Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i2.4393

EMERGENCE OF EXTENSIVE DRUG RESISTANCE TYPHOID IN HOSPITALIZED COVID-19 PATIENTS IN SOUTH PUNJAB, PAKISTAN

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Abstract

Introduction: In Pakistan, one of the main causes of mortality and morbidity is typhoid fever. Notably, extended drug-resistant (XDR) typhoid has been misdiagnosed widely due to many physicians continued use of Widal and Typhi-dot as serological tests for diagnosis during the COVID-19 pandemic. There were increased cases of extensively drug-resistant typhoid due to the use of extended drugs during the COVID-19 pandemic without confirming the antimicrobial susceptibility of *Salmonella typhi*.

Methodology: In this study, 118 patient blood samples after the COVID-19 pandemic was analyzed by automated blood culture through BacT/ALERT 3D, and antimicrobials susceptibility was performed. And compared with the antimicrobial susceptibility before the COVID-19 pandemic samples of the same patients.

Results: Out of 118 blood cultures of Post-COVID-19 isolates, 34 cultures were positive for *Salmonella typhi.* On antibiotic susceptibility test profiling of these 34 isolates, 44.12% were extensive drug-resistant (XDR), 38.24% were multidrug-resistant (MDR), 8.82% were non-MDR, non-XDR and 8.82% were resistant to all antibiotics. While in Pre-COVID-19 isolates 21.74% were XDR, 60.87% were MDR, 17.39% were non-MDR, non-XDR and 0.0% were resistant to all antibiotics. There were an increased XDR typhoid ratio as compared to MDR typhoid in Post-COVID-19 isolates.

Conclusions: There is an increased emergence of XDR typhoid cases in Post-COVID-19 isolates of *Salmonella typhi* as compared to the Pre-COVID-19 *Salmonella typhi* isolates. Moreover,

implementing antimicrobial susceptibility profiling and proper diagnosis before using any antibiotic against *Salmonella typhi* prevent the XDR typhoid outbreak in the near future.

Keywords: XDR, COVID-19, typhoid, *Salmonella typhi,* S. Typhi, MDR

Introduction

Typhoid fever is an infectious disease caused by a specific strain of bacteria *Salmonella enterica serotype typhi*. It is a systemic illness that primarily affects the gastrointestinal system but can also spread to other organs in the body. Typhoid fever is characterized by a high and prolonged fever, along with various other symptoms including headache, high-grade fever, abdominal pain, loss of appetite, fatigue, weakness, and gastrointestinal disturbances such as diarrhea or constipation. It is an illness that can spread throughout the body and harm different organs, including the intestines, liver, spleen, and gallbladder [1]. Typhoid fever exhibits a significant global burden, with an estimated annual incidence of approximately 21 million cases and a resultant mortality rate of 200,000 deaths per year. The disease burden of enteric fever in Pakistan is significantly elevated due to inadequate sanitation and densely populated urban areas, resulting in an annual incidence rate of 493.5 cases per 100,000 individuals [2]. S. Typhi is a serotype or strain of the bacterium *Salmonella enterica*. It is a gram-negative bacterium belonging to the Enterobacteriaceae family. S. Typhi is a rod-shaped bacterium, typically around 2-3 micrometers in length and 0.5 micrometers in diameter [3].

The primary route of S. Typhi infection is through the ingestion of contaminated food or water. In some cases, direct person-to-person contact can also lead to transmission [4]. The healthcare systems have experienced disruptions due to the COVID-19 pandemic, which may cause a delay in typhoid fever diagnosis as well as treatment. For example, lockdowns and some other restrictions have led to changes in behavior, stress, depression, and feelings of sadness have consistently been linked to heightened food consumption and suboptimal dietary decisions, often leading to a preference for sugary treats over healthier snack options [5].Due to fear of COVID-19 misuse of antibiotics while neglecting the multi-drug resistant typhoid. Moreover, these conditions can facilitate the propagation of the disease, resulting in a surge in the number of confirmed cases. Now once again need for an enhanced typhoid fever diagnosis as well as treatment [6].

