

Antimicrobial susceptibility of bacteraemic isolates of *Salmonella enterica* serovar typhi and paratyphi infection in Pakistan from 2017-2020

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Abstract

Objective: To determine the antibacterial susceptibility pattern of bacteraemia isolates of *Salmonella enterica* serovar typhi and paratyphi.

Method: The retrospective descriptive observational study was conducted at the Microbiology section of Dow Diagnostic Research and Reference Laboratory, and comprised blood culture reports from January 1, 2017, to Dec 30, 2020, which were screened for the presence of *Salmonella typhi* and paratyphi growth. The frequency of the isolates and their antibiotic resistance patterns were analysed. Data was analysed using SPSS 20.

Results: Of the 174,190 blood culture samples, 62,709 (36%) were positive for bacterial growth. *Salmonella* were isolated in 8,689 (13.8%) samples of which 8,041 (92.5%) were *Salmonella typhi*, 529 (6%) were *Salmonella paratyphi* A and 119 (1.3%) were *Salmonella paratyphi* B. There was a drastic increase in resistance to third-generation cephalosporin in *Salmonella typhi* from 71 (12.8%) in 2017 to 1,420 (71%) in 2018, 2,850 (74.6%) in 2019 and 1,251 (77%) in 2020. All isolates were sensitive to meropenem and azithromycin.

Conclusion: A high number of extensively drug-resistant typhoid cases due to *Salmonella typhi* were found. All isolates were sensitive to meropenem and azithromycin.

Keywords: *Salmonella typhi*, Multidrug resistance, Extensively drug-resistant (JPMA 73: 505; 2023) DOI: 10.47391/JPMA.6083

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Introduction

Enteric fever is still the main health problem in Pakistan. Typhoid is caused by gram-negative bacteria called *Salmonella enterica* serovars typhi and paratyphi, which is acquired through the ingestion of faecally-contaminated food and water. The clinical presentation of the acute infection is nonspecific and indistinguishable from other infectious diseases. However, if not treated timely, it could progress to serious complications that can result in mortality. The case fatality rate of typhoid fever is 10-30% and it goes down to 1-4% with proper therapy. *Salmonella* (S.) can be isolated from various specimens, such as bone marrow, blood and other body fluids.¹ According to updated statistics, it accounts for 11-21 million cases and is responsible for the mortality of around 128,000 to 161,000 per year globally.²⁻⁵ In addition, typhoid is a major cause of bacteraemic illness in children, especially among children aged 2-15 years with an estimated rate of >451.7 per 100,000.⁶ Antibiotic has been used to cure enteric fever and has decreased the

mortality related to complicated cases. In most of South Asia, 50-80% of *S. typhi* isolates have acquired multidrug resistance (MDR), defined as resistance to the three first-line classes of antimicrobial agents; chloramphenicol (C), ampicillin (AMP) and trimethoprim/sulphamethoxazole (SXT).⁷ Next option that was used to treat these infections was the usage of fluoroquinolones. Yet again in early 2000, it lost its efficacy and bacteria acquired resistance against it, becoming multidrug-resistant, especially in the southern part of Asia.^{1,8,9} This has led to the usage of third-generation cephalosporin, like ceftriaxone (CRO) as the recommended first-line treatment. Similarly, high level of fluoroquinolones and third-generation cephalosporin resistance in *S. typhi* has been reported from Pakistan.⁷ Recently, an outbreak of extensive drug resistance (XDR) isolates that have acquired resistance to the first line of antibiotics, fluoroquinolones and third-generation cephalosporin, has been reported by the World Health Organisation (WHO). Most of the cases were from Karachi and Hyderabad.² Recently, from November 2016 to December 2018, Provincial Disease Surveillance and Response Unit reported an unusually high number of XDR *S. typhi* cases (5274 out of a total of 8188 typhoid isolates) from several regions in the Sindh province of Pakistan.

The current study was planned to find out the

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antibacterial susceptibility pattern of bacteraemia isolates of *Salmonella enterica* serovar typhi and paratyphi.

Materials and Methods

The retrospective descriptive observational study was conducted at the Microbiology section of Dow Diagnostic Research and Reference Laboratory (DDRRL), and comprised blood culture reports from January 1, 2017, to Dec 30, 2020.

Data was retrieved after approval from the institutional ethics review board using the laboratory information system (LIS) on March 1, 2021. The Dow laboratory received patient samples from several collection points across the province. All blood samples identified as *S. typhi* and paratyphi were included. All blood samples showing growth of organisms other than *S. typhi* and paratyphi were excluded.

