**ORIGINAL ARTICLE**

# Evaluation of the Current Trends in the Antimicrobial Susceptibility Patterns of *Typhoid Salmonellae*

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## ABSTRACT

**Background:** Typhoid fever, being severe systemic illness is controlled in developed world due to enhanced preventive modalities like sanitation, while it continues to plague the South Asian countries. *Salmonella typhi* is the most widespread etiological agent of enteric fever, with a current rise in *Salmonella paratyphi A* strains as well. Due to continuing steady rise in resistance, it is essential to find out the current spectrum of these pathogens so as to rationalize the empiric therapy.

**Aims:** To find out in vitro antimicrobial susceptibility patterns of Typhoid *Salmonellae* isolated from blood culture confirmed cases of typhoid fever, to build an imminent approach to improvise empiric therapy.

**Methods:** A total of 141 Typhoid *Salmonellae* were isolated from 5115 blood cultures received from different departments of Pakistan Institute of Medical Sciences from July 2011 to October 2012. All isolates were further processed for identification using API & serological tests. Drug susceptibilities were performed as per CLSI 2012 criteria by Kirby-bauer disc diffusion and MIC's of selected isolates.

**Results:** *S.typhi* (71) & *S.paratyphi A* (70) were isolated in almost equal number. Predominantly males between 11-30 years were affected. Patients between 1-20 years had *S.typhi* as the predominant organism. Combined susceptibilities of Typhoid *Salmonellae* showed Chloramphenicol (70.9%) as the most sensitive antibiotic, followed by Amoxicillin (70.2%) and SXT (65%). More than 90% of resistance was seen with Quinolones, both in *S.typhi* and *S.paratyphi A*. Third generation Cephalosporins (Ceftriaxone) and Carbapenems revealed 100% sensitivity. Multi Drug Resistant isolates (21.2%) & Extreme Drug Resistant isolates (12.02%) were all *S.typhi*. 21% of isolates were β-lactamase producers whereas no ESBL production was seen. Azithromycin susceptibility was also done but could not be interpreted due to non-availability of interpretive guidelines.

**Conclusions:** Reversion of susceptibility pattern of first line anti-typhoid drugs noted, but still don't qualify as empirical drugs. At present third generation Cephalosporins and Carbapenems are the only empirical treatment options. Flouroquinolones is no more recommended for the treatment of typhoid fever. As no interpretive guidelines are available for Azithromycin, so it can be evaluated in future on the basis of its clinical efficacy in therapeutic trials and available guidelines.

**Keywords:** *S.typhi, S.paratyphi A*, Minimum Inhibitory Concentration.

## INTRODUCTION

Typhoid fever is a grave, potentially lethal multisystem illness caused by *Salmonella typhi*, and less often by *S. paratyphi A, S. paratyphi B and S. paratyphi C*[1]. The protean manifestation of the diseases makes it a true diagnostic challenge. It is a primeval disease and is thought to be responsible for the Great Plague of Athens around 426-430 BC[2].

Due to the advances in public health, hygiene & antibiotic treatment, it is almost eradicated in developed world, but still remains endemic in most of the developing countries[3] especially the tropical world with 80%-90% of burden residing in South East Asian countries like China, India, Pakistan and Vietnam[4].

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According to WHO, global burden of the disease is 22 million new cases/year and 217,000 to 600,000 deaths/year[6]. *S. paratyphi A*, which previously was responsible for only about 15-20% cases of typhoid fever in Asia, is increasingly becoming a pathogen in India,[7] probably due to non-availability of vaccination. Multi drug resistant (MDR) *S.typhi* has also become endemic, causing epidemics in many parts of Southeast Asia[8].

Appropriate antimicrobial therapy is known to reduce significantly in mortality from typhoid fever[9]. Chloramphenicol was introduced as an effective cure of enteric fever during 1948[10], followed by Trimethoprim-sulfamethoxazole (Co-trimoxazole) and Ampicillin in 1962[11]. Before 1970's typhoid fever strains were sensitive to all the first line drugs[12] but resistance started developing soon. By 1990, there were reports of MDR *S.typhi* strains from India & Pakistan, limiting the usefulness of conventional anti-typhoid drugs[13]. Hence Fluoroquinolones were