Several factors can predispose individuals to typhoid fever after COVID-19. Firstly, COVID-19 can directly or indirectly, effects the immune system, making individuals more susceptible to secondary infections such as typhoid fever [7]. Health care systems have also been affected by the COVID-19 epidemic, which can lead to delays in typhoid diagnosis and its treatment. Despite an order given by the NIH commonly known as the National Institute of Health, Pakistan to discontinue the use of antibody tests to diagnose typhoid fever, it was recommended during COVID-19 by many practitioners. Furthermore, economic hardship and food insecurity resulting from the COVID-19 pandemic can contribute to the spread of typhoid. Ultimately, several indirect factors may impact the rise the extensively drug-resistant (XDR) typhoid cases during the COVID-19 pandemic. With COVID-19 pandemic there were remarkable changes such as increased fear, stress and other restrictions have led to changes in behavior due to lockdown [8]. These things are common in human health, and the COVID-19 pandemic messed up a lot of things, including the changed ways of antimicrobials usage [9, 10].

Chloramphenicol, which was introduced in 1948, was the initial antibiotic utilized for the cure of typhoid cases [11]. S. Typhi which is Chloramphenicol resistant documented in Pakistan dates back to 1987 [10]. Since 1961 to 1967, the effectiveness of ampicillin as an antimicrobial treatment for S. Typhi was widely recognized [13]. In the reaction of Ampicillin-resistant S. Typhi, the combination of Trimethoprim-Sulfamethoxazole was found to be equally effective as Ampicillin. Consequently, it became the preferred choice of treatment for typhoid cases affected by ampicillin resistance S. Typhi strains [14].

S. Typhi isolates that exhibit resistance to three or more distinct groups of drugs is categorized as MDR S. Typhi (multidrug-resistant). In 1987, the first documented case of MDR (Multi-drug resistant) S. Typhi emerged from Pakistan [15]. Before 1990, ciprofloxacin exhibited notable

effectiveness against S. Typhi strains that showed resistance to initial treatments for typhoid fever. In 1993, Pakistan reported its first documented case of reduced susceptibility to ciprofloxacin, highlighting the growing prevalence of this alarming situation [16]. The impact of the COVID-19 pandemic on antibacterial usage primarily stems from the widespread prescription of anti-bacterial to individuals with COVID-19 to address potential secondary or co-bacterial infections. Among the third-generation cephalosporins, ceftriaxone is widely recognized as the potential drug for managing typhoid cases affected by fluoroquinolone-resistant or MDR (Multi-drug resistant) S. Typhi [17].
A significant incidence of typhoid fever, which was resistant to ceftriaxone, commenced in the city of Hyderabad in November 2016. The microbe in question was identified as extended drug-resistant S. Typhi and was found to possess resistance to five distinct categories of antimicrobial agents, the namely first line of drugs (trimethoprim-sulfamethoxazole, ampicillin chloramphenicol) and extended drugs (cephalosporins, fluoroquinolones) As a result of this resistance profile, it was classified as an XDR(extended drug resistant) strain of S. Typhi. The extensively drug-resistant strain of *Salmonella typhi* under consideration exhibited susceptibility towards azithromycin and meropenem [18, 19]. The patients of COVID-19 have treated with azithromycin in Pakistan, despite the potential presence of extended drug-resistant (XDR) typhoid. The potential consequences could be significant if XDR develops resistance to azithromycin. It is mentioned that XDR typhoid fever has been subjected to significant misdiagnosis as a result of the use of Typhi DOT and Widal as serological tests by several medical practitioners [20]. The excessive utilization of azithromycin in the management of COVID-19 has the potential to compromise one of the limited remaining therapeutic options. A large number of doctors have continued to recommend the Widal and Typhi Dot as antibody tests to diagnose typhoid fever, which is the major reason for the misdiagnosis of typhoid fever. Antibody tests are still used in clinical settings despite instructions from the NIH to stop doing so [21].

