# Growth of Antimicrobial Susceptibility in S Typhi

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## Abstract

Salmonella typhi, the principal culprit in typhoid fever, is still a major threat to public health in third world nations. However, there is a lack of information on the prevalence of the disease in poor and middle-income nations where it is prevalent. The growth and spread of antibiotic-resistant strains pose a danger to the efficacy of antimicrobial treatment. Salmonella enterica serovars are the causative agents of typhoid and paratyphoid fever. Paratyphi and Typhi About 120 million cases of typhoid fever and 700,000 fatalities occur each year throughout the globe. In our investigation, a total of 107 patients were suspected to be suffering typhoid fever.

**Keywords:** Salmonella typhi, Multidrug resistant Salmonella typhi, antimicrobial susceptibility pattern. Blood; Stool

## 1. INTRODUCTION

Salmonella typhi, the principal culprit in typhoid fever, is still a major threat to public health in third world nations. Typhoid fever is remains common in the poor world despite significant progress in our knowledge of the disease's pathophysiology and risk factors. Population-based estimates of typhoid in underdeveloped nations vary from 150 to 1000 cases per 100,000 people, albeit these numbers are notoriously difficult to nail down with precision. More than 90% of the world's typhoid-related fatalities and illnesses in 2000 were reported in Asia, when an estimated 2.16 million cases were reported. New data from South Asia show that, contrary to common belief, typhoid fever has a disproportionately high impact on children younger than five, leading to increased rates of hospitalisation and illness. Typhoid fever has been much decreased in the industrialised world due to improvements in sanitation, the availability of clean drinking water, and the use of antibiotics, but it is still widespread in the Third World.

Salmonella enterica serovar Typhi and Paratyphi are the causative agents of enteric fever, a potentially fatal systemic infection (A, B, and C). However, there is a lack of information on the prevalence of the illness in poor and middle-income nations where it is widespread. Culture-confirmed typhoid cases in India occur at a rate of around 377 per 100,000 people, with a mortality rate of about 1%. Although antibiotics are successful in treating most of them, a shifting resistance profile in typhoidal Salmonella strains presents a challenge to illness management. Ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole were formerly the go-to drugs for treating enteric fever. As multidrug-resistant (MDR) S. Typhi emerged in the 1970s, however, fluoroquinolones replaced penicillin as the go-to therapy for typhoid fever. However, a decline in ciprofloxacin-susceptible (DCS) S. Typhi in endemic areas of South Asia and Southeast Asia has been reported often during the 2000s [8, 9]. There is a growing trend toward using the third-generation cephalosporin ceftriaxone (intravenously) and the macrolide azithromycin (orally) to treat difficult and simple typhoid fevers, respectively. A first bout of severe typhoid fever is also responsive to the combination of ceftriaxone and azithromycin.

Disease caused by Salmonella enterica serovar Typhi (S Typhi) continues to be a significant public health problem worldwide,1 accounting for 11 million new cases and over 100,000 fatalities each year. Seventy percent of the world's illness burden is concentrated in south Asia, although the regions of sub-Saharan Africa, southeast Asia, and Oceania all experience significant morbidity and death. 4 The growth and spread of antibiotic-resistant strains pose a danger to the efficacy of antimicrobial treatment. In the 1970s, multidrug-resistant strains initially appeared; by the 2000s, a single lineage linked with multidrug-resistance among the H58 haplotype had become worldwide prevalent. These strains carried genes encoding resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. In the 1990s, fluoroquinolones replaced penicillin as the standard treatment for multidrug-resistant S Typhi. In contrast, by the 2010s, most S Typhi in south Asia had

mutations in the quinolone resistance-determining areas (QRDR). A widespread epidemic of S Typhi with plasmid-mediated resistance to 3rd generation cephalosporins and fluoroquinolones and chromosomally placed genes encoding multidrug-resistance was discovered in Pakistan in 2016. This strain was classified as extremely drug-resistant (XDR). In 2021, it was reported that a single polymorphism in the AcrB efflux pump imparting resistance to azithromycin had independently developed in various lineages of S Typhi, posing a danger to the effectiveness of all oral antimicrobials for typhoid therapy.