After venepuncture, 10ml of blood sample was collected from adult patients (from children according to their body weight). Blood culture was done in automated BacT/ALERT instrument for the detection of positive culture growth. BacT/ALERT bottles with green and yellow tops were used for adults and paediatric patients, respectively. All positive samples were gram-stained followed by inoculation on MacConkey agar, 5% sheep blood agar and chocolate agar. Then plates were

incubated at 37°C for 18-24 hours. A standard bacteriological technique was applied for the identification of isolates. It was based on gram stain, colonial morphology and biochemical tests, such as oxidase, triple sugar iron tests, urease, and citrate and indole test. It was confirmed by serological identification for *Salmonella* species with specific O and H antisera (BD Laboratory) and API20E. Antimicrobial susceptibility of all *Salmonella* isolates was performed by Kirby-Bauer disk diffusion method on Muller Hinton agar with standard antimicrobial disk, according to Clinical Laboratory Standard Institute (CLSI) guidelines.¹⁰ Eight antimicrobials were included in the study for antimicrobial susceptibility patterns, including AMP (10µg), C (30µg), SXT (1.25/23.75µg), CRO (30µg), cefixime (CFM) (5µg), ciprofloxacin (CIP) (5µg), azithromycin (AZM) (15µg) and meropenem (MRM) (10µg).

Data was analysed using SPSS 20. Data was expressed as frequencies and percentages of the isolated organisms and their antibiotic susceptibility pattern.

Results

Of the 174,190 blood culture samples, 62,709(36%) were positive for bacterial growth. *Salmonella* were isolated in 8,689(13.8%) samples of which 8,041(92.5%) were *S. typhi*, 529(6%) were paratyphi A and 119(1.3%) were paratyphi B.

Table-1: Age distribution of *Salmonella typhi* and *Salmonella paratyphi* A and B isolates from blood culture (N=8689)

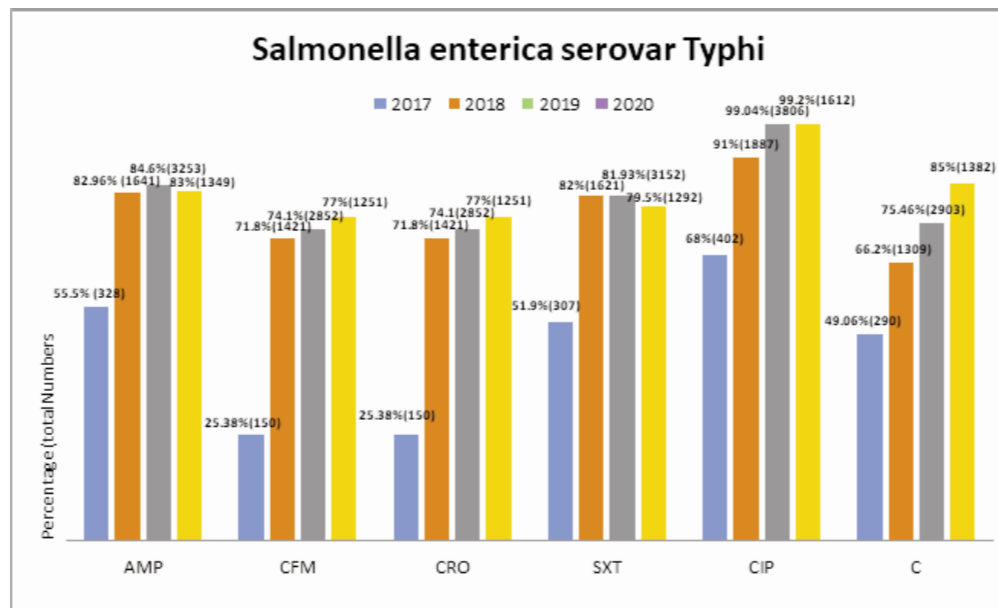
Age groups (Years)	2017 (n :%)	2018 (n :%)	2019 (n :%)	2020 (n :%)	*P-value
0 to 5	111(18.8%)	623(31.5%)	1592(41.4%)	526(32.4%)	<0.001
>5 to 10	195(33.0%)	669(33.8%)	1120(29.1%)	520(30.9%)	
>10 to 15	114(19.3%)	308(15.6%)	489(12.7%)	212(13%)	
>16 to 30	122(20.6%)	298(15.1%)	547(14.2%)	299(18.4%)	
>30	49(8.3%)	80(4.0%)	99(2.6%)	86(5.3%)	
Total	(100%)	1978(100%)	3847(100%)	1625(100%)	
paratyphi A	2017	2018	2019	2020	*P-value
0 to 5	7(4.5%)	12(14.1%)	23(16.5%)	23(15.3%)	0.003
>5 to 10	32(20.6%)	21(24.7%)	22(15.8%)	41(27.3%)	
>10 to 15	28(18.1%)	16(18.8%)	23(16.5%)	25(16.7%)	
>16 to 30	52(33.5%)	27(31.8%)	56(40.3%)	43(28.7%)	
>30	36(23.2%)	9(10.6%)	15(10.8%)	18(12%)	
Total (N)	155(100%)	85(100%)	139(100%)	150(100%)	
paratyphi B	2017	2018	2019	2020	*P-value
0 to 5	6(13.1)	14(28%)	9(50%)	5(100%)	<0.001
>5 to 10	15(32.6%)	9(18%)	0(0)	0	
>10 to 15	13(28.3)	14(28%)	2(11.1)	0	
>16 to 30	10(21.7%)	12(24%)	4(22.2%)	0	
>30	2(4.3%)	1(2%)	3(16.7%)	0	
Total (N)	46(100%)	50(100%)	18(100%)	5(100%)	