Methodology

Post-COVID-19 Pandemic Typhoidal Patient's Sampling

One hundred and eighteen samples were collected from September 2022 to February 2023. In summary, blood samples ranging from 1 to 4 mL were collected from each suspected child, taking into consideration their age and body weight. For adults, blood samples of 5-10 mL were collected. These samples were then transferred to BacT/ALERT bottles and incubated for a maximum of 5 to 6 days. The bottles were filled with BacT/ALERT media, which was supplemented with (APB) absorbent polymeric beads to effectively neutralize antimicrobial substances [22].

Sampling History Criteria

Blood samples were collected from various hospitals of South Punjab, Bahawalpur Victoria Hospital, Nishtar Hospital Multan, DHQ Hospital D.G Khan, and Layyah. These patients were suspected to be suffering from typhoid during September, 2022 to Feb, 2023 with a previous history of typhoid infection before the COVID-19 from April, 2019 to Sep, 2019. They visited the hospital during COVID-19 pandemic and were referred for Typhoid fever screening tests such as the Widal test and Typhi Dot.

Pre and Post-COVID-19 Social Demographic of the Study for Typhoid Fever

This study was conducted on patients of different hospitals in south Punjab, including the city Bahawalpur, Multan, D.G Khan, and Layyah. Demographic history of patients with the symptoms of *Salmonella typhi* before, during, and after COVID-19 were entered on the history performa. Analyzing this history performa by age groups 0-9 years, 10-60 years and > 60 years across all cities. During COVID pandemic, Widal and Typhidot were used as serological test for diagnosis of typhoid fever from Feb, 2019 to Sep, 2023 instead of Microbiological Culture.

Post-COVID-19 blood cultures for Salmonella typhi

Microbiological culture of blood samples of the same patients after the COVID-19 pandemic was performed using an automated system (BacT/ALERT) at all sites following the collection of a single

aerobic bottle. The proprietary media utilized in this study consist of a tryptic soy broth that has been supplemented with complex amino acids and carbohydrates. These media have been specifically formulated to facilitate the growth of microorganisms and promote optimal carbon dioxide (CO_2) production. The bottles are filled with a volume of 30 ml of the medium that contains an anticoagulant known as sodium poly anethole sulfonate, present in a concentration of 0.035%.

Any bottle which triggers the fluorescence sensor due to an increase in CO_2 produced as a result of microbial growth was flagged up [22]. The blood culture bottle was detected as positive by transferring the broth from a glass slide to a wet slide to confirm the positive culture. Catalase test indicated by the production of bubbles by mixing H_2O_2 with the culture was used to aid the identification of enterobactericae. Subculture a loopful of blood/broth onto Salmonella Shigella (S.S) agar used as selective medium for the isolation of Slamonella spp. and rule out members of enterobactericae, as *Salmonella typhi* isolates produced typical colony with blackening at center due to H_2S production. After overnight incubation at $35^0C\pm2$, carried out sub culturing on to TSI (Triple Sugar Iron) agar slants. On the basis of H_2S indicated as light black colour, absence of gas production, yellow colour of butt and red slant, *Salmonella typhi* was differentiated from other species of *Salmonella* that cause typhoid in human. Slant of Simmons citrate agar used for confirmation of S. Typhi as it remained negative in case of S. Typhi while positive in others species of *Salmonella* that cause typhoid in human. Antimicrobial susceptibility test was performed according to Clinical Laboratory Research Institution (CLSI-20) standards.

