

Susceptibility to Fluoroquinolones among Salmonella Enterica Serovars in Blood Culture

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ABSTRACT

Background and Aim: Enteric fever is a severe bloodstream infection contributing to higher rate of morbidity and mortality around the globe caused by *S. Typhi* and Paratyphi A. Fecal-oral route is the prevalent transmission route of enteric fever that manifests with various clinical outcomes such as nausea, fever, transient rash, chills, hepatosplenomegaly, abdominal discomfort, and malaise. Assessment of susceptibility to fluoroquinolones in *Salmonella enterica* serovars in blood cultures was the prime objective of the current study.

Methodology: This cross-sectional study was carried out on 326 suspected enteric fever patients attending the outpatient department of HBS Dental and General Hospital, Islamabad for the duration from July 2021 to June 2022. Prior to study conduction, ethical approval for the study was taken and each individual signed the written informed consent. BACTEC culture bottles were used for the incubation of blood. Standard procedures were followed to identify the bacterial growth in the obtained isolates from the vials. *Salmonella enterica* serotyping of identified isolates was done. *Salmonella Typhi* and *Salmonella Paratyphi* isolation and identification were processed. All the isolates were then subjected to Kirby-Bauer disc diffusion method for antibiotic susceptibility test. Clinical Laboratory Standards Institute guidelines were used for the interpretation of results. All the data from laboratory analysis were analyzed in SPSS version 25.

Results: Of the total 326 suspected enteric fever patients, the incidence of positive *Salmonella enterica* was found in 28 (8.6%), out of which *Salmonella Typhi* and *Salmonella Paratyphi A* were 15 (54%) and 13 (46%) respectively. *Salmonella enterica* growth was not observed in patients with age >51 years. However, patients of age group 11-20 years and 21-30 years showed higher proportion of *Salmonella enterica* growth. Male patients had higher *Salmonella enterica* growth than females. All the isolates of *Salmonella enterica* exhibited resistance to ampicillin and nalidixic but sensitivity to chloramphenicol and ceftriaxone. The *Salmonella Paratyphi A* and *Salmonella Typhi* isolates presented reduced susceptibility to ofloxacin and ciprofloxacin.

Conclusion: The present study found that reduced susceptibility and higher level of resistance to fluoroquinolones are the major reasons for not considering the fluoroquinolones as a treatment option for *Salmonella* infections. Ceftriaxone is still the better drug choice in *Salmonella Typhi* and Paratyphi A.

Keywords: Fluoroquinolones, *Salmonella Typhi*, *Salmonella Paratyphi*, Enterica serovars, Blood culture

INTRODUCTION

Salmonella enterica serovars Typhi and Paratyphi A are responsible for enteric fever, a continuous health issue in developing countries especially Pakistan [1]. Enteric fever is a severe bloodstream infection contributing to higher rate of morbidity and mortality around the globe caused by *S. Typhi* and Paratyphi A. Fecal-oral route is the prevalent transmission route of enteric fever that manifests with various clinical outcomes such as nausea, fever, transient rash, chills, hepatosplenomegaly, abdominal discomfort, and malaise [2, 3]. Globally, the estimated enteric fever and mortality reported were 15 million and 136 thousand respectively in 2017 [4, 5]. *Salmonella enterica* was responsible for 77% cases of enteric fever [6]. Diagnosis is made based on organism isolation from bone marrow, body fluid, and blood; however, blood culture yielded 60% to 80% [7]. Resistance to antimicrobial agents such as ampicillin, chloramphenicol, and trimethoprim /sulfamethoxazole is referred to as Multidrug resistance. Enteric fever associated complications and mortality could be prevented by antibiotics mainstay therapy [8].

