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# Current Antibiotics Resistance Patterns of *Salmonella typhi* in District Peshawar, Khyber Pakhtunkhwa, Pakistan

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## Abstract

*Salmonella infection is most common in developing countries. In the current study, the prevalence and resistance pattern of Salmonella typhi from April, 2021 to March, 2022 in District Peshawar, Pakistan was identified/traced. Out of a total of 210 patients, 89 (43%) were found positive for S. typhi. Of these, 47 (52.8%) were men and 42 (47.2%) were women. The current study was conducted in four different sessions. It showed that nalidixic acid is the leading resistant drug. No significant differences were observed in antibiotics resistance with respect to age and gender (p≥0.05). To conclude, highly observed prevalence of S. typhi is a serious health concern. It was also concluded that season has no effect on the resistance pattern.*

**Keywords:** antibiotic resistance, antimicrobial susceptibility, *Salmonella typhi*, typhoid fever

## Introduction

*Salmonella* species is a member of family *Enterobacteriaceae*. It has a potentially confusing taxonomy, so its membership has been revised and updated [1]. The *Salmonella* genus consists of two species: *Salmonella enterica* and *S. Salmonella bongori* [1, 2]. *Salmonella* have more than 2500 serotypes on the basis of flagella H antigens and somatic O (lipopolysaccharide). Further, they have been classified in clinical and

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epidemiological studies by performing antimicrobial sensitivity and molecular biological techniques, such as whole genome sequencing, pulsed-field gel electrophoresis, and multi-locus sequence typing. Historically, *Salmonella* has been categorized as invasive (typhoidal) or non-invasive (non-typhoidal *Salmonella*) based on host preference and disease manifestations in human beings [3].

*Salmonella* pathogenesis includes gastroenteritis in human beings. Approximately, 94 million cases of salmonellosis are recorded all over the world each year [4]. *Salmonella* infection primarily spreads through contaminated foods including eggs, meat, fish, and water. It leads to symptoms including abdominal pain, diarrhoea, nausea, vomiting, and fever [5, 6]. *Salmonella* are one of the most common pathogens isolated from the stool culture of diarrhoea patients [7]. In USA, nearly 26% of all food related diseases are caused by *Salmonella* sp. and its economic burden on the society was estimated to be over a billion USD [8, 9]. All over the world, medical expenses and productivity loss associated with salmonellosis have been estimated to cost a tremendous amount of money [10]. The World Health Organisation (WHO) records an incidence of 16 to 33 million typhoid fever patients all over the world every year, with 0.5 to 0.6 million deaths [11]. Asia has the highest death rate of 93% due to typhoid fever [12].While in the subcontinent, Pakistan has its largest occurrence, that is, 451.7 per 100,000 persons/year of typhoid fever [13].

In light of these reports, the current study was conducted to estimate the frequency distribution of *S. Typhi* and its resistance pattern. Furthermore, molecular identification of its associated resistance genes was also performed.

## Materials and Methods

### Study Area and Sample Collection

The study was conducted from April, 2021 to March, 2022 in District Peshawar, KP, Pakistan. The ethical committee of Public Health Laboratory Division, National Institute of Health (NIH), Islamabad, Pakistan approved the study. Blood samples from symptomatic typhoid fever patients were collected and transported for processing to Microbiology Laboratory, Public Health Laboratories Division, NIH, Islamabad, Pakistan. The entire sample collection was performed under standard and sterile measures. Before sampling, a patient's complete

medical history was recorded containing information regarding patient's age, gender, and clinical history on a proper consent form.

### Isolation and Identification of *S. typhi*

About 02ml of blood sample was inoculated in to a Tryptone Soy Broth (TSB) and incubated at 37ºC for 72 hrs. After incubation, 01 ml of TSB was taken from the primarily cultivated sample, poured on blood and MacConkey agar (Oxoid,USA) plates, and incubated at 37ºC for 24 hours. The plates with growth were kept separately. For identification, the isolates were subjected to Gram staining, colony morphology, triple sugar iron, citrate, indole, and urease tests. API 20E strips were utilized for the confirmation of *S. typhi*.