Antimicrobial Susceptibility Profiling of Pre-COVID-19 Salmonella typhi Isolates

Antimicrobial susceptibility profiling of same patients before the COVID-19 pandemic were obtained from hospitalized data. The present study utilized bacterial strains that were isolated specifically for the purpose of treatment. Furthermore, it should be noted that the study was conducted in a fully anonymous manner, ensuring that no data or identifiable information was obtained. As a result, the need for informed consent or ethics approval is not necessary in accordance with the local legislation governing this type of study.

Antimicrobial Susceptibility Profiling of Post-COVID-19 Salmonella typhi Isolates

The antimicrobial susceptibility testing was conducted by applying the Kirby-Bauer disc diffusion method, following the zone size interpretation guidelines provided in the most recent CLSI recommendations. Specifically, Mueller-Hinton (MH) agar was used, and an approximate 0.5 McFarland standard colony suspension was prepared. The incubation process occurred at a temperature of 35°C ± 2°C in ambient air for a duration of 16 to 18 hours, as specified by CLSI guidelines in 2020 [23]. The antibiotic discs used in this study were purchased from Oxoid, a reputable supplier based in the United Kingdom.

XDR Typhoid Emergence in Pre and Post-COVID-19 Salmonella typhi Isolates

A variety of antimicrobial agents were examined for all the strains that were isolated, although the specific agents used varied depending on the site and the antimicrobials that were accessible to the clinical teams. Multi-drug resistance (MDR) S. Typhi refers to the state of being non-susceptible to amoxicillin, chloramphenicol, ampicillin and co-trimoxazole. Extensive Drug Resistance (XDR) is characterized by a lack of susceptibility to various antibiotics, including ampicillin and amoxicillin, chloramphenicol and co-trimoxazole, fluoroquinolones (specifically ciprofloxacin), and 3rd generation cephalosporins (such as ceftriaxone). The aforementioned antibiotic discs were used to classify the isolates as MDR or XDR according to established criteria [24].

Statistical Association Pre and Post-COVID-19 Emergence of XDR

Analyzed the emergence of extensive drug-resistant typhoid between Pre-COVID-19 and Post-COVID-19 isolates of *Salmonella typhi* on the basis of their antibiotic susceptibility profiling criteria. Antibiotic susceptibility profiling was measured in percentage using descriptive statistics by using SPSS version-22 [25].

Results

One hundred and eighteen samples were collected between September 2022 to February 2023 from various hospitals of South Punjab, like Bahawalpur Victoria Hospital, Nishtar Hospital Multan, DHQ Hospital D.G Khan, and Layyah after COVID-19 pandemic. The age range of 10-60 emerged as the most prominent frequency (54.2%), with the highest frequencies observed in Multan (26.3%), Bahawalpur (9.3%), D.G Khan (9.3%), and Layyah (9.3%) describe in Table 1. (Table1)

Table 1: Social Demographic of the Study for Typhoid Fever in South Punjab.

City		Age 0-9 Frequency (%)	Age 10-60 Frequency (%)	Age >60 Frequency (%)
Bahawalpur	Count	5	11	10
	% of Total	4.2%	9.3%	8.4%
Multan	Count	13	31	11
	% of Total	11.0%	26.3%	9.3%
D.G Khan	Count	3	11	6
	% of Total	2.5%	9.3%	5.0%
Layyah	Count	4	11	2
	% of Total	3.4%	9.3%	1.7%
Total n=118		25	64	29
Total Frequency (%)		21.2%	54.2%	24.57%

Age group 10-60 constituted a significant proportion of the population in these cities, which confirmed that a large number of individuals risk of contracting and transmitting *Salmonella typhi* due to their active involvement in work, activities, and mobility. The blood culture testing for *Salmonella typhi* patients before the COVID-19 pandemic.

The study revealed that the percentage of positive blood cultures increases with age, with the highest percentage observed in the age group of 10-60 (9.3%). While after the COVID-19 pandemic the percentages of positive blood cultures increase with age, the highest percentage observed similar age group as before COVID-19 but the percentage was high about (14.4%) describe in Table 2. (Table2) This indicates a higher prevalence of *Salmonella typhi* infection in age group 10-60 individuals before and after the COVID-19 era.