introduced for treatment of typhoid fever in 1990.[14] By the year 2000 high level of resistance to the second line drugs was seen in *S.typhi* strains, particularly due to indiscriminate use of antibiotics[15] rising up to 90% during the year 2005[6]. With the development of Quinolone resistance, third-generation Cephalosporins were introduced[16] but unfortunately there are already reports of high-level of resistance to Ceftriaxone in *S. typhi* and *S. paratyphi A,* leading to treatment failures[17]. In West a new class of antibiotic, Azithromycin (macrolide) is currently being used in children with typhoid fever, showing excellent therapeutic effects, but it is still under trials.[18] The emergence of such high level of resistance in anti-typhoid drugs and spread of broad-spectrum β-lactamase have greatly limited the therapeutic options, leaving expensive drugs like Carbapenems & Tigecycline as secondary antimicrobial drugs.

Pakistan being endemic for the disease, hence this study was designed to evaluate the cases of typhoid fever in a tertiary care hospital, in order to see the changes in the susceptibility profiles and resistance patterns over a period of time, so as to find better and targeted treatment options.

## Objectives

1. To isolate *Typhoid Salmonellae* from blood culture and find out the current in vitro antimicrobial susceptibility patterns of clinical isolates against the in use anti-typhoid drugs.
2. To detect phenotypically the mechanism of resistance to antimicrobials used in the treatment of enteric fever.

## MATERIALS & METHODS

It was a prospective, non-randomized, descriptive, observational study conducted from July 2011 to October 2012 in a clinical setting. A structured questionnaire was designed to get relevant data in a uniform manner. The blood culture samples from out-patients, wards and emergency department cases at PIMS, Islamabad were collected under aseptic conditions in Bactec Plus Aerobic/F Culture Vials (Bactec Dickinson, USA) and incubated in Bactec 9240 Automated Blood culture System. Duplicate samples from same patients were excluded from the study. All positive cultures were included in the study and processed for isolation of pathogens by sub-culturing on Blood agar and MacConkey agar (Oxoid, USA) and incubated aerobically at 35±2$^{o}$C for 18-24 hrs. Pathogens were identified on the basis of their colonial morphology, color imparting change in media, Gram staining, biochemical tests (API 20E) and serotyping (Remel Europe Ltd U.K) according to standard methods[19]. Antimicrobial disc testing, Minimum Inhibitory Concentration testing (MIC) and Extended Spectrum β-Lactamase (ESBL) screening were done as per CLSI 2012 recommendations.[20] Agar disc diffusion was performed by modified Kirby-Bauer method. MIC's of the selected isolates were recorded using E-strips (Liofilm). Screening of ESBL's was done by double disc diffusion method. Phenotypic detection of β-lactamase production was done by comparing the disc diffusion results of Amoxicillin and Amoxicillin-Clavulanic Acid. A Panel of 10 antibiotics was used to establish the antibiograms. Quality control was established by using *E.coli* 25922, which was within range. Recommendations for the zone sizes and MIC range for Azithromycin was taken from BSAC 2012[21], as there were no established breakpoints for Azithromycin in CLSI. Means, percentages and P value of the present research data were derived. The graphic representation and statistical analysis were determined by using SPSS version 16.

## RESULTS

According to the inclusion criteria, a total of 141 clinical isolates of *Typhoid Salmonellae* were obtained of which 71 were *Salmonella typhi* & 70 *Salmonella paratyphi A.* Male to female ratio was 1.8:1. Age ranged from 2-80 years, with a mean of 22 years. It was established that in age group of less than 20 years, 61% of isolates were *S.typhi*, contrasting with the older age group of 21-40 years where *S.paratyphi A* was the predominant organism (62.8%).

The combined susceptibility profile of *Typhoid Salmonellae* on disc diffusion showed that the first line drugs were up to 70.9% sensitive, and Chloramphenicol was the most sensitive of the three. The percentage sensitivity amongst the second line drugs i.e., Nalidixic acid and Ciprofloxacin was only 7.1%. Third generation Cephalosporins and Carbapenems were 100% sensitive. Azithromycin was sensitive in 10.6% of cases only following the criteria of BSAC 2012 (Table I). Multi drug resistance (MDR) was defined as isolates resistant to all three first line anti-typhoid drugs. (i.e. Amoxicillin, Chloramphenicol, Trimethoprim-sulfamethoxazole) and Extreme drug resistant (XDR) as isolates resistant to first line and second line anti typhoid drugs (6 fluorinated quinolone i.e., Ciprofloxacin). 21.2% MDR's & 12% XDR's were isolated and all were amongst *S.typhi*.