World Health Organization (WHO) recommended antibiotics such as ampicillin, chloramphenicol, fluoroquinolones, cotrimoxazole, azithromycin, ceftriaxone, and cefixime for enteric fever management. However, the *Salmonella enterica* isolates reduced susceptibility to frequently used antibiotics are still a key issue for enteric fever operative remedy prolonging the fever duration and increasing risk for further complications [9]. Ofloxacin and ciprofloxacin like Fluoroquinolones is now a backbone for managing the *Salmonella* severe infections [10]. However, *Salmonella* straining increases resistance to fluoroquinolones as reported in various studies [11, 12]. In Pakistan, the fluoroquinolone resistance is an increasing trend for *S. Typhi* and *S. Paratyphi* has been reported [13]. The fundamental risk factors

for antimicrobial resistance cause different diagnosis shortfalls due to antimicrobial agent's easy availability, fluoroquinolones, cheap substandard formulations, and lack of laboratory facilities [14].

METHODOLOGY

This cross-sectional study was carried out on 326 suspected enteric fever patients attending the outpatient department of HBS Dental and General Hospital, Islamabad for the duration from July 2021 to June 2022. Prior to study conduction, ethical approval for the study was taken and each individual signed the written informed consent. BACTEC culture bottles were used for the incubation of blood. Standard procedures were followed to identify the bacterial growth in the obtained isolates from the vials. *Salmonella enterica* serotyping of identified isolates was done. *Salmonella Typhi* and *Salmonella Paratyphi* isolation and identification were processed. All the isolates were then subjected to Kirby-Bauer disc diffusion method for antibiotic susceptibility test. Clinical Laboratory Standards Institute guidelines were used for the interpretation of results. Details of clinical history such as age and gender were recorded. Improper labeling, patients with previous antibiotic therapy of 1 week, and insufficient blood volume were excluded. For BACTEC culture, blood of 3mL to 5 mL were collected especially in culture vials. Till the growth indication, about 7 days incubation was done in BACTEC. Microbial growth indications were routinely observed in culture bottles. Red alarm in BACTEC machine is indicator for growth.

Biochemical tests and gram staining were used for identification of *Salmonella* spp based on bacterial isolated colonies taken on BA and MA analysis. For gram-negative rods, various tests such as oxidase test, methyl red test, triple sugar iron (TSI) test, biochemical tests- catalase test, urease test, triple sugar iron (TSI) test, and citrate test were performed. *Salmonella*

enterica serotyping was carried out and confirmed with different antisera by antigen antibody agglutination reactions. Modified Kirby Bauer Disc diffusion technique was used. For the antibiotic susceptibility testing on Mueller Hinton Agar (MHA) plate following CLSI for levofloxacin (Levaquin) and ciprofloxacin (Cipro). SPSS version 25 was used for data analysis. Mean and standard deviation was used to express the quantitative variables. Qualitative variables were described as frequency and percentage. Different variables were compared based on Chi-square test taking 95% confidence interval and 5% level of significance.

RESULTS

Of the total 326 suspected enteric fever patients, the incidence of positive Salmonella enterica was found in 28 (8.6%), out of which Salmonella Typhi and Salmonella Paratyphi A were 15 (54%) and 13 (46%) respectively. Salmonella enterica growth was not observed in patients with age > 51 years. However, patients of age group 11-20 years and 21-30 years showed higher proportion of Salmonella enterica growth. Male patients had higher Salmonella enterica growth than females. All the isolates of Salmonella enterica exhibited resistance to ampicillin and nalidixic but sensitivity to chloramphenicol and ceftriaxone. The Salmonella Paratyphi A and Salmonella Typhi isolates presented reduced susceptibility to ofloxacin and ciprofloxacin. Distribution or Incidence of salmonella isolates in different age groups are shown in Figure-1. Incidence of Salmonella Typhi and Salmonella Paratyphi A is illustrated in Figure-2. Figure-3 shows the gender distribution of Salmonella isolates. Nalidixic acid susceptibility pattern of S. enterica Typhi (n = 15) and S. enterica Paratyphi A (n = 13) is shown in Figure-4. Table-I represent the concentration of Minimum inhibitory of ciprofloxacin against Salmonella isolates.