Table 2: Pre and Post-COVID-19 Positive Blood Culture for *Salmonella typhi* with age group.

Age Group		Positive Blood Culture (%) Pre-COVID-19 Isolates	Positive Blood Culture (%) Post-COVID-19 Isolates
Group 0-9		4.2%	7.6%
Group 10-60		9.3 %	14.4 %
Group >60		5.9%	8.5%
Total n=118	Count	23	36
	Percentage (%)	19.5%	30.5%

Antimicrobial Susceptibility profiling on Pre-COVID-19 *Salmonella typhi* isolates indicate that Ampicillin and Co-trimoxazole was not effective against the tested bacterial strain, while Chloramphenicol exhibits limited effectiveness.

On the other hand, Ciprofloxacin, Ceftriaxone, and Azithromycin demonstrate a higher level of susceptibility.

These results highlighted that there was no emergence of extended drugs and azithromycin was totally susceptible in Pre-COVID-19 times presented in Table 3. (Table3)

Table 3: Antimicrobials susceptibility in Pre-COVID-19 *Salmonella typhi* isolates.

Antimicrobial Agent n=23	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin	0(0.00)	0(0.00)	23(100)
Co-trimoxazole	0(0.00)	2(8.70)	21(91.30)
Chloramphenicol	4(17.39)	0(0.00)	19(82.61)
Ciprofloxacin	17(73.91)	0(0.00)	6(26.09)
Ceftriaxone	13(56.52)	0(0.00)	10(43.48)
Azithromycin	23(100)	0(0.00)	0(0.00)

Antimicrobial susceptibility profiling on Post-COVID-19 *Salmonella typhi* isolates shown that ampicillin has no susceptibility among the 34 isolates tested, indicating complete resistance. Similarly, none of the isolates were susceptible to Co-trimoxazole, with the majority (94.12%) displaying resistance. Chloramphenicol demonstrated limited effectiveness, as only one isolate (2.94%) showed susceptibility, while the majority (97.06%) exhibited resistance. In contrast, Ciprofloxacin displayed a moderate level of susceptibility, with 29.41% of isolates being susceptible, and 67.65% showing resistance. Ceftriaxone fared slightly better, with 38.24% of isolates being susceptible, while 58.82% showed resistance. Azithromycin exhibited the highest level of susceptibility, with 91.18% of isolates being susceptible and 8.82% showing resistance describe in Table 4. (Table4)

Table 4: Post-COVID-19 antimicrobials susceptibility profiling of *Salmonella typhi* isolates.

Antibiotics	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin	0(0.00)	0(0.00)	34(100)
Co-trimoxazole	0(0.00)	2(5.88)	32(94.12)
Chloramphenicol	1(2.94)	0(0.00)	33(97.06)
Ciprofloxacin	10(29.41)	1(2.94)	23(67.65)
Ceftriaxone	13(38.24)	1(2.94)	20(58.82)
Azithromycin	31(91.18)	0(0.00)	3(8.82)

Out of 118 blood culture samples 34 cultures were positive for *Salmonella typhi*. On antibiotic susceptibility test profiling of 34 positive isolates, 44.12% were extensive drug-resistant (XDR), 38.24% were multidrug-resistant (MDR), 8.82% were non-MDR, non-XDR and 8.82% were resistant to all antibiotics. There was an increased XDR typhoid ratio as compared to MDR typhoid in Post-COVID isolates shown in Figure 1.

