



MULTIDRUG-RESISTANT DECREASED SENSITIVITY TO THE ANTIBIOTIC CIPROFLOXACIN IN SALMONELLA TYPHI ISOLATE_s

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ABSTRACT

Salmonella enterica serovar Typhi causes a potentially fatal systemic infection known as enteric fever. One hundred S. Typhi isolates were tested against various antibiotics using a quality-controlled disk diffusion method in a cross-sectional investigation. Multidrug-resistant infections were defined as those caused by isolates that were immune to several antibiotic classes (MDR). The MIC E-test was performed on 30 MRSA isolates that had previously been chosen at random.

KEYWORDS: S. Typhi, chloramphenicol, chloramphenicol, isolates.

INTRODUCTION

Infectious diarrhea, often known as Typhoid fever. It's caused by Salmonella species and is found in foods all around the globe. Typhoid fever is a major public health issue that has been recognized as such by the World Health Organization. Intestinal and extraintestinal infection may occur after intake. Bimodal incubation periods with maxima at about 8-18 days indicate the equilibrium between the infectious dosage and the host immunological defense systems once Salmonella typhi (S. typhi) has invaded the gastrointestinal tract and been taken up by the reticuloendothelial system (RES).

Since 1989, S. a. l m o n e l l a typhi with resistance to many antimicrobials has become a major health problem in India and its neighbors. Initially proposed in 1990 by Rave and coworkers, ciprofloxacin has now become a popular alternative treatment. S typhi's reduced sensitivity to ciprofloxacin in the United Kingdom grew from 2.7 percent in 1995 to 5.0 percent in 1996–1997, and then to 21.0 percent in 1998; most of the patients from whom the isolates were generated had returned from India and Pakistan. the spread of S typhi strains resistant to nalidixic acid and with reduced sensitivity to ciprofloxacin, currently prevalent in India and Pakistan and maybe elsewhere in the Indian subcontinent.

We report on our findings since there seems to be a dearth of official information on the subject. Since late 1989, cases of S. typhi that are resistant to several antibiotics, including ampicillin, chloramphenicol, and co-trimoxazole, have been documented in our region. To most Indian doctors, ciprofloxacin's effectiveness against typhoid has diminished with time. The longer defervescence time observed⁴ after ciprofloxacin treatment for typhoid seems to be the basis for this thinking. Although it has been reported that ciprofloxacin's MIC values for S. typhi rise, we discovered a substantially greater rate of decreasing susceptibility for S. typhi.

Other *Salmonella* spp. with MICs of 2 mg/mL or above showed a more dramatic reduction in susceptibility to ciprofloxacin (14 of 25 isolates). In these situations, a different pharmacological treatment strategy is required. Our data on enteric fever isolates support doing MIC testing, but doing so in India would be impractical. Because of the decline in resistance to commonly used antibiotics including chloramphenicol, ampicillin, and co-trimoxazole, they may once again be used for certain conditions. It's also possible to utilize cephalosporins.

Controlling morbidity and preventing deaths from typhoid fever requires effective antibiotic treatment. Since the formation of the MDRTF, medical professionals have been searching for alternatives to Cm, Am, and SMX-TMR. Since *S. typhi*'s susceptibility to several antimicrobials was widely studied in labs. These medications were used by pediatricians. However, medicines such as furazolidone, gentamicin, and cephalexin, either alone or in combination, failed to provide consistently positive outcomes. Rifampicin, at serum quantities that are easily obtainable, has been proven to be effective in vitro against *S. typhi* strains. Since rifampicin resistance only requires a single point mutation to emerge, it is very improbable that it will be effective as a stand-alone medication in the treatment of MDRTF. Some employees have tried aztreonam. Unfortunately, the newer medicine has not consistently outperformed the older one either. Therefore, the choices are now limited to fluoroquinolones and third-generation cephalosporins.