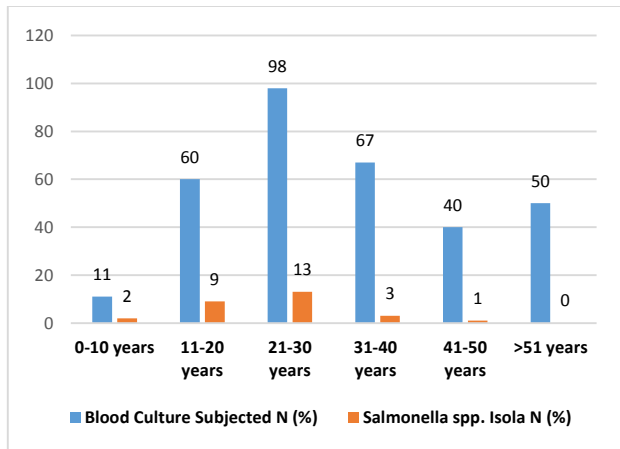


Figure-1: Salmonella isolates distribution based on age (n=326)

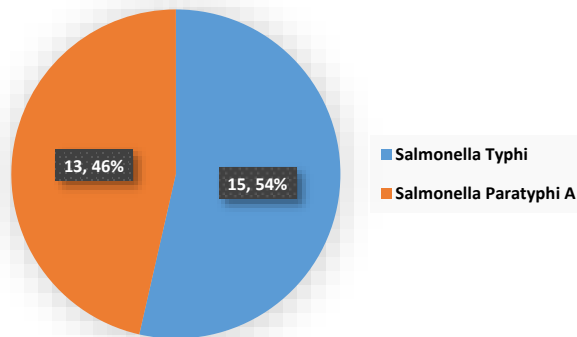


Figure-2: Occurrence of Salmonella Typhi and Salmonella Paratyphi A (n=28)

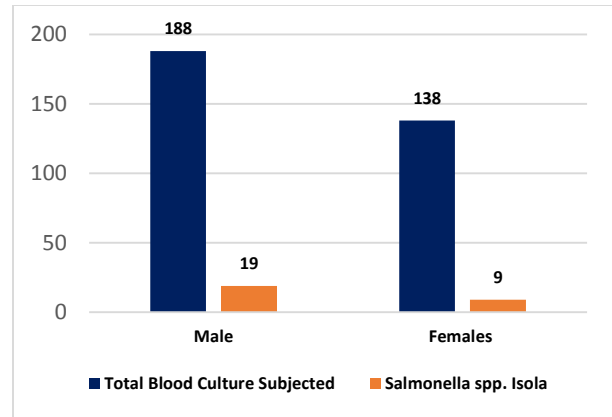


Figure-3: Gender distribution of salmonella isolates (n=326)

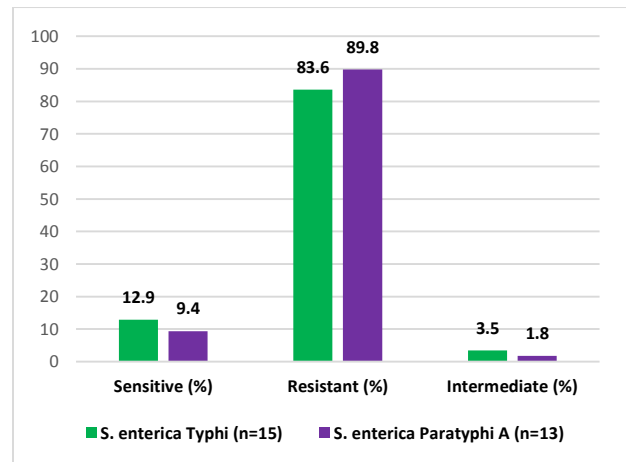


Figure-4: susceptibility pattern of Nalidixic acid to S. enterica Typhi (n = 15) and S. enterica Paratyphi A (n = 13)