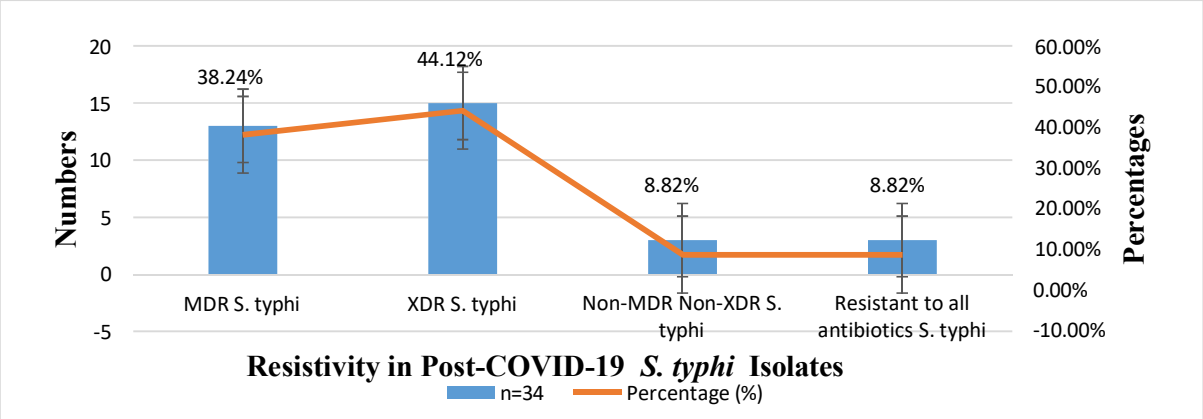

Figure 1: XDR, MDR, and Resistant to all antibiotics of *S. typhi* isolates in the Post-COVID-19 era.

While positive cultures of *Salmonella typhi* isolates before the COVID-19 pandemic showed that out of the 23 isolates tested, 21.74 % were classified as extensive drug-resistant XDR, 60.87% were multidrug-resistant (MDR), 17.39% were non-MDR, non-XDR and 0.0% were resistant to all antibiotics S. Typhi and the comparison between Pre and Post-COVID-19 sampling show in Table 5. (Table5), resistivity in Pre-COVID-19 isolates show in Figure 2.

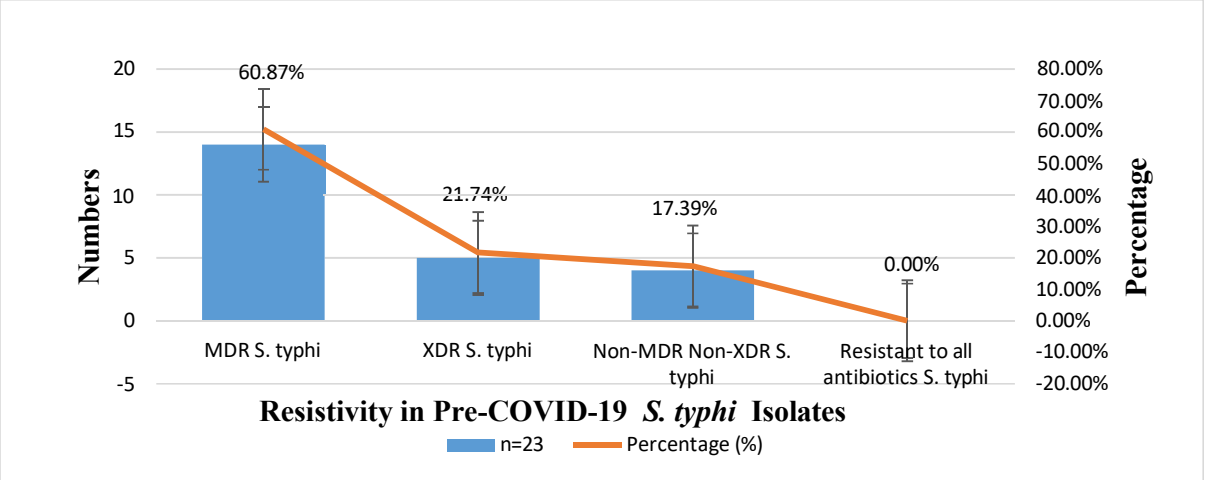

Figure 2: The Emergence Ratio of XDR and MDR in Pre-COVID-19 *Salmonella typhi* isolates.