In recent years, researchers have conducted a number of surveillance studies in endemic areas of India to assess the prevalence of enteric fever and the progression of antimicrobial resistance (AMR). While these studies have documented cases of enteric fever in tertiary care facilities, they have yet to examine the condition outside of major medical hubs. Data on the prevalence of enteric fever and its antibiotic resistance profile throughout India is needed to understand the causes, consequences, and treatment choices for typhoid fever in the country.

LITERATURE AND REVIEW

AnandManoharan et al (2022) One of the leading causes of community-acquired bloodstream infection in children in India is enteric fever brought on by *Salmonella enterica* serovar typhi and *Salmonella enterica* serovar Para typhi A. The emergence of multidrug resistance is worrying since it limits treatment options for infectious diseases like enteric fever. This work used in-silico homology modeling and molecular docking to gain insight into the widespread quinolone resistance that has been seen over the last decade. From 2011 to 2020, the study hospital had a total of 1010 cases of enteric fever confirmed by blood culture (*S. typhi* n=849; *S. Para typhi A* n=161). For *Salmonella typhi*, multidrug resistance was 2.12%, but for *Salmonella Para typhi A*, it was 0%. Over 95% of the time period under investigation, resistance to fluoroquinolones was very strong. The percentage of *S. typhi* patients that were resistant to ampicillin, chloramphenicol, and co-trimoxazol was very low (3%). Nobody who participated in the research died. Molecular docking research revealed that, for both *S. typhi* and *S. Para typhi A*, the binding affinity of quinolone to gyrase A mutants was lower than that of the wild type. Enteric fever patients had a significant level of quinolone resistance, but no such resistance was shown in relation to third-generation cephalosporins. The steady rise in ciprofloxacin resistance seen over the research period was suggested by in vitro analyses to be the result of a mutation in the gyrase A gene.

Kashmira A. Date et al (2016) Concerning shifts in worldwide patterns of antibiotic resistance have been linked to the rise of enteric fever in the United States, which has been linked mostly to international travel. In this, the first ever complete release of NTPFS data, we cover the period between 2010 and 2015, when typhoid and paratyphoid fevers were most prevalent in the United States (2008–2012). We analyzed information on laboratory-confirmed cases reported to NTPFS, as well as related antimicrobial susceptibility results of *Salmonella Typhi* and *Para typhi A* isolates sent for testing by participating public health laboratories to the Centers for Disease Control and Prevention's National Antimicrobial Resistance Monitoring System laboratory. About 2,341 instances of enteric fever were recorded between 2008 and 2012, with typhoid accounting for 80% and paratyphoid A for 20%. Paratyphoid A now accounts for 22% of all cases, up from 16% in 2008. (2012). Of the 1961 patients surveyed, 86% of those with typhoid and 92% of those with paratyphoid A reported having traveled internationally during the 30 days before to their sickness. A large percentage of people (82% for typhoid, 97% for paratyphoid A) traveled to southern Asia. Resistance to nalidixic acid (NAL-R) rose from 2008 to 2012 (Typhi, 60% to 68%; Para typhi A, 91% to 94%) among 1091 (58%) typhoid and 262 (56%) paratyphoid A isolates evaluated for antimicrobial resistance. In regards to ciprofloxacin, almost all NAL-R isolates were resistant or exhibited reduced

sensitivity. Typhi isolates, most of which were obtained in southern Asia (13%), were the only ones shown to be resistant to at least ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (multidrug resistant [MDR]). The majority of the MDR-I was NAL-R. Treatment difficulties for enteric fever are compounded by the fact that Americans are becoming resistant to the antibiotics used to combat the disease, which is typically contracted by travelers returning from southern Asia. A vaccine that protects against both typhoid and paratyphoid is urgently required.