*Pearson Chi-square test for association

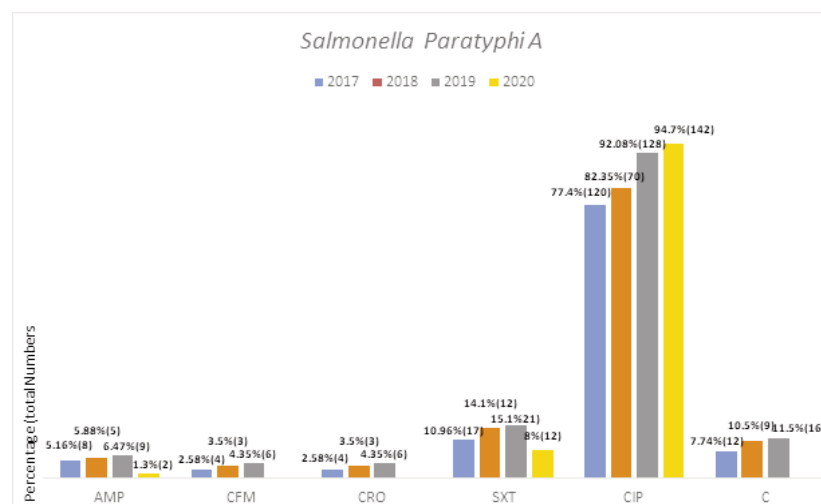
Table-2: Location-wise distribution of Salmonella isolates.

District	2017		2018		2019		2020	
Name	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
KHI-East	283	47.9	747	37.8	1563	40.6	484	29.8
KHI-Malir	36	6.2	250	12.6	671	17.4	140	8.6
KHI-South	26	4.4	194	9.8	503	13.1	252	15.5
KHI-Central	84	14.2	345	17.4	489	12.7	301	18.5
KHI-Korangi	26	4.3	136	6.9	277	7.2	120	7.4
KHI-West	19	3.3	124	6.3	179	4.7	139	8.6
Out side Karachi City	117	19.78	182	9.2	165	4.3	189	11.6
Total	591	100.00	1978	100.0	3847	100.0	1625	100.0

KHI: Karachi.

**Figure-1:** Antibacterial resistance pattern of Salmonella typhi in blood culture from 2017 to 2020 (N=8041).

AMP: Ampicillin, CFM: Cefixime, CRO: Ceftriazone, SXT: Trimethoprim/Sulfamethoxazole, CIP: Ciprofloxacin, C: Chloramphenicol

**Figure-2:** Antibacterial resistance pattern of Salmonella paratyphi A in blood culture from 2017 to 2020 (N=529).

AMP: Ampicillin, CFM: Cefixime, CRO: Ceftriazone, SXT: Trimethoprim/Sulfamethoxazole, CIP: Ciprofloxacin, C: Chloramphenicol.

Increase in the number of *S. typhi* was observed from 591(7.3%) in 2017 to 1,978(24%) in 2018, 3,847(47%) in 2019, followed by decrease in 2020 1,625(20%). The most common age group in which *S. typhi* was observed was 5-10 years in both 2017 and 2018 compared to 2019 and 2020 in which a slight upper shift was observed to age group 0-5 years.