### Antibiotic Sensitivity Testing

Cefixime(5 µg), imipenem/meropenem (10 µg), ampicillin (10 µg), erythromycin (15 µg), ceftriaxone (30 µg), ceftazidime (30 µg), levofloxacin (5 µg), azithromycin (15 µg), ofloxacin (5 µg),co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), gentamicin (10 µg),and chloramphenicol (30 µg) were used for antibiotic sensitivity testing. The Kirby-Bauer method of disc diffusion was utilized to find the sensitivity of the pathogen, following the guidelines of Clinical and Laboratory Standards Institute (CLSI) [14].

### Statistical Analysis

Data were analyzed with the help of SPSS software (Statistical Package for the Social Sciences Inc. Chicago, Illinois USA, version22). Two-way ANOVA was applied to observe the season's effect on antibiotic resistance pattern. Cross-tabulations were applied to the study variables.

## Results

Out of 210 patients, 89 (43%) were found to be positive for *S. typhi*. Among the positive patients, 47 (52.8%) were men and 42 (47.2%) were women. Urban to rural distribution of patients was 61 (59.2%) and 28 (40.8%), respectively. Overall, the difference between resistance to antibiotics among age groups and gender was not significant ($p=0.302$ or $p\geq0.05$and $p=0.976$ or $p\geq0.05$). Furthermore, a three-month session wise distribution of resistance pattern was followed.

**First Session (April 2021 – June 2021)**

In the 1st season, 37 positive samples were processed using the disc diffusion method. It was observed that these 37 isolates were most sensitive to cephalosporins, as shown in Table 1. Among the class of cephalosporins, ceftazidime was 75.67% susceptible drug, followed by cefixime and ceftriaxone showing 72.97% and 67.56% activity, respectively. Nalidixic acid showed 89.18% leading resistance among the selected drugs, while imipenem/meropenem, ciprofloxacin, levofloxacin, erythromycin, co-trimoxazole, and chloramphenicol showed 81.08%,78.37%, 48.64%, 64.86%, 62.16%, and 62.16% resistance, respectively.

**Table 1.** Antibiotic Susceptibility Studies of *S. typhi* in the First Session

| Antibiotics | Susceptibility | | Intermediate | | Resistance | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| **Penicillin** | | | | | | |
| Ampicillin | 13 | 35.13 | - | - | 24 | 64.86 |
| **Carbapenems** | | | | | | |
| Imipenem/Meropenem | 7 | 18.91 | - | - | 30 | 81.08 |
| **Quinolones** | | | | | | |
| Ciprofloxacin | 6 | 16.21 | 2 | 5.4 | 29 | 78.37 |
| Levofloxacin | 19 | 51.35 | - | - | 18 | 48.64 |
| Nalidixic Acid | 4 | 10.81 | - | - | 33 | 89.18 |
| **Aminoglycosides** | | | | | | |
| Gentamicin | 25 | 67.56 | - | - | 12 | 32.43 |
| **Cephalosporin** | | | | | | |
| Cefixime | 27 | 72.97 | - | - | 10 | 27.02 |
| Ceftriaxone | 25 | 67.56 | 3 | 8.1 | 9 | 24.32 |
| Ceftazidime | 28 | 75.67 | - | - | 9 | 24.32 |
| **Macrolides** | | | | | | |
| Erythromycin | 10 | 27.02 | 3 | 8.1 | 24 | 64.86 |
| Azithromycin | 21 | 56.75 | - | - | 16 | 43.24 |
| **Others** | | | | | | |
| Co-trimoxazole | 9 | 24.32 | 5 | 13.51 | 23 | 62.16 |
| Chloramphenicol | 14 | 37.83 | - | - | 23 | 62.16 |

**Second Session (July 2021 – September 2021)**

In the 2nd session, 26 isolates were subjected to antibiotic susceptibility using the disk diffusion method. Among these 26 isolates, ceftriaxone was found to be the most effective antibiotic with 80.76% susceptibility. Furthermore,76.92% of the samples were susceptible to cefixime and ceftazidime, as shown in Table 2. The nalidixic acid showed 73.07%resistance, followed by ciprofloxacin (65.38%), levofloxacin (57.69%) co-trimoxazole (57.69%), gentamycin (53.84%), and erythromycin (53.38%), respectively.

