6.5% to 44.9% according to a study conducted in India.[5]

For the management of enteric fever, tailored antibiotic therapy constitutes an integral part, since the mortality rate without adequate treatment can be as high as 30%.[6,7] Since the isolation of multidrug resistant (MDR) *S. typhi* strains which show resistance to the classical first-line anti-typhoid agents (Ampicillin, Chloramphenicol, Co-trimoxazole) used in the 1980's, the Fluoroquinolone class of antibiotics and two of the third generation Cephalosporins have become the choice for empirical therapy of suspected typhoid fever.[1,2,7] However, the rampant use of Fluoroquinolones, which were introduced in the mid 1990's,[8] has led to the emergence of documented resistance to this class of drugs.[3,9,10] This is a major setback in empirical management of enteric fever.

The aim of this study was to determine the current sensitivity and resistance profile of *Salmonellae* isolates in a laboratory setting so that the new profile of antimicrobial resistance can be determined.

METHODOLOGY

This study was carried out on isolates (n=217) of *S. typhi*, *S. paratyphi-A* and *B* grown from routine clinical blood culture samples referred to different branches of Dr.Essa`s Laboratory in Karachi Metropolis, during 2 years ending October 2010. Inclusions of the study were bacteraemia documented in all blood samples positive for *S.typhi*, *S. paratyphi-A* and *B*, collected during November 2008 till October 2010 from Dr. Essa`s Laboratory and Diagnostic centre. Excluded were all samples other than blood and blood samples -ve for *S.typhi, S. paratyphi-A* and *B* during the same period. These samples, 5-10 ml from adults and 2-3 ml from children, were collected by venepuncture using aseptic technique and inoculated directly into blood culture bottles containing 50 ml brain

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heart infusion broth (Oxoid), incubated at 37°C for 7 days. Positive blood samples were further cultured on sheep blood agar, chocolate and McConkey agar (Oxoid) plates and incubated at 35-37°C for 24-48 hours. Suspected non-lactose fermenting colonies were screened using API20E and their identity confirmed using specific agglutination antisera (Difco).

Antibacterial susceptibility testing was done on Mueller-Hinton agar (Oxoid) by the Kirby-Bauer disc diffusion technique[11] using Amoxicillin (10 ug), Aztreonam (10 ug), Cefixime (5 ug), Cefotaxime (10 ug), Ceftriaxone (30 ug), Chloramphenicol (30 ug), Co-trimoxazole (1.25/23.75 ug), Ciprofloxacin (5 ug), Enoxacin (5 ug), Fosfomycin (30 ug) and Ofloxacin (5 ug) discs.

The data was extracted from the laboratory and transferred to SPSS 16.0 (SPSS Inc. Chicago, IL, USA), for statistical analysis. Frequencies and percentages as descriptive statistics were calculated. The comparison of positive isolates for *Salmonellae* species were made according to age and gender, using chi-square test. Antibiotic sensitivity pattern of *Salmonellae* species was calculated. MDR was calculated and compared between the 2 organisms (*S. typhi* and *S. paratyphi-A*). Comparison with *S. paratyphi-B* was not made because of its quite small number of cases as compared to the others. Multidrug resistance (MDR) of isolates was defined as the isolates showing resistance to all conventional anti-typhoid agents i.e. Chloramphenicol, Amoxicillin and Co-trimoxazole. Level of significance was taken as $p < 0.05$.

RESULTS

Four hundred and eighty one cases of bacteraemia were documented during the study period, out of which 217 (45.11%) isolates were found to be positive for enteric fever. The predominant *Salmonellae* species identified were *S. typhi* (n=131, 60.36%) followed by *S. paratyphi-A* (n=71, 32.71%) and *S. paratyphi-B* (n=15, 6.9%).

S. typhi (84.73%, n=111) were isolated more often in < 18 years age group as compared to *S. paratyphi-A* (66.25%, n=47) and *S. paratyphi-B* (66.67%, n=10), (Table I), with children more frequently affected than adults thereby constituting 77.41% (n=168) of the total cases.

Overall, males were predominantly affected (58.06%, n=126) than females. Also, the predilection for male sex was noted in all the age groups except in infants and pre-school children (0-5 years), where the gender distribution was nearly equal.

A larger number (28.11%, n=61) of the cases of enteric fever were encountered in the hot months of April, May and June, as well as the Monsoon season (28.11%, n=61) after which the number of patients decreased during Autumn and Winter (October - December 25.35%, n=55, January - March 18.43%, n=40).