Salmonella enterica serovars Typhi (S. typhi) and Paratyphi are responsible for typhoid and paratyphoid fever, respectively (S. Paratyphi). The typhoid virus (TF) is a serious threat to public health worldwide and a leading cause of mortality and disability, especially in poorer regions of the world. Worldwide in 2017, there were 14.3 million cases of typhoid fever and paratyphoid fever, with 136,000 fatalities as a result [1]. The greatest incidence rate of 549 occurrences per 100 000 Population [1] was recorded in South Asia, where 103 million cases of typhoid were reported in 2017. This accounted for 72% of the worldwide burden in 2017. Incidence rates of 451.7 cases of typhoid fever per 100,000 people per year [2] indicate that typhoid fever is widespread in Pakistan. In Karachi, there are as many as one thousand instances of TF diagnosed in every one hundred thousand kids each and every year. The public health crisis that might result from S. typhi's more common antimicrobial resistance pattern in the future is quite real. There have been reports of MDR Salmonella typhi and Salmonella paratyphi with decreased susceptibility to fluoroquinolone in numerous countries since 2000.

Wherever there is a lack of access to clean water and proper sanitation, typhoid disease is a severe threat to public health. The majority of sub-Saharan Africa and most of Latin America are also considered endemic zones. Salmonella enterica serovar Typhi is responsible for this illness (S. Typhi). Studies of Egyptian populations have shown yearly incidence rates of 13/100,000 in the Nile Delta's Belbis District [d 61/100,000 in the south's Fayoum Governorate. Equally tragic, restricted access to fresh water and sewage dumping into the rivers affects thousands of Iraqis annually, resulting in a 10-20% death rate]. Although infections are widespread in Jordan, the country's Ministry of Health has only conducted a few epidemiological studies, and the country's overall incidence rate is not clearly characterized. The high rate of typhoid fever infection in Pakistan (451/100,000) lends credence to the results of Kothari et al., who had previously asserted that the typhoid fever burden in Asia is greater than in Africa. However, Uzbekistan is seeing a rise in typhoid cases as a result of the deteriorating water treatment and distribution infrastructure in the former Soviet republics [7]. Most infectious diseases in Qatar are brought in by expat employees and tourists from the endemic Indian subcontinent and the Far East.

## 2. LITERATURE REVIEW

**Amit Katiyar et.al (2020)** Increased prevalence of multidrug-resistant Salmonella enterica serovar The presence of Typhi is presently a key hurdle in the fight against enteric fever. In areas where it is common, as well as for those returning from underdeveloped nations, this presents a serious health risk. Ceftriaxone has replaced fluoroquinolones as the medicine of choice, while azithromycin is now the treatment of choice for mild cases of typhoid fever. Antimicrobial resistance genes and their putative links with their phenotypes need to be thoroughly analysed to aid in the selection of future treatment regimens after an isolated case of diminished susceptibility to the most recent medication regime. 133 typhoid patient clinical isolates were subjected to whole genome sequencing (WGS). Pan-genome analysis and gene prediction for antibiotic resistance were performed using the sequence output files. In all cases, the WGS analysis found mutations in the gyrA, gyrB, parC, and parE genes that conferred resistance to fluoroquinolones. CatA1 genes for chloramphenicol resistance, dfrA7 and dfrA15 for trimethoprim-sulfamethoxazole resistance, sul1 and sul2 for sulfamethoxazole resistance, and blaTEM-116 and blaTEM-1B for amoxicillin resistance were all recognised as pathways for acquired resistance. Ceftriaxone and cefixime resistance determinants were not identified. Moreover, chloramphenicol, ampicillin, co-trimoxazole, ciprofloxacin, and ceftriaxone characteristics were linked with genotypes. Genotypes and phenotypes of S. Typhi isolates were shown to be highly correlated. According to the results of the genome-wide study, the metabolic activities of core genes were significantly enriched, whereas the accessory genes were significantly associated with disease and antibiotic resistance. According to Heap's law analysis, the S. Typhi pan-genome is complete (Bpan = 0.09). With a Simpson's diversity score of just 0.51, genetic diversity among S. Typhi isolates was found to be minimal. Over all, the results of this study add to the existing knowledge that WGS can aid in the prediction of resistance genotypes and their eventual correlation with phenotypes, opening the door to the possibility of identifying AMR determinants for rapid diagnosis and prioritising antibiotic use directly from sequence.