Table 5: The Emergence of MDR, XDR, and Resistant to all antibiotics typhoid Comparison in Pre and Post-COVID-19 Sampling.

Resistivity in Positive Cultures of *Salmonella typhi* during Pre & Post-COVID-19 isolates				
Cultures	Pre-COVID-19		Post-COVID-19	
Isolates of S. Typhi	n=23	Percentage (%)	n=34	Percentage (%)
MDR S. Typhi	14	60.87%	13	38.24%
XDR S. Typhi	5	21.74%	15	44.12%
Non-MDR Non-XDR S. Typhi	4	17.39%	3	8.82%
Resistant to all antibiotics S. Typhi	0	0.00%	3	8.82%

Discussion

The World Health Organization, commonly known as the WHO, claims that the world experiences up to twenty-one million cases of typhoid infection annually, with a fatality rate ranging from around more than 128,000 to 161,000 mortalities per year as a result of the disease. In under develop countries systemic infection is a common kind of sickness. The highly resistant strain affects both the native populations and individuals traveling to regions where typhoid fever is endemic [26]. Typhoid fever exhibits a significant global burden, with an estimated annual incidence of approximately 21 million cases per year. The disease burden of enteric fever in Pakistan is significantly elevated due to inadequate sanitation and densely populated urban areas, resulting in an annual incidence rate of 493.5 cases per 100,000 individuals [2].

The emergence of extensively drug-resistant typhoid is increasing in Post-COVID-19 isolates of *Salmonella typhi*. The main reason is the use of extended drugs without culture sensitivity tests for typhoid treatment during the COVID-19 pandemic. The emergence of extensively drug-resistant typhoid into the azithromycin resistant which is the last effective drug against *Salmonella typhi* infection. Azithromycin use during COVID-19 was the most common. [27].

Several indirect factors may impact the rise the extensively drug-resistant (XDR) typhoid cases during the COVID-19 pandemic. For example, changes in life style, behavior and consumption of food and water resources during lockdowns due to COVID-19 pandemic. Some direct factors for XDR typhoid cases such as COVID-19 directly or indirectly effect the immune system, making individuals more susceptible to infections such as typhoid fever. Healthcare systems have also been affected by the COVID-19 epidemic, which can lead to delays in typhoid diagnosis and treatment [28].

In COVID-19 diversion of resources and healthcare staff may result in delayed diagnosis, inadequate treatment, and increased emergence of XDR typhoid [29]. XDR typhoid fever has been misdiagnosed by the use of Typhi DOT and Widal as serological tests by several medical practitioners during COVID-19, leading to the emergence of XDR typhoid [30]. In COVID-19 patients with secondary infection have been treated with extended drugs as well as azithromycin in Pakistan, which was the main cause of extensive drug-resistant (XDR) typhoid [31].

A significant incidence of typhoid fever, which was resistant to ceftriaxone, commenced in the city of Hyderabad in November 2016. The onset of the epidemic originated in Hyderabad and subsequently disseminated to neighboring urban centers, such as Karachi. In the cities of Hyderabad and Karachi, more than 10,000 cases of extensive drug-resistant (XDR) typhoid were registered in August 2019 [18, 19].

In Pakistan, a significant proportion of *Salmonella typhi* cases, approximately 95%, exhibited resistance to ciprofloxacin, which is commonly prescribed as the initial treatment option. There was a notable prevalence of resistance observed against ciprofloxacine and the injectable antibiotic, ceftriaxone, in the context of *Salmonella typhi*. The drugs Azithromycin and Imipenem exhibited a sensitivity exceeding 90% [32].