However, in the case of *Salmonella paratyphi*, the most common age group was 16-30 years consistently in all the four years.

Salmonella paratyphi B was present in different age groups each year (Table1). *Salmonella typhi* isolates were predominantly recovered from males 4712(58.6%) compared to females 3320(41.3%). Location-wise distribution of *Salmonella* isolates was also noted (Table 2).

Increasing trend of resistance to AMP, C and SXT was observed in *S. typhi* and *paratyphi A* and B (Figure 1). Also noted was a drastic increase in resistance to third-generation cephalosporin in *S. typhi* from 71(12.8%) in 2017 to 1,420(71%) in 2018, 2,850(74.6%) in 2019 and 1,251(77%) in 2020. However, a low level of cephalosporin resistance in *Salmonella paratyphi A* and B was observed from 2017 to 2020. All isolates of *S. typhi* were sensitive to MRM and AZM.

Resistance to CIP in *S. typhi* increased from 401(68%) in 2017 to 1,799(91%) in 2018 to 3,731(97%) in 2019 and 1,612(99.2%) in 2020. Similarly,

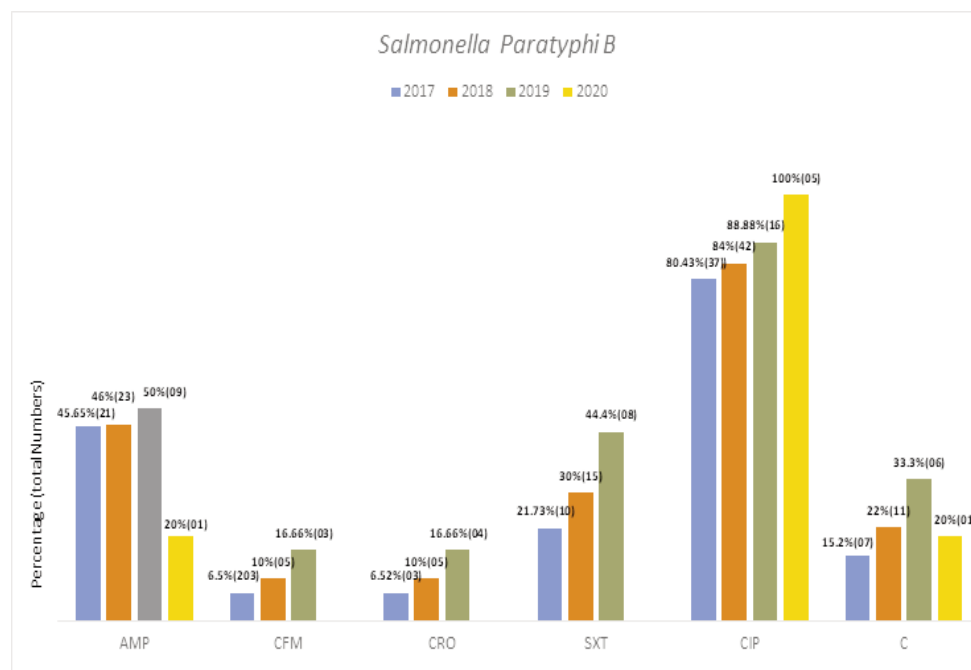


Figure-3: Antibacterial resistance pattern of *Salmonella paratyphi B* in blood culture from 2017 to 2020 (N=119).

AMP: Ampicillin, CFM: Cefixime, CRO: Ceftriaxone, SXT: Trimethoprim/Sulfamethoxazole, CIP: Ciprofloxacin, C: Chloramphenicol.

in *Salmonella paratyphi A* and *B*, CIP resistance increased from 119(77%) and 37(80%) in 2017 to 69(82%) and 42(84%) in 2018 to 127(92%) and 16(88%) in 2019 to 142(94.7%) and 5(100%) in 2020 (Figure2-3).