**Rokshana Akhtar (2021)** Salmonella Typhi and Salmonella Paratyphi A are the leading causes of typhoid fever in the poor countries. Emergence of multidrug-resistant Salmonella (MDR) is a major health threat.

Salmonella typhi and Salmonella paratyphi A blood culture isolates were tested from September 2018 to August 2019 to determine their antibiotic susceptibility patterns at Bangabandhu Sheikh Mujib Medical University. Blood culture samples were analysed to see whether they contained the bacteria suspected to be Salmonella spp. The agar dilution technique was used to calculate the ciprofloxacin MIC, and the Kirby Bauer disc diffusion method was used to test for antimicrobial susceptibility in accordance with CLSI's 2018 guidelines. Sixty-two (38.5%) of the 160 isolates were S. Paratyphi A and 98 (61.3%) were S. Typhi. Eighty-three percent were sensitive to ampicillin, eighty-seven percent to chloramphenicol, and eighty-nine percent to cotrimoxazole. By minimum inhibitory concentration (MIC) testing, 83.8% of the Salmonella isolates were shown to be intermediately sensitive to ciprofloxacin. The results of a pefloxacin disc diffusion test showed that 95.6% of the isolates tested were resistant. Ceftriaxone, cefixime, and cefepime were all effective against every single isolate, while a total of 31.9 percent of the samples tested positive for azithromycin. The sensitivity of the isolates to ampicillin, cotrimoxazole, and chloramphenicol was rather high. This suggests that these antibiotics may be of benefit in the treatment of typhoid fever once again.

**Anu Maharjan et.al (2021)** Introduction: Systemic Salmonella enterica Typhi and S. enterica Paratyphi infections, known as enteric fever, are widespread in low-income areas like Nepal. Mechanisms for the emergence of resistance to nalidixic acid and decreased susceptibility include, but are not limited to, mutations in chromosomal genes encoding DNA gyrase and Topoisomerase IV and via plasmid mediated quinolone resistant (PMQR) genes. ciprofloxacin resistant Although enteric fever is quite common in Nepal, there is a lack of research on antibiotic resistance and the pathogen's frequency of occurrence. Therefore, the purpose of this research was to evaluate the antibiotic Identify the susceptibility profile ofSalmonella strains and calculate the minimal inhibitory concentration of ciprofloxacin. We collected 1298 blood samples from individuals who were suspected of having patient with enteric fever who was treated at Sukraraj Tropical and Infectious Disease Hospital (STIDH) between March and It's August of this year (2019). Immediately after collection, blood samples were injected into BACTEC culture bottles. Salmonella Typhi and S. Paratyphi were isolated and identified during processing. Ancient Axenian civilisations antimicrobial susceptibility testing (AST) was performed on the isolates using the revised the CLSI-recommended Kirby-Bauer disc diffusion technique. Minimum inhibitory concentration the minimum inhibitory concentration (MIC) of ciprofloxacin was evaluated using the agar-dilution technique. Conclusions: Based on the data from Only 40 out of 1298 (3%) blood cultures were positive for Salmonella spp. 11 (27.5%) of the isolates were S. Paratyphi A and 12 (75%) were S. Typhi. In AST, 12.5% (5/40), 15% (6/40), and 26 (75%) of the isolates were S. Typhi Nalidixic acid, ofloxacin, and levofloxacin were effective against 20% (8/40) of the Salmonella isolates. but ciprofloxacin was ineffective against all of the isolates. Calculating the MIC for ciprofloxacin concentrations were found to be between 0.06 and 16 g/mL, with 5% (2/40) and 52.5% (21/40) of Both ciprofloxacin-susceptible and -resistant strains were found among the isolates. This investigation found no evidence of MDR among the isolates. Overall, the results of this investigation demonstrated that quinolone-resistant Salmonella spp. were quite common, whereas susceptibilities to first-line medicines had re-emerged in a significant way. In light of this, traditional first-line drugs and third-generation cephalosporins may be useful. use as a stand-in for scientifically proven treatments of enteric fever. Despite the fact that there were no MDR strains in our sample, thorough monitoring, improved diagnostics, and a revised treatment strategy with the help of AST report are suggested for combating the country's growing medication resistance.