There has been a notable increase in the prevalence of highly resistant fluoroquinolone infections, particularly in South Asia, where they now add up the majority of typhoid cases. This emergence creates a significant threat to global public health, as there is a potential increase in transmission. Consequently, healthcare professionals have come to rely on azithromycin and cephalosporins as the final resort in treatment, supported by clinical trial evidence [16].

The emergence of extensively drug-resistant (XDR) typhoid fever has raised significant concerns due to the limited treatment options available. According to available reports, there has been a noted decline in the susceptibility to azithromycin [33]. According to Saderi *et al.* (2015) study before the COVID-19 there was, 48 (54.5%) and 29 (33%) isolates were characterized as Multidrug resistant and extensive drug resistant, respectively [34].

This study shows that there was less extensive drug resistant as compare to the Multidrug resistant. The present study results also showed that before the COVID-19 pandemic there was less emergence of extensive drug resistance typhoid as compare to the Multidrug resistant typhoid. This study showed that 23 positive cultures of *Salmonella typhi* isolates obtained before the COVID-19 pandemic. Among these isolates, 14 (60.87%) were identified as multidrug-resistant strains, while 5 (21.74%) were extensively drug-resistant (XDR) strains. The results justified that before COVID-19 extensive drug-resistant typhoid was less as compared to the Multidrug-resistant.

But when this study performed on Post-COVID-19 isolates of *Salmonella typhi* there was an increased number of extensive drug-resistant typhoid as compared to multidrug-resistant typhoid. Out of these isolates, 13 (38.24%) were found to be multidrug-resistant strains, while 15 (44.12%) were extensively drug-resistant strains. Only 3 isolates (8.82%) were identified as non-MDR non-XDR strains, and 3 isolates (8.82%) were resistant to all tested antibiotics. According to the Britto *et al.* (2018) study specifically, the emergence of extensive drug-resistant rates has risen from 34.2% to 48.5% during this period of COVID-19 [35].

This research with azithromycin resistance and increased XDR strains of *Salmonella typhi* after COVID-19 pandemic is an alarming situation. This resistance occurred due to antibiotic prescriptions without the microbiological culture and antibiotic susceptibility profiling of *Salmonella typhi* during COVID-19. To prevent future outbreaks of all antibiotic-resistant *Salmonella typhi*, it was significant that additional therapeutic alternatives and urgent implication of action plan against irrational use of antimicrobial agents.

Conclusion

There is increase emergence of XDR typhoid cases in Post-COVID-19 isolates of *Salmonella typhi* as compare to the Pre-COVID-19 *Salmonella typhi* isolates. All this due to misdiagnosis during of *Salmonella typhi* during COVID-19, additional use of extensive drugs against typhoid fever and secondary infections during COVID-19 pandemic without antimicrobial susceptibility profiling against causative agent. Emergence of azithromycin resistant typhoid is due to common use of azithromycin in treating the COVID-19 patients while neglecting the XDR typhoid pandemic. To prevent future outbreaks of all antibiotic-resistant *Salmonella typhi*, it is significant that additional therapeutic alternatives and urgent implication of action plan against antimicrobial agents. Moreover, implementation of antimicrobial susceptibility profiling and proper diagnosis before use of any antibiotic against *Salmonella typhi* prevent the XDR typhoid outbreak in near future.

Contributions of the writers

The final manuscript was reviewed and approved by all writers. Study designed, write up and lab experiments performed by Safdar Ali. Muhammad Moazam Jalees and Waqas Ashraf supervised all experiments and helped in review. Maimona Sadia and Rafia Anwar helped in sampling. Saleha Zafar and Tariq Mahmood did help in data collection. Muhammad Rizwan helped in statistical analysis. Abdul Mannan did help in patient history and sample transportation.

Acknowledgments

Chlolistan University of Veterinary and Animal Sciences researcher Mr. Safdar Ali awardee the master's degree with the notification number CE/DMC/PG/1949 with registration number 21-CUVAS-0143 at Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan.

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