Of the classical first line antityphoid drugs, resistance to Chloramphenicol was observed in 62.03% (n=144) of the cases of enteric fever isolates (*S. typhi*: 71.33%, n=92, *S. paratyphi-A*: 61.98%, n=44, and *S. paratyphi-B*: 52.78%, n=8) (Table II). Also, resistance was 29.91% (n=78) to Co-trimoxazole and 96.48% (n=128) to Amoxicillin. Additionally, the isolates were 98.19% sensitive to third generation Cephalosporins [Cefixime: 96.62% (n=202), Cefotaxime: 99.17% (n=208), Ceftriaxone: 98.79% (n=195)], and to Fosfomycin (90.52%, n=195).

Table I: Age and gender of patients positive for *S. typhi* and *S. paratyphi.*

Characteristics	*S. typhi* n (%)	*S. paratyphi-A* n (%)	*S. paratyphi-B* n (%)	p-value
Age (years):				
< 18	111 (84.73%)	47 (66.2%)	10 (66.67%)	0.006
> 18	20 (15.27%)	24 (33.8%)	05 (33.33%)	
Gender:				
Male	76 (58.02%)	41 (57.75%)	09 (60%)	0.987
Female	55 (41.98%)	30 (42.25%)	06 (40%)	

Table II: Antibiotic sensitivity pattern of *S. typhi* (n=131), *S. paratyphi-A* (n=71) and *S. paratyphi-B* (n=15) isolates

Antibiotics	*S. typhi*	*S. paratyphi-A*	*S. paratyphi-B*
Amoxicillin	01 (0.76%)	01 (1.41%)	01 (8.33%)
Aztreonam	46 (90.68%)	45 (100%)	Not tested
Cefixime	123 (96.45%)	66 (97.4%)	13 (93.75%)
Cefotaxime	129 (98.74%)	65 (100%)	12 (100%)
Ceftriaxone	127 (98.7%)	66 (98.68%)	15 (100%)
Chloramphenicol	37 (28.67%)	27 (38.02%)	07 (47.22%)
Ciprofloxacin	109 (97.39%)	58 (92.84%)	15 (100%)
Co-trimoxazole	70 (53.43%)	56 (86.15%)	10 (72.22%)
Enoxacin	43 (100%)	31 (87.91%)	07 (100%)
Fosfomycin	123 (94.23%)	59 (84.65%)	13 (86.11%)
Ofloxacin	126 (98.7%)	63 (88.34%)	14 (100%)

The resistance to Fluoroquinolones was approximately 4.27%, as seen individually with Enoxacin (5.04%, n=4), Ciprofloxacin (3.53%, n=8) and particularly Ofloxacin (4.26%, n=10).

Enoxacin was noted to be 100% effective on *S. typhi* and *S. paratyphi-B* isolates, with some indifference offered by *S. paratyphi-A* (12.09%, n=4). Ciprofloxacin and Ceftriaxone were almost similar in activity on all three *Salmonellae*.

Multidrug resistance (MDR) by the *Salmonellae* species was exerted by 136 of the 217 isolates (62.64%). Of the strains encountered, *S. typhi* (72.37%), *S. paratyphi-A* (58.14%) and *S. paratyphi-B* (57.41%) strains were MDR's. Also, Co-trimoxazole was comparatively more effective than Chloramphenicol and Amoxicillin respectively as shown in Table II.

DISCUSSION

Approximately 60.36% (n=131) of culture-proven febrile episodes of enteric fever in this study was caused by *S. typhi*, an observation which closely correlated with the isolation rate (61.62%) reported in Shimla, India during

2000 - 2006.[12] Additionally, the association rate of *S. paratyphi-A* was increased as compared to *S. typhi* in several studies conducted in Pakistan.[1,6,8] However, comparing the proportion of *S. typhi* among *Salmonellae* isolates in positive cultures, the frequency of *S. paratyphi-A* was appreciable (32.71%, n=71) but lesser than the former findings. Possibly, the replacement of bivalent TA vaccine with monovalent vaccines effective only against *S. typhi* may have contributed to the increase in the prevalence of *S. paratyphi-A*[5,7] which raises concerns about the impact of anti-typhoid vaccines currently in use on enteric fever rates.[3] Furthermore, the blood culture-based estimate of typhoid fever frequency possibly accounts for only half of the actual occurrence of cases.[13] The isolation of *Salmonellae* is compromised by the logistic challenges of enteric fever surveillance techniques and an improper usage of antibiotics preceding the provision of patient blood specimens for culture and drug-sensitivity testing.

A greater burden of disease was observed in the summer and monsoon seasons, a penchant also recorded in several other studies.[1,6,7,12,13] The logical risk factors associated with this seasonality of enteric fever episodes include the consumption of unhygienic beverages in the summer months. Also, the ground water-table level sets progressively lower, becomes more stagnant, and its quality deteriorates increasing the likelihood of people ingesting viable *Salmonellae* in drinking water.[7] The drainages and over-packed rubbish dumps in Karachi, especially following rainfall, also contribute to the higher incidence. It is hence imperative to ensure safe tap-water supply, and to identify and treat chronic carriers of the implicated bacteria.