Minimum inhibitory concentration for all the antibiotics was performed on selected isolates (Table: IV), according to the selection criteria given in the table II. MIC results along with the interpretive

criteria are shown in table III. P values were calculated by applying Pearson t-test and it was established that the MIC results were in concordance with the disc diffusion results, with significant P value <0.00001, except for Azithromycin. (P value= 0.011). (Table: IV). All the isolates were studied for β-lactamase production. Out of 42 Amoxicillin resistant isolates, 29 were sensitive to Amocillin-Clavulanic acid combination, concluding that 20.56% of *Typhoid Salmonellae* had β-lactamase production as the mechanism of resistance to Amoxicillin. No isolate of Typhoid *Salmonellae* was ESBL producer.

| Antibiotics | Sensitive (%) | Intermediate (%) | Resistant (%) |
|---|---|---|---|
| Amoxicillin | 99 (70.2) | 11 (7.9) | 31(21.9) |
| Amoxicillin-Clavulanic acid | 128 (90.8) | 5 (3.5) | 8(5.7) |
| Trimethoprim-sulfamethoxazole | 92 (65.2) | 2 ( 1.5) | 47(33.3) |
| Chloramphenicol | 100 (70.9) | 7 (4.9) | 34(24.2) |
| Nalidixic acid | 10 (7.1) | 0 (0) | 131(92.9) |
| Ciprofloxacin | 10 (7.1) | 57 (40.4) | 74(52.4) |
| Ceftazidime | 141 (100) | 0 | 0 |
| Ceftriaxone | 141 (100) | 0 | 0 |
| Imipenem | 14 (100) | 0 | 0 |
| Azithromycin | 15 (10.6) | 0 | 126 (89.3) |

Table I: Susceptibility Profile of *Typhoid Salmonellae* on disc diffusion n= 141(*S.typhi*=71 & *S.paratyphi A*=70)

## DISCUSSION

Enteric fever has been a peril and threat to society for decades and a persistent problem of South-East Asia including Pakistan. Keeping this in view, especially the endemic nature of disease, this study was conducted to evaluate the current susceptibility profiles of *Typhoid Salmonellae* in order to establish empirical and therapeutic guidelines and to measure the magnitude of problem. Endemic nature of typhoid in Pakistan is an established fact[22] and this study further proves this finding, as the organisms were isolated throughout the year

Table II: Selection Criteria for MIC Testing

| Drugs | Selection Criteria |
|---|---|
| Amoxicillin | Isolates sensitive to Amoxicillin & resistant to Chloramphenicol and Trimethoprim-sulfamethoxazole on disc diffusion. |
| Chloramphenicol | Isolates sensitive to Chloramphenicol & resistant to Amoxicillin and Trimethoprim-sulfamethoxazole on disc diffusion. |
| Trimethoprim-sulfamethoxazole | Isolates sensitive to Trimethoprim-sulfamethoxazole & resistant to Amoxicillin and Chloramphenicol on disc diffusion. |
| Nalidixic acid | All the Nalidixic acid and Ciprofloxacin sensitive isolates on disc diffusion. |
| Ciprofloxacin | Nalidixic acid and Ciprofloxacin sensitive isolates on disc diffusion/<br>Nalidixic acid resistant and Ciprofloxacin intermediate isolates on disc diffusion. |
| Ceftriaxone | XDR isolates (Resistant all three first line drugs and to 6-fluorinated quinolones on disc diffusion). |
| Imipenem | XDR isolates (Resistant to all three first line drugs and to 6-fluorinated quinolones on disc diffusion). |
| Azithromycin | XDR isolates (Resistant to all three first line drugs and to 6-fluorinated quinolones on disc diffusion). |