**Table 2.** Antibiotic Susceptibility Studies of *S. typhi* in the Second Session

| Antibiotics | Susceptibility | | Intermediate | | Resistance | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| **Penicillin** | | | | | | |
| Ampicillin | 13 | 50 | 1 | 3.84 | 12 | 46.15 |
| **Carbapenems** | | | | | | |
| Imipenem/Meropenem | 9 | 34.61 | - | - | 17 | 65.38 |
| **Quinolones** | | | | | | |
| Ciprofloxacin | 8 | 30.76 | 1 | 3.84 | 17 | 65.38 |
| Levofloxacin | 11 | 42.3 | - | - | 15 | 57.69 |
| Nalidixic Acid | 6 | 23.07 | 1 | 3.84 | 19 | 73.07 |
| **Aminoglycosides** | | | | | | |
| Gentamicin | 12 | 46.15 | - | - | 14 | 53.84 |
| **Cephalosporin** | | | | | | |
| Cefixime | 20 | 76.92 | 2 | 7.69 | 4 | 15.38 |
| Ceftriaxone | 21 | 80.76 | 2 | 7.69 | 3 | 11.53 |
| Ceftazidime | 20 | 76.92 | 2 | - | 4 | 15.38 |
| **Macrolides** | | | | | | |
| Erythromycin | 12 | 46.15 | - | - | 14 | 53.84 |
| Azithromycin | 16 | 61.53 | - | - | 10 | 38.46 |
| **Others** | | | | | | |
| Co-trimoxazole | 11 | 42.3 | - | - | 15 | 57.69 |
| Chloramphenicol | 16 | 61.53 | - | - | 10 | 38.46 |

### Third Session (October 2021 – December 2021)

The 3rd session revealed that cefixime (82.35%) was the most susceptible drug, followed by ceftriaxone (76.47 %), ceftazidime (76.47 %), and chloramphenicol (64.70%), as shown in Table 3. The nalidixic acid resistance was 70.58%, followed by ciprofloxacin, levofloxacin, erythromycin, and co-trimoxazole which showed 58.82%, 41.17%, 64.7%, and 47.05% resistance, respectively.

**Table 3.** Antibiotic Susceptibility Studies of *S. typhi* in the Third Session

| Antibiotics | Susceptibility | | Intermediate | | Resistance | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| **Penicillin** | | | | | | |
| Ampicillin | 10 | 58.82 | - | - | 7 | 41.17 |
| **Carbapenems** | | | | | | |
| Imipenem/Meropenem | 9 | 52.94 | 2 | 11.76 | 6 | 35.29 |
| **Quinolones** | | | | | | |
| Ciprofloxacin | 6 | 35.29 | 1 | 5.88 | 10 | 58.82 |
| Levofloxacin | 9 | 52.94 | 1 | 5.88 | 7 | 41.17 |
| Nalidixic Acid | 4 | 23.52 | 1 | 5.88 | 12 | 70.58 |
| **Aminoglycosides** | | | | | | |
| Gentamicin | 9 | 52.94 | 1 | 5.88 | 7 | 41.17 |
| **Cephalosporin** | | | | | | |
| Cefixime | 14 | 82.35 | - | - | 3 | 17.64 |
| Ceftriaxone | 13 | 76.47 | - | - | 4 | 23.52 |
| Ceftazidime | 13 | 76.47 | - | - | 4 | 23.52 |
| **Macrolides** | | | | | | |
| Erythromycin | 5 | 29.41 | 1 | 5.88 | 11 | 64.7 |
| Azithromycin | 8 | 47.05 | 1 | 5.88 | 8 | 47.05 |
| **Others** | | | | | | |
| Co-trimoxazole | 9 | 52.94 | - | - | 8 | 47.05 |
| Chloramphenicol | 11 | 64.7 | - | - | 6 | 23.29 |

### Fourth Session (January 2022 – March 2022)

In the 4thseason, 9 isolates were subjected to antibiotic susceptibility tests. During this time period, ceftazidime (88.9%) and cefixime (78%) showed

the highest sensitivity, as depicted in Table 4. The nalidixic acid and imipenam showed 78.8% resistance, while ciprofloxacin, levofloxacin, erythromycin, co-trimoxazole, and chloramphenicol showed 67% resistance.