**Rabiu Sahal Muhammed (2018)** Clinical specimens with multidrug-resistant Salmonella typhi were studied for their characterization. Patients at a few different hospitals in Bauchi's metropolitan area provided a total of 364 clinical specimens, including faeces and blood. Isolates were determined after being cultivated from the samples using standard medium, and then subjected to biochemical and serological testing for somatic H and O antigens. In order to test for antibiotic resistance, the isolates were subjected to the conventional disc diffusion procedure. Of the 364 samples taken, 9 (2.4% of the total) tested positive for Salmonella typhi; of them, 55.5 percent were from females and 44.4 percent were from males. Children accounted for more cases of typhoid fever (55.5%) than adults. Isolates from various hospitals have similar p values (>0.05), suggesting no discernible variation among them. Each and every isolate tested positive for resistance to at least three (3) different antibiotics. Of the total isolates, 88.8% were resistant to ampicillin, 77.7% to cotrimoxazole, and 88.8% to chloramphenicol. Nonetheless, no evidence of ciprofloxacin resistance was observed among the isolates. Clinicians should exercise caution when prescribing ciprofloxacin for severe cases of typhoid fever, and fluoroquinolone antibiotic therapy should be reserved for patients with laboratory-confirmed cases of the disease and Salmonella-associated bacteremia.

**Felix Mills-Robertson et.al (2018)** Fifty-eight strains of Salmonella typhi were obtained from patients at Ghana's Korle-Bu Teaching Hospital and the Noguchi Memorial Institute for Medical Research who were suspected of having typhoid fever. The disc diffusion assay was used to test the antibiotic susceptibility of each

isolate against ampicillin, chloramphenicol, streptomycin, tetracycline, and trimethoprim/sulfamethoxazole. Ten of the isolates were resistant to the 'first line' antibiotics used to treat typhoid fever, including ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole, although only five were resistant to all five. There were 34 isolates that showed resistance to at least one antibiotic, and 62% of those isolates had Inc HI conjugable plasmids. Ninety percent of the conjugable plasmids caused strains to develop resistance to multiple drugs. In addition, 14 of the strains had transformable plasmids, and 6 of those plasmids encoded for resistance to several drugs. Our results suggest that S. typhi multidrug resistance to 'first line' antibiotics is more widespread in Africa than was previously believed.

## 3. RESEARCH AND METHODOLOGY

From August 2006 to September 2007, 84 S. Typhi isolates were collected from blood cultures of patients with probable typhoid fever who sought treatment at local outpatient clinics or were hospitalised to private and public hospitals in the area. Eleven more environmental isolates, found in things like sewage, water, and food, were collected. S. Typhi was tested for in 1,200 blood samples and 50 environmental samples. Blood samples were enriched using bile salt broth (broth culture) [19] and streptokinase broth (clot culture) [20], whereas environmental samples were enriched using Selenite F-broth. Mac-Conkey, XLD, and Wilson Blair media were streaked with the enriched samples when the turbidity became evident. Conventional biochemical testing with API20E identified the isolates that produced typical colonies, and agglutination with Salmonella O9, Vi specific, and Hd antisera purchased from the King Institute of Preventive Medicine in Guindy, Chennai, verified the identification.