Table III: Comparison of Disc Diffusion and MIC Results of Selected Isolates

| Drugs (No. of Isolates tested) | MIC range of the E- strip | Disc Diffusion results | MIC results | P-value |
|---|---|---|---|---|
| Amoxicillin (9 isolates) | 0.016-256ug/ml | 9 isolates (S) | 9 isolates (S) | <0.00001 |
| Chloramphenicol (3 isolates) | 0.016-256ug/ml | 3 isolates (S) | 3 isolates (S) | <0.00001 |
| Trimethoprim-Sulfamethoxazole (2 isolates) | 0.002-32ug/ml | 2 isolates (S) | 2 isolates (S) | <0.00001 |
| NA (8 isolates) | 0.016-256ug/ml | 8 isolates (S) | 8 isolates (S) | <0.00001 |
| Ciprofloxacin (8 isolates) | 0.016-256µg/ml | 8 isolates (S) | 8 isolates (S) | <0.00001 |
| Ciprofloxacin (9 isolates) | 0.002-32ug/ml | 9 isolates (I) | 9 isolates (I) | |
| Ceftriaxone (17 isolates) | 0.002-32ug/ml | 17 isolates (S) | 17 isolates (S) | <0.00001 |
| Imipenem (11 isolates) | 0.002-32ug/ml | 11 isolates (S) | 11 isolates (S) | <0.00001 |
| Azithromycin (7 isolates) | 0.016-256ug/ml | 5 isolates (S) | 7 isolates (S) | 0.011 |

The male to female ratio was comparable with other studies from Pakistan and rest of endemic countries.[23] It is understood that males are affected more due to increased risk of exposure related to their employment and living conditions, which affects their eating habits and life style making them more vulnerable to contract the disease.

It was found that, out of the etiological agents of enteric fever i.e. *Salmonella typhi, Salmonellae paratyphi A, B & C,* in the current study only *Salmonella typhi* and *S.paratyphi A* were isolated. There are numerous reasons of *Salmonella paratyphi A* coming back as a major etiological agent of typhoid fever. Firstly the high MIC's of this organism against Fluoroquinolones as observed by Hanan. *et* al. in 1988 and secondly the indiscriminate deployment of Quinolones, leading to the suppression of *S.typhi*, permitting the comparatively resistant *S.paratyphi* A to occupy the niche vacated by *S.typhi*. On the other hand changing host susceptibility mainly through vaccination, and enhanced virulence of the pathogen could also be responsible for emergence of this pathogen. The substitution of bivalent typhoid vaccine with monovalent vaccine effective only against *S.typhi* may have also added upon to the relative increase in the prevalence of *S.paratyphi A*[24].

When the susceptibility of study isolates against the first line anti-typhoid drugs was accessed, a changing trend was observed contrasting with the situation in past, in which there was more than 80% resistance seen against these drugs, whereas in the present study the resistance to first line drugs was decreased to significantly both in *S.typhi* and *S.paratyphi A*[25]. On evaluation, *S.paratyphi* was found to be more sensitive to the conventional anti-typhoid drugs as compared to *S.typhi* isolates. All *S.paratyphi A* isolates were 100% sensitive to Chloramphenicol, followed by Amoxicillin (88.5%) and Trimethoprim-sulfamethoxazole (81.4%) respectively.

Amongst the *S.typhi* the percentage sensitivity to Amoxicillin was (52.2%), whereas almost less than 50% of the isolates were found to be sensitive to Trimethoprim-sulfamethoxazole (49.2%) and Chloramphenicol (42.3%). Comparable findings were reported from PIMS hospital in 2008.[26] Hence it is a positive trend towards improvement amongst first line antibiotics, and the best drug is Chloramphenicol, but these percentages are not good enough to choose these drugs at present empirically, especially in adult patients.

Analyzing the susceptibilities to the second line drugs, only 7 isolates of *S.typhi* (9.8%) and 3 of *S.paratyphi A* (4.3%) were found to be fully susceptible to both Nalidixic acid and Fluoroquinolone, concluding that Fluoroquinolones can no longer be used as empirical or therapeutic regimen in typhoid fever. A comparable trend has been seen for quite some time in rest of the world as well[27].

All the isolates were found to be fully susceptible to third generation Cephalosporins and Carbapenems. Study from the same institution (PIMS) conducted in 2008 showed almost similar results [26]. A study from Agha Khan University Hospital of 2011 showed completely sensitive MIC of Ceftriaxone but 3 isolates showed higher resistant MIC value of 2µg/ml[28] depicting the impending resistance in the coming years.