Table-1: Concentration of Lowest inhibitory of ciprofloxacin

Specimen/ Organism	Sensitive ≤ 0.0625	Intermediate 0.125–0.5	Resistant ≥ 1	Total n (%)
Salmonella spp.	2 (7.1%)	9 (32.1%)	17 (60.7%)	28 (100%)

DISCUSSION

The present study found that the reduced susceptibility and higher level of resistance to fluoroquinolones are the major reasons for not considering the fluoroquinolones as a treatment option for Salmonella infections. Salmonella enterica growth was not observed in patients with age > 51 years. Growth was observed in the age group of 11-20 and 21-30 years. Salmonella was found higher in male patients than females. Salmonella spp. Isolates were present in 28 patients. In case of antibiotic susceptibility the prevalence of sensitive, intermediate and resistant isolates were 7.1%, 32.1%, and 60.7% respectively. Salmonella typhi and paratyphi was found in 46% and 54% respectively.

Among the suspected cases 8.6% showed positive blood cultures for Salmonella enterica which is analogous to the findings in earlier studies [15, 16]. In contrast, Kayastha et al [17] and Sah et al [18] reported lower rates of bacterial growth 6.9% and 5.1% respectively in their study. Another study conducted by Zellweger et al [19] reported 2.3% growth rate among children. Reduction in growth rate might be due to therapy during fever.

In the present study, male patients are more susceptible to Salmonella growth compared to females. These findings were similar to the study conducted by Joshi et al [20]. Another study also reported similar results according to which male patients had a higher enteric fever than females [21]. Majority of patients in the

age group 21-30 years had positive *Salmonella* isolates followed by 11-20 years. Manoharan et al [22] found in their study that 14% of patients had positive *Salmonella* results in the age group 5 to 18 years. Unhygienic food consumption, mobility, and street water vendors were the common causes for higher enteric fever in these age group patients.

Serotypes such as *Salmonella* Typhi (54%) and *S. Paratyphi* A (46%) were identified in the present study. Similar results were reported in a study conducted by Yamba et al [23], according to which *S. typhi* was 66.7% and *S. paratyphi* A was 33.3%. Another study by Dilip et al [24] reported *S. typhi* and *S. paratyphi* A in 54% and 46% respectively.

In our study, nalidixic acid resistant (NAR) were found 100% in all the isolates which is significantly higher than reported in previous studies [25, 26]. This nalidixic acid resistance increasing trend was reported in a study conducted by Rahman et al. [27] in which *S. Typhi* and *S. paratyphi* A were 73.3% and 90.2% respectively. Self-medication, poor hygiene, and antimicrobial drugs are other causes for nalidixic acid higher resistance in developing countries.

Of the total 28 NAR isolates, MIC test (MIC 1 µg/mL) found that 17 (60.7%) showed resistance by disc diffusion. However, the susceptibility to ciprofloxacin was reduced in MIC value 0.125-0.5 µg/mL cases. The nalidixic acid resistance was significantly associated with ciprofloxacin reduced susceptibility in *S. typhi* and *S. paratyphi*. Similar findings were reported in a study conducted by Wong et al [28], according to which fluoroquinolones reduced susceptibility were correlated to the reduced fluoroquinolones susceptibility in *Salmonella* isolates.

CONCLUSION

The present study found that there is an increasing resistance to fluoroquinolones in *Salmonella* infections and should not necessary be used as a first line antibiotic. To reduce the development of resistant strains and their spread misuse and abuse of fluoroquinolones should be avoided and prudently used, only where there is a clinical need. Ceftriaxone is still the better drug choice in *Salmonella* Typhi and *Paratyphi* A Ceftriaxone is still the better drug of choice in *Salmonella* Typhi and *Paratyphi* A.

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