The Evaluation of Susceptibility to Antimicrobials Ampicillin (10 g/disk), chloramphenicol (30 g/disk), cotrimoxazole (1.25-23.75 g/disk), ciprofloxacin (5 g/disk), ceftriaxone (5 g/disk), nalidixic acid (30 g/disk), and imipenem (10 g/disk) were used in the Kirby-Bauer disc diffusion technique for antibiotic As a negative control, Escherichia coli ATCC 25922 was employed, while S. Typhi MTCC 734 was used as a positive control to see how well the antibiotic discs worked. Disks with a diameter of six millimetres were utilised (produced by Himedia Laboratories in Mumbai). For the agar dilution test [22], we used pure antibiotic powders to estimate the minimum inhibitory concentrations (MICs) of chloramphenicol, ampicillin, and nalidixic acid resistant isolates (Himedia Laboratories, Mumbai). A total of 20 nalidixic acid-resistant isolates were chosen at random for ciprofloxacin MIC testing. Multidrug-resistant isolates are those that are resistant to at least three different antibiotics.

## 4. DATA ANALYSIS

There were a total of 95 S. Typhi isolates found in the 1,250 samples tested, for an incidence rate of 7.6%; the highest rate was found in environmental samples (11 total), at 22%, followed by blood samples (84 total), at 7%.

The antibiogram showed that all of the S. Typhi strains were susceptible to imipenem. Nalidixic acid resistance was highest (31.57 percent), followed by ampicillin (29.47 percent) and chloramphenicol (28.42 percent). There was some resistance to the remaining antibiotics among the S. Typhi isolates, although it was generally at a low level (Table1)

Ten percent (N = 9) of the isolates showed resistance to three or more drugs. Forty percent (N = 38) of the isolates showed sensitivity to at least one of the antibiotics used in the study. Ampicillin, chloramphenicol, and co-trimoxazole were the three antibiotics most often not effective against MDR isolates. Of the 28 S. Typhi isolates that were resistant to ampicillin, eight had a minimal inhibitory concentration (MIC) of 64 g/ml, sixteen had a MIC of 128 g/ml, and the remaining four had a MIC of 256 g/ml. Many S. Typhi strains have developed resistance to the antibiotic 27-chloramphenicol.

Table 2. MIC value of resistant S.Typhi to various antibiotics.

| Antibiotic | No | Range (µg/mL) |
|---|---|---|
| Nalidixic acid<br>(N = 30) | 15<br>13<br>2 | 128<br>64<br>32 |
| Ciprofloxacin<br>(N = 20) | 18<br>1 | 0.125<br>1 |

| | | |
|---|---|---|
| | 1 | 4 |
| Ampicillin<br>(N = 28) | 4<br>16<br>8 | 256<br>128<br>64 |
| Chloramphenicol<br>(N = 27) | 3<br>24 | 256<br>32 |

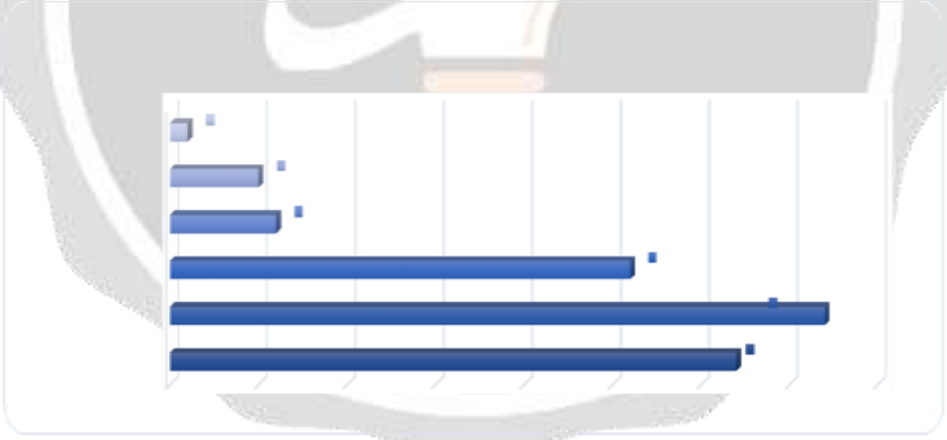
**20 Nalidixic acid resistant isolates were tested for ciprofloxacin MIC**

isolates, 24 have a minimum inhibitory concentration (MIC) of 32 g/ml, while the other 3 have a MIC of 256 g/ml.