None of the study isolate was found to be ESBL producer. Contrasting this, there are already reports of high-level resistance to Ceftriaxone with MIC as high as 64µg/ml, in *S. typhi* in Pakistan and across the globe due to ESBL production.[29] The isolates in the present study were also tested for β-lactamase production, in order to identify the cause responsible for Amoxicillin resistance. β-lactamase production was seen in 20.5% of Typhoid *Salmonellae*. Comparable with these findings a 5 year retrospective (2002-2006) study from Nigeria revealed that almost 31% of Amoxicillin resistant *Typhoid Salmonellae* were sensitive to Amoxicillin[30].

As there were no breakpoints for Azithromycin in CLSI 2012, so MIC of ≤16 µg/ml was taken as the cut off for *S.typhi* from BSAC 2012. It was observed that there was no positive parallel association of Azithromycin zone sizes and MIC in *S.typhi* isolates. As it was seen that two isolates of *S.typhi* resistant to Azithromycin on disc diffusion were found to be falling in sensitive range of MIC according to the criteria applied i.e. as recommended in BSAC 2012. *Salmonella paratyphi A* zone sizes and MIC were done but it was not possible to interpret them, as no available guidelines at present. They can be evaluated in future on the basis of their clinical efficacy in therapeutic trials.

## CONCLUSIONS

The study is clear evidence to the fact that the percentage of resistance among *Typhoid Salmonellae* and the mechanism behind them, diverge among different population groups both in Pakistan and at international level. A lot of work has been done on this subject previously, but as typhoid is a disease endemic in this region, so this subject needs continuous work and surveillance in order to look in to the varying pathogenicity amongst the typhoid bacilli, so as to formulate new and up dated guidelines for management.

| Drugs No. of Isolates (MIC range) | Isolates | MIC range µg/ml | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.008 | 0.012 | 0.016 | 0.023 | 0.032 | 0.047 | 0.064 | 0.094 | 0.125 | 0.19 | 0.25 | 0.5 | 0.75 | 1 | 1.5 | 2 | 3 | 4 | 6 | 8 | 16 |
| Amoxicillin 9 (0.016-256ug/ml) | *S.typhi=03 (S)* | | | | | | | | | 2 | 1 | | | | | | | | | | | |
| | *S.paratyphi A = 06 (S)* | | | | | | | | | | | | 1 | 2 | 3 | | | | | | | |
| Chloramphenicol 3 ( 0.016-256ug/ml) | *S.typhi=02 (S)* | | | | | | | | | | | | | | | 1 | | | 1 | | | |
| | *S.paratyphi A= 01 (S)* | | | | | | | | | | | | | | | | | | 1 | | | |
| Trimethoprim-Sulfamethoxazole 2(0.002-32ug/ml) | *S.typhi=01 (S)* | | | | | 1 | | | | | | | | | | | | | | | | |
| | *S.paratyphi A = 01 (S)* | | 1 | | | | | | | | | | | | | | | | | | | |
| Nalidixic acid 8 (0.016-256ug/ml) | *S.typhi = 07 (S)* | | | | | | | | | | | | | | | | 3 | 3 | | 1 | | |
| | *S.paratyphi A = 01 (S)* | | | | | | | | | | | | | | | | | 1 | | | | |
| Ciprofloxacin 17(0.002-32ug/ml) | *S.typhi=14 (7 S & 7 I)* | 1 | 2 | 3 | | | 1 | / | | | | 5 | 2 | | | | | | | | | |
| | *S.paratyphiA= 03(1S&2I)* | | | | | 1 | | | | | | 1 | 1 | | | | | | | | | |
| Ceftriaxone 17(0.002-32ug/ml) | *S.typhi =11(S)* | | | | 2 | 2 | 5 | 1 | | 1 | | | | | | | | | | | | |
| | *S.paratyphi A =6 (S)* | | | | | | 4 | 2 | | | | | | | | | | | | | | |
| Imipenem 11(0.002-32ug/ml) | *S.typhi=10 (S)* | | | | | 1 | 3 | 5 | 1 | | | | | | | | | | | | | |
| | *S.paratyphi A= 1 (S)* | | | | | | | | 1 | | | | | | | | | | | | | |
| Azithromycin 10 (0.016-256ug/ml) | *S.typhi= 7 (2R & 5 S)* | | | | | | | | | | | | | | | 1 | | 2 | 1 | 2 | 1 | |

Table: IV Minimum Inhibitory Concentrations of Typhoid *Salmonallae* (Red marks are break points)

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