**Table 4.** Antibiotic Susceptibility Studies of *S. typhi* in the Fourth Session

| Antibiotics | Susceptibility | | Intermediate | | Resistance | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| **Penicillin** | | | | | | |
| Ampicillin | 41 | 46.06 | 1 | 1.12 | 47 | 52.8 |
| **Carbapenems** | | | | | | |
| Imipenem/Meropenem | 27 | 30.33 | 2 | 2.24 | 60 | 67.41 |
| **Quinolones** | | | | | | |
| Ciprofloxacin | 23 | 25.84 | 4 | 4.49 | 62 | 69.66 |
| Levofloxacin | 42 | 47.19 | 1 | 1.12 | 46 | 51.68 |
| Nalidixic Acid | 16 | 17.97 | 2 | 2.24 | 71 | 80 |
| **Aminoglycosides** | | | | | | |
| Gentamicin | 52 | 58.42 | 1 | 1.12 | 36 | 40.44 |
| **Cephalosporin** | | | | | | |
| Cefixime | 68 | 76.4 | 2 | 2.24 | 19 | 21.34 |
| Ceftriaxone | 67 | 75.28 | 5 | 5.61 | 17 | 19.1 |
| Ceftazidime | 69 | 77.52 | 2 | 2.24 | 18 | 20.22 |
| **Macrolide** | | | | | | |
| Erythromycin | 30 | 33.7 | 4 | 4.49 | 55 | 62 |
| Azithromycin | 49 | 55.05 | 1 | 1.12 | 39 | 43.82 |
| **Others** | | | | | | |
| Co-trimoxazole | 32 | 36 | 5 | 5.61 | 52 | 58.42 |
| Chloramphenicol | 44 | 49.43 | - | - | 45 | 50.56 |

## Discussion

Antibiotic resistance is one of the major global public health issues. *Salmonella* infection is a widespread condition among resources constrained countries [4]. Asia, specifically Pakistan, has a high number of *Salmonella* cases due to poor potable water use [15, 16].Typhoid fever is a noticeable disease in the Sindh province of Pakistan [17, 18]. The current study reported a majority of patients with less than 20 years of age. This fact is in agreement with the previously established fact that typhoid is more

common in the younger population than the older age groups in Pakistan. There were reported 1000 cases per 100,000 in children only in Karachi. Furthermore, it was found that the resistance pattern has no effect on age and gender (that was proved statistically) and parallel reporting was observed in previous studies as well [19, 20].

A study reported 21.2% frequency of the positive cases of *S. typhi* in Pakistan in 2022 [16]. The reported frequency is higher as compared to the cases reported by the current study. Rayan et al. reported 44% nalidixic acid resistance in 22 out of 50 isolates[21].The current study reported the highest (89%) resistance in the 1st session of the isolated samples. This could be due to the spread of resistance strains and overuse of antibiotics in human beings [16]. Another study reported antibiotic resistance in Shanghai, China in 38 isolates, revealing 100% resistance in ceftazidime, while ampicillin also showed a high resistance to nalidixic acid (97.37%). Similar was the case with chloramphenicol (28.95%) [22]. Comparing with these results, the current study found the resistance of ceftazidime as 75.67%, ampicillin as 64%, and chloramphenicol as 62%.

### Conclusion

The current study concludes that high prevalence of *S. typhi* is a serious threat for public health. Furthermore, high resistance of nalidixic acid (Quinolone) was identified in all the sessions. It was also observed that season has no effect on the resistance pattern.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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