Twenty nalidixic acid resistant isolates were selected to test for ciprofloxacin MIC. Eighteen of the isolates had a MIC of 0.125 g/ml, while the other two had MICs of 1 g/ml and 4 g/ml (Table 1).

This research used a total of 968 S. Typhi isolates from various countries all over the world, including Egypt (n = 654), Uzbekistan (n = 123), Pakistan (n = 65), Iraq (n = 59), Qatar (n = 43), and Jordan (n = 24). In comparison to the other countries in this investigation (13-17%, p 0.01; Table 1), the prevalence of MDR S. Typhi isolates was substantially greater in Iraq (49/59, 83%) and Pakistan (34/65, 52%). Most of the Egyptian isolates were from the governorates of Fayoum (41%), Cairo (25%), Aswan (10%), and Alexandria (7%). When compared to the other governorates, where the prevalence of MDR was between 0% and 7% (Table 1), the prevalence of MDR among Fayoum isolates was much higher at 29%

In our investigation, a total of 107 patients were suspected to be suffering typhoid fever, of male and female gender between the age group of 5 months to 60 years old were recruited between the study period from September 2019 to December 2019 (Figure 1a). In our analysis sixty-seven patients (63%) were male and forty patients (37%) were female (Figure 1b). Male having predominance over females as male patients were more affected (63%) than female



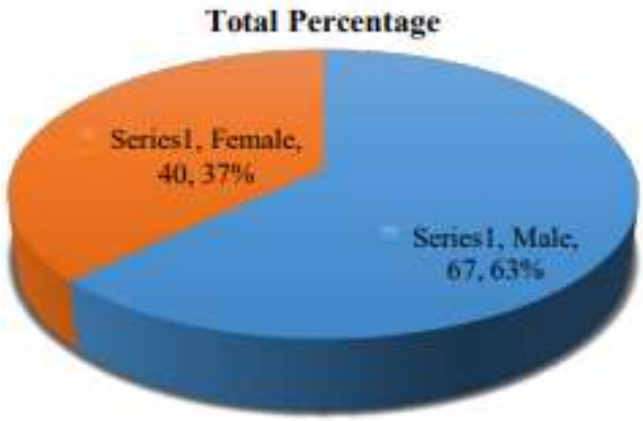


**Figure 1. a: Distribution of age groups among respondents. b: Distribution of gender groups among respondents**

patients (37%). Moreover, patients' ages ranged from 11-20 (Figure 1a)years showing the highest degree of nsusceptibility pattern (37n) for the typhoid fever followed by 21-30 years (26n), 5m-10y (32n). The patients' age sort/classify from 1 year to 30 years was more affected in terms of male and female patients whereas the lowest incidence rate of typhoid fever was between 41 to 60 years.

In the present study 38 isolates were found to be resistant to at least 2 antibiotics, 18 isolates were found to be resistant to 3 antibiotics, 11 isolates were resistant to 4 antibiotics and 7 isolates were resistant to 5 or more antibiotics (Table 3).

**Table 3. Prevalence of multidrug-resistant isolates**

| No. of ineffective antibiotics | No. of *S. typhi* isolates (n=74) |
|---|---|
| **Two (2)** | 38 |
| **Three (3)** | 18 |
| **Four (4)** | 11 |
| **Five (5) and above** | 7 |

## 5. CONCLUSION

It's safe to say Salmonella typhi is still the leading cause of diarrheal illness here. We demonstrate that ciprofloxacin resistance has emerged among typhoidal Salmonella isolates from India, despite the fact that these strains have recovered sensitivity to first-line antibiotics. Salmonella enterica serovar Typhi (S Typhi) infection, which causes typhoid fever, is still a serious global health problem. Many S. typhi isolates showed XDRST, showing solely Azithromycin and Imipenem sensitivity. Most infectious diseases in Qatar are brought in by expat employees and tourists from the endemic Indian subcontinent and the Far East. The findings of the present study indicated that Salmonella typhi in clinical blood specimens is seriously turning out to be a matter of concern due to their multi-drug resistance (MDR) pattern.

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