Krishma Laxmi Shrestha, et al (2016) In India, enteric fever is a major health concern. The rise of multidrug-resistant strains of *Salmonella* spp. has resulted in a shift away from using ampicillin, chloramphenicol, and cotrimoxazole as empiric therapy for the treatment of enteric fever during the last two decades. Instead, fluoroquinolones have become the medications of choice. The purpose of this investigation was to examine the antibiotic resistance profiles of *Salmonella* spp. Standard microbiological procedures were used to grow a total of 620 blood samples from individuals suspected of having enteric fever. *Salmonella* spp. antibiotic susceptibility testing was conducted using the Kirby Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI). Ciprofloxacin, ofloxacin, and nalidixic acid's minimal inhibitory concentrations were evaluated using the agar dilution technique. *Salmonella typhi* made up 48 of the 83 isolates (57.83%) while *Salmonella Para typhi A* accounted for 35 (42.26%) of the total. Ninety-eight percent of 83 *Salmonella* spp. isolates tested positive for chloramphenicol and co-trimoxazole susceptibility, while almost 96% tested positive for ampicillin susceptibility. Similar numbers of nalidixic acid-resistant isolates (69) were found. For whatever reason, only 16.9% of the isolates tested positive for ciprofloxacin susceptibility. Multidrug resistance was found in a single *S. Typhi* isolate. In this study, researchers found that *S. Typhi* and *S. Para typhi A* were less susceptible to fluoroquinolones than previously thought, making these drugs unsuitable for use as an empirical treatment for enteric fever. Additionally, the isolates' increased resistance to first-line medicines including ampicillin, chloramphenicol, and cotrimoxazole implies that these treatments may be used for empirical therapy.

Inusa Titus et al (2018) Researchers looked at the possibility of characterizing multidrug-resistant *Salmonella typhi* from clinical samples. Patients from a few different hospitals in Bauchi city's metro area had their excrement and blood taken as clinical specimens. Isolates were identified by culture on standard medium, followed by biochemical and serological assays for somatic H and O antigens. Isolates were tested for antibiotic resistance by a disk diffusion technique standard susceptibility test. Among the 364 samples taken, 9 (2.4% of the total) tested positive for *Salmonella typhi*; of them, 5 (55.5% of the total) were taken from females and 4 (44.4% of the total) were taken from males. Children accounted for more cases of typhoid fever (55.5%) than adults. Isolates collected from various hospitals and clinics did not vary statistically (p values > 0.05). Of the total isolates, 88.8 percent were found to be resistant to ampicillin, 77.7 percent were found to be resistant to cotrimoxazole, and 88.8 percent were found to be resistant to chloramphenicol. However, no evidence of ciprofloxacin resistance was observed among the isolates. Therefore, ciprofloxacin remains the drug of choice for severe cases of typhoid fever, although clinicians should exercise caution in their prescription such that fluoroquinolones antibiotics therapy is used only in laboratory-proven cases of the disease and *Salmonella*-associated bacteremia to preserve its efficacy.

Shu-Kee Eng et al (2015) One of the most often identified food-borne diseases is *Salmonella*. Globally, it's responsible for 93.8 million cases of food poisoning and 155,000 annual fatalities, making it a serious issue in public health. *Salmonella enterica* subsp. *enterica* accounts for the vast majority of *Salmonella* infections in people and is one of over 2500 *Salmonella* serotypes discovered to date. Invasive serotype *Salmonella* infections are potentially fatal and need prompt medical attention and treatment. Increased death rates from *Salmonella* infections may result from the spread of multi-drug-resistant (MDR) strains of the bacteria. Patients infected with multidrug-resistant *Salmonella* serotypes tend to suffer from more severe and protracted illness than those infected with susceptible strains, supporting the findings of epidemiological research. In order to stop the spread of *Salmonella*, preventative procedures have been recommended. Food safety and water cleanliness must always be prioritized, but other measures, such as limiting the unnecessary use of antibiotics in livestock, are also crucial. This article summarizes the current understanding of *Salmonella* infection by covering its nomenclature, pathophysiology, clinical symptoms, epidemiology, and antibiotic resistance.