This study also quantifies a higher incidence of enteric fever in the younger age group, an observation which concurs with views in published literature[13,14] and also demonstrates that age-specific attack rates reach a peak in children between 5-12 years.[7,13] Also, the presentation of typhoid fever may be atypical and hence, misdiagnosed in pre-school age group due to an underdeveloped reticuloendothelial system.[15] Interestingly, *S. typhi* was isolated also from a 1-day-old infant; this transmission may have occurred vertically as suggested by Prajapati *et al*.[16]

Though, there were seemingly a higher number of male patients, the male-to-female ratio (1.38:1) was not of significance, an inspection in agreement with a study done in Karachi, where the ratio was also stated to be 1.38:1.[1] Incidentally, this slight preponderance of infections in males was also observed in Shimla[12] and Nepal.[16]

In the mid 90`s, the rampant use of first-line agents for the treatment of enteric fever led to the emergence of MDR strains of *S. typhi*, which posed a perplexing factor in the routine selection of effective antibiotics for the treatment. Several strains are sensitive to Chloramphenicol (MIC 2 mg/l), Ampicillin (MIC 1-8 mg/l) and Co-trimoxazole (MIC 1-3 mg/l); however, the degree of resistances to individual drugs depends on the serotype and country of origin of *Salmonellae*.[15-17] Formerly, Chloramphenicol was considered the most effective agent for the treatment of Typhoid fever, however, by 1990, 20% of *S. typhi* strains isolated in the UK were found resistant to it and > 90% of Chloramphenicol-resistant isolates were also indifferent to Trimethoprim and Ampicillin,[18] thus reducing the efficacy of these drugs. Possibly, the practice of self-medication and insufficient therapy meted may have encouraged the increased incidence of MDR's and also chronic carriers.

In this study, multidrug resistance of the three *Salmonellae* to the first-line agents was 62.64% (n=136). Of these, the prevalence of MDR *S. typhi* alone was 72.37%, an estimate which is substantial but lesser than that determined in Nigeria (80%) during 1997-2003.[19] This decline in the numbers of MDR *S. typhi* suggests a re-susceptibility to the first-line drugs following their restricted use.[19] But increased MDR *S. paratyphi-A* is itself becoming a major health concern, its percentage escalating from 14% in 1996 to 44% in 2003 and as much as 58.14% in 2008-10.[1,8]

Improved management of enteric fever and MDR strains have been observed with Fluoroquinolone compounds and also third generation Cephalosporins[20] because of their satisfactory half-life and adequate distribution in the infected host.[21] In this study, there was an average 98.19% sensitivity of isolates to the three frequently prescribed Cephalosporins, Cefotaxime (99.17%), Ceftriaxone (98.79%) and Cefixime (96.62%). Also, *S. paratyphi-A* isolates were found to be adequately sensitive to Cefotaxime (100%) and Ceftriaxone (98.68%); similar results have also been observed in Nepal in 2008.[16]

Nonetheless, possibly due to socioeconomic constraints and indiscriminate drug use, the prevalence of quinolone resistance has gradually increased.[22,23] The first case of Fluoroquinolone treatment failure in a Typhoid fever patient in Pakistan indeed was reported in 1993,[8] and the resistance in this study currently is 4.27%.

The successful confrontation by microorganisms to the Fluoroquinolones suggested altered DNA gyrase, and the transfer of plasmid-mediated Quinolone resistance has become accepted as an important mode of transmission of resistance between bacteria.[3,24]

Fluoroquinolone antibiotics are still under strict prescription of clinicians in advanced countries[19] and further encouraged for strict prescription due to reported deleterious effects on the developing tendons which make them contraindicated in the young and in pregnant

mothers. Surprisingly, Ciprofloxacin is freely marketed in syrup form for children in Pakistan in spite of possible harmful consequences.

This study was limited to blood samples that were found to be positive for *S. typhi*, *S.paratyphi-A* and *B* within Karachi.

CONCLUSION

The frequency of *S. paratyphi-A* isolates was found to be increasing than previously reported. Also, differences in drug sensitivity patterns of the etiological agents of enteric fever are presented. Empirical ciprofloxacin prescription could reduce the prevalence of enteric fevers, despite gradual decline in Fluoroquinolone susceptibility. Regular evaluation of antibiotic sensitivity profiles and its incorporation in hospital antibiotic policy can control enteric fever incidence.

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