Discussion

In the past, the invention of unique antimicrobials against typhoid infection saved many lives across the globe. Dismally, the inappropriate usage of antibiotics has led to the expansion of MDR and XDR strains of *S. typhi*. The current study 92.5% of the strains were *S. typhi* and only 6% were *Salmonella paratyphi A* and 1.3% were *Salmonella typhi B*. Similarly, a recent study conducted in Lahore reported *S. typhi* at 94%, and *Salmonella paratyphi* at 6% of the *Salmonella* strains.¹¹ The current study found a substantial increase in the number of cases. A similarly higher number of *S. typhi* cases were reported by other studies.^{1,11} However, the decrease in 2020 could be due to the emergence of coronavirus disease-2019 (COVID-19), leading to decreased surveillance secondary to the lack of resources during the pandemic. In addition, the decrease in the number of cases in 2020 is in line with the findings of the Federal Disease Surveillance and Response Unit of the Field Epidemiology Disease Surveillance report of the National Institute of Health (NIH).¹²

In the current study, higher numbers of isolates were from the East district of Karachi. It is most likely because of the

location of DDRRL in this district. Several residential areas in the East district include slums around residential areas. The increased number of cases from these areas could be because of the unhygienic condition, improper sewerage amenities, and mixing of drinking water with the sewage.

The most commonly affected age group in the study 5-10 years with a male preponderance. This may be because children are the most vulnerable group exposed to poor hygienic conditions as well as due to lack of medical facilities. Moreover, males are more prone to be

infected because of their exposure to the external environment. This finding is in line with earlier studies.^{13,14} First outbreak of resistant strains of *S. typhi* against the first line of antibiotics was reported in 1980. Several studies have documented a higher prevalence of MDR typhoid from 12% in 1987 to 75% in 1995 in Pakistan.¹⁵ Moreover, a study documented the rise in MDR rate of *S. typhi* from 34% to 48.5% from 2001 to 2006 along with a higher level of quinolone resistance from 1.6% to 64.1%.¹⁶ Another study reported an increasing trend of MDR in *S. typhi* from 2001 (34%) to 2006 (48%).¹⁵ The current study also reported a high level of resistance of *S. typhi* to first-line antibiotics. This correlates with earlier studies.^{8,15,17} Fluoroquinolones were the treatment of choice after the resistance against first-line conventional antibiotics, but due to their excessive use, they turned out to be ineffective. The current study also observed a higher level of fluoroquinolones resistance in *S. typhi*. This finding is in accordance with other studies.^{8,18}

The development of MDR *S. typhi* with resistance to fluoroquinolones is distressingly leading to narrowing down of therapeutic choices in high-burden countries, such as Pakistan. However, CRO resistance has stayed uncommon, with <1% of isolates resistant from 2009 to 2014 in Pakistan. It continues to be the first therapeutic option for inpatient management of typhoid.^{1,8} However, in 2016 the initial outbreak of CRO-resistant *Salmonella*

was observed in two sub-districts of Hyderabad, Sindh. The genome sequencing analysis of these resistant isolates was associated with the H58 haplotype. It contained many resistant elements, including the *qnrS* fluoroquinolones resistance gene and extended-spectrum β -lactamase *bla*CTX-M-15 genes that made these isolates non-susceptible to fluoroquinolones and CRO. This clone holds a substantial propensity for worldwide spread and was capable of transforming MDR to XDR strains.¹⁰ The two main therapeutic options for XDR infection are MRM and AZM. The current study also found increased trend of XDR isolates of *S. typhi*. This finding is in line with a WHO report on the emergence of XDR *S. typhi* in Hyderabad, Sindh. According to the report, 5,274 cases of XDR typhoid fever were reported from November 2016 to December 2018 and till August 2019, the number increased exponentially, with more than 10,000 documented cases of XDR typhoid. A recent study documented 64% prevalence of XDR *S. typhi*.^{2,19} However, the declining pattern of antibiotic resistance of *S. typhi* has also been reported. It could be because the XDR *S. typhi* outbreak was more prevalent in the province of Sindh compared to the rest of the country. All isolates of *S. typhi* were sensitive to MRM and AZM in the current study.

The *S. typhi* and *Salmonella* paratyphi A and B distribution was similar to literature,⁹ and so was the pattern of resistance.^{20,21} The main reasons for this high-level resistance are injudicious usage and easy over-the-counter availability of drugs.

Antibiotics should be used very cautiously, and prevention and control of the spread of typhoid are crucial which may be attained through upgrading water and sanitation conditions, hand hygiene awareness, effective vaccination programmes, and availability of standardised laboratory facilities.

Limitation: The present study has limitations, due to its retrospective analysis design. We analyzed and interpreted laboratory data records. This has brought in limited information regarding prior treatments, including antibiotic use before giving blood samples for culture and sensitivity testing.

Conclusion

There was a high number of XDR *S. typhi* cases. The most common age group infected was 5-10 years with a male preponderance. However, all isolates of *S. typhi* were sensitive to MRM and AZM.

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Conflict of Interest: None.

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