Saroj Khadka et al (2021) Effective antibiotics may greatly decrease the morbidity and mortality caused by typhoid fever. Treatment for typhoid fever now makes use of azithromycin, fluoroquinolones, and third-

generation cephalosporins. *Salmonella Typhi* and *Salmonella Para typhi* are common causes of enteric fever, but their fluctuating antibiotic susceptibility presents unique difficulties for treating the condition. This study set out to see how different *Salmonella Typhi* isolates responded to different antibiotic treatments. From June through September of 2018, 706 blood samples were taken from feverish patients visiting the outpatient department at Kathmandu Model Hospital. Nalidixic acid, ciprofloxacin, ofloxacin, levofloxacin, cefixime, ceftriaxone, cefotaxime, azithromycin, cotrimoxazole, chloramphenicol, and amoxicillin were among the 11 medications tested for susceptibility using the disk diffusion technique. In addition, the agar dilution technique was used to calculate the MIC for ciprofloxacin, ofloxacin, and azithromycin. PCR-RFLP was used to identify a mutation at *gyrA ser83* that is related with lower sensitivity to fluoroquinolones. Culture-positive for *Salmonella enterica* (*S. Typhi*, $n = 46$) was found in 6.94% ($n = 49$) of 706 blood samples. The results showed that almost all *S. Typhi* isolates (97.2%) responded to the first-line antibiotics ampicillin, chloramphenicol, and cotrimoxazole, while nearly all (97.3%) responded to cephalosporins, and nearly all (95.7%) responded to azithromycin. Among the fluoroquinolones tested, 97.8% of *S. Typhi* were resistant to ciprofloxacin, 91.3% were resistant to ofloxacin, and 89.1% were resistant to levofloxacin. Bacteria strain *S. Typhi* has MICs for ciprofloxacin ranging from 0.008 to 32 g/mL, ofloxacin ranging from 0.03 to 16 g/mL, and azithromycin ranging from 2 to 8 g/mL. *S. typhi gyrA ser83* mutation was found in 44 out of 46 isolates (95.60%). However, fluoroquinolones are not very effective against *Salmonella Typhi*. As the MIC value of azithromycin continues to rise among *S. Typhi*, it may no longer be effective in treating typhoid fever. Due to their in vitro efficacy, traditional first-line antibiotics may be put into clinical practice after extensive research.

RESEARCH METHODOLOGY

One hundred *S. Typhi* isolates were analyzed from cross-sectional research. The blood samples of typhoid patients at Hospital and Apollo Hospital were used to culture the isolates. The purity of the isolated strains stored away was evaluated by subculturing them on MacConkey agar. In order to preserve the integrity of the *S. Typhi* isolates prior to analysis, they were frozen at 70 °C on freezing medium.

Evaluation of Antibiotic Susceptibility Antimicrobial susceptibility testing for individual *S. Typhi* isolates was performed using a quality-controlled disk diffusion method in accordance with CLSI standards. Antibiotics like ampicillin (10 micrograms), amoxicillin-clavulanic acid (30 micrograms), cefuroxime (30 micrograms), ceftriaxone (30 micrograms), cefotaxime (30 micrograms), ciprofloxacin (5 micrograms), nalidixic acid (10 micrograms), tetracycline (30 micrograms), chloramphenicol (30 micrograms), cotrimoxazole (25 micrograms) Based on CLSI recommendations, the findings were classified as sensitive, moderate, or resistant. The standard *Escherichia coli* strain was ATCC 25922. Isolates resistant to ampicillin, chloramphenicol, and cotrimoxazole were considered multidrug-resistant strains.

Data Analysis

Of the 100 *S. Typhi* isolates analyzed, 71 came from the Hospital and 29 came from the Apollo Hospital, both of which are archived and verified strains. Twenty-three of the isolates were completely resistant to every antibiotic tested. The majority of the investigated isolates showed a high sensitivity to ceftriaxone (94%), gentamicin (97%), cefotaxime (83%), and amoxicillin-clavulanic acid (81%). Resistant rates were particularly high for first-line antibiotics such as ampicillin (72%), chloramphenicol (72%), and cotrimoxazole (70%). While only 6% of the isolates were completely resistant to ciprofloxacin, 69% indicated intermediate susceptibility. Table 1 summarizes the results of antibiotic susceptibility tests conducted on the 100 isolates.

Multi- drug resistant *S. Typhi*

About two-thirds (68%) of the isolates showed multidrug resistance. Those resistant to four medicines (98.6%) had the greatest MDR phenotype. Table 2 shows that the number of multidrug-resistant (MDR) isolates has decreased in both institutions.

Table 1 Antibiotic susceptibility patterns among the 100 S. Typhi isolates

Drug	Susceptible Number (%)	Intermediate Number (%)	Resistant Number (%)
Amoxicillin-clavulanic acid	57 (81)	4 (6)	9 (13)
Ampicillin	17 (24)	3(4)	50 (72)
Cefotaxime	58 (83)	8 (11)	4 (6)
Ceftriaxone	67 (94)	1(2)	2 (4)
Chloramphenicol	19 (27)	0 (1)	50 (72)
Cefuroxime	48 (68)	18 (27)	4 (6)
Ciprofloxacin	17 (24)	49 (69)	4 (6)
Gentamicin	69 (97)	0 (0)	2 (3)
Nalidixic acid	50 (71)	16 (22)	5 (7)
Tetracycline	18 (26)	0(1)	51 (73)
Cotrimoxazole	19 (29)	0(1)	49 (70)

Minimum inhibitory concentration

Amoxicillin-clavulanic acid was effective against all 30 (100%) of the isolates used for the MIC test. Approximately 97% were ceftriaxone-resistant. There were five cefotaxime-resistant (17%) isolates and fourteen chloramphenicol-resistant (47%) isolates. Out of the nine ciprofloxacin-resistant isolates tested by disc diffusion, only four (13%), with a minimum inhibitory concentration (MIC) 1 g/mL, showed full resistance to ciprofloxacin. Seventy percent of the isolates showed intermediate susceptibility, with MIC values ranging from 0.12-0.5 g/mL. Ten of the isolates tested were resistant to nalidixic acid by disc diffusion, and another six isolates showed resistance at a higher concentration (MIC 32 g/mL), while the other 24 isolates were entirely responsive at a lower concentration (MIC 16 g/mL). (Table 3).

Table 2 Distribution of MDR S. Typhi

Parameter	AIMS Hospital N = 71 (%)	Apollo Hospital N = 29 (%)	Total no. of MDR isolates
No. of MDR S. Typhi	46 (65%)	23 (79%)	69 (68%)
Year of isolation			
2004 (n = 15)	8 (53%)	4 (29%)	12 (81%)
2005 (n = 47)	25 (53%)	4 (9%)	29 (62%)
2006 (n = 37)	19 (51%)	2 (5%)	21 (56%)
2007 (n = 1)	0 (0%)	0 (0%)	0 (0%)
No. of resistant antibiotics			
3 (n = 8)	3 (37.5%)	0 (0%)	3 (37.5%)
4 (n = 69)	58 (84%)	10 (14.5%)	68 (98.6%)
5 (n = 22)	10 (45.5%)	10 (45.5%)	20 (91%)
6 (n = 4)	3 (75%)	1 (25%)	4 (100%)
7 (n = 2)	0 (0%)	1 (50%)	1 (50%)
9 (n = 1)	1 (100%)	0 (0%)	1 (100%)
11 (n = 1)	0 (0%)	1 (100%)	1 (100%)

Table 3 Minimum inhibitory concentrations to antimicrobials

Antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
Cefotaxime	23 (77)	2 (6)	5 (17)
Chloramphenicol	16 (53)	—	14 (47)
Ciprofloxacin	5 (17)	21 (70)	4 (13)
Ceftriaxone	29 (97)	1 (3)	0 (0)
Nalidixic acid	24 (80)	—	6 (20)
Amoxicillin-clavulanic acid	30 (100)	0 (0)	0 (0)

The results of this research corroborated the findings of a previous examination of *S. Typhi* susceptibility trends, which found that just 16% of the isolates were sensitive to all 11 medicines tested, while 96% were resistant to at least one of the eleven antibiotics used in the study. After being initially reported in 1997–1998, when the frequency of the MDR phenotype was estimated at 50–65%, the incidence of MDR *S. Typhi* in Kenya has steadily increased over the years. Previous research indicate that the incidence has been rising steadily since then. MDR *S. Typhi* was reported to have a prevalence of 70-82% in 2001-2002, while Mengo and colleagues in 2010 found that it was 70%. According to the results of this investigation, 68% of the isolates were multidrug-resistant (Table 2). Despite this, research conducted in a tertiary care hospital in Coastal Karnataka, India, found that 1.94 percent of *S. Typhi* isolates from blood samples were MDR, but earlier studies conducted in India found no MDR strains. The constant rise in the occurrence of MDR strains may have been fueled by the over-the-counter prescription, self-medication, and unrestricted usage of these medications. Farmers' use of antibiotics as growth promoters in animal production—including tetracyclines, sulfonamides and trimethoprim, nitrofurans, aminoglycosides, β -lactams, and quinolones—may also predispose people to resistant infections.

Seventy-two percent of the ampicillin-resistant, 70 percent cotrimoxazole-resistant, and 72 percent chloramphenicol-resistant isolates in our investigation were resistant to the most commonly used antibiotics. High rates of resistance to ampicillin (75%), cotrimoxazole (73%), and chloramphenicol (74%), were previously documented by Mengo et al. in Kenya. Since these antibiotics have not been used for a long time to treat typhoid fever, new study on *S. Typhi*'s antibiogram shows that the bacteria is displaying complete susceptibility to them. The susceptibility rates to chloramphenicol, co-trimoxazole, and ampicillin were found to be 98.8, 98.8, and 97.6%, respectively, in Indian research. These numbers are encouraging because they suggest that drug-resistant forms of typhoid fever may be on the rise again. Ciprofloxacin is one antibiotic that has been used to treat MDR patients.

Despite this, there has been a noticeable rise in isolates resistant to or recording lower sensitivity to ciprofloxacin, which is why CLSI is now reviewing breakpoints for this drug. Thirteen percent of the isolates demonstrated resistance based on ciprofloxacin MIC data in this investigation. The development of resistance to ciprofloxacin is a major health concern, particularly in underdeveloped nations where typhoid fever is endemic. The results were the same in other African nations. In Malawi, all of the isolates that were put through the nalidixic acid resistance test showed a level of resistance of at least 10%. One-third of the bacteria in the Democratic Republic of the Congo were multidrug-resistant, with 15% exhibiting nalidixic acid resistance and reduced sensitivity to ciprofloxacin. Blood culture isolates of *Salmonella typhi* were shown to be 98% resistant to the antibiotic nalidixic acid in one Indian investigation. During the research period, the isolates were shown to be more resistant to ampicillin and cotrimoxazole, according to the same study (2008–2013).

The establishment of fluoroquinolones resistance may have been hastened by the recommendation to use fluoroquinolones for empirical therapy rather than first line antibiotics. Given the minimal prevalence of resistance shown in this research, ceftriaxone may be utilized as an option in the treatment of typhoid fever. However, the CTM-X ESBL-producing gene family has been found in studies conducted in Germany, India, and Nigeria, indicating that it imparts resistance to ceftriaxone. Ceftriaxone resistance has recently been reported in a number of countries, highlighting the need for frequent screening of such isolates.

CONCLUSION

The percentage of multidrug-resistant and ciprofloxacin-resistant *S. Typhi* isolates was rather high. Future treatment of typhoid may be complicated by the emergence of resistance to fluoroquinolones. In clinical settings, regular observation of this trait is required. The findings of this comprehensive surveillance investigation corroborate the downward trend of MDR *Salmonella* isolates in India. Ciprofloxacin resistance is more common, thus other antibiotics, including azithromycin and ceftriaxone, should be used with caution when treating enteric fever.

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