CURRENT PREVALENCE RATE OF ESBL PRODUCTION IN *SALMONELLA TYPHI* AMONG MULTIDRUG-RESISTANT (MDR) STRAINS AT CITI LAB AND RESEARCH CENTER (CRC)

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ABSTRACT

Background: This study is conducted to establish the epidemiology of rate of occurrence of extended-spectrum β-lactamase (ESBL) production among multidrug-resistant (MDR) *Salmonella typhi* isolates during the whole period of the year.

Methods: Among the 327 blood cultures samples 153 found to be MDR *S. typhi* isolates. For the identification of isolates API-20 E system was used, and Kirby-Bauer method of disc diffusion for antibiotic sensitivity was applied. Double-disc synergy test was performed to check ESBL-production.

Results: Susceptibility among anti-typhoid drugs of first-line was as follows: chloramphenicol (27.3%), ampicillin (21.8%), and trimethoprim/sulfamethoxazole (18.8%). Among the quinolone ciprofloxacin susceptibility was lower at 31.5%. The number of MDR *S. typhi* isolates significantly increased from Jan 2020 to December 2024. Only five isolate 3.0% were identified as an ESBL producer.

Conclusion: Throughout the year typhoid fever stays endemic. The highest incidence was observed during hot seasons and was lowest in chilly season Although cephalosporins of 3rd generation have decreased the incidence of MDR *S. Typhi*. These cephalosporins, along with gentamycin and carbapenems were highly effective for MDR*S. typhi.*

Key words: Salmonella, Typhoid, S. typhi, Prevalence, Multi Drug Resistance, ESBL, Pakistan, Enteric fever, Antibiotics, Resistance, Lahore.

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INTRODUCTION

Typhoid fever remains one of the significant public health issues, especially in Pakistan. According to WHO data 2019, typhoid causes illness in 9 million people and kills 110,000 of them annually. In Pakistan, typhoid fever ranks as the fourth leading cause of death, with mortality rates ranging between 16% and 36% in different Asian countries.[1, 2].

The burden of typhoid fever is often under reported in developing nations because of the lack of culture facilities along with improper reporting systems[3]. However, this disease can be controlled effectively through proper medical treatment, clean drinking water, improved sanitation, and better hygiene practices [4].

The course of typhoid fever was transformed when in 1948 chloramphenicol was introduced; illness was reduced to days from weeks and death rate to below 1% [5]. It immediately became a gold standard for treatment around the world because it had efficacy that was reproducible over time [6].

Ampicillin was introduced in the 1960s as a very effective alternative for the treatment of chloramphenicol-resistant infections [7]. Later, trimethoprim/sulfamethoxazole was also proved effective, especially in cases resistant to both chloramphenicol and ampicillin [8].

MDR S. typhi was first documented in Pakistan in 1987 and quickly increased to more than 80% during the 1990s [9]. For MDR isolates Floro quinine was used which proved effective but after a short time it also

become resistant. 3rd Generation cephalosporin were used against the resistance strains of MDR S typhi which were prove excellent for treatment.[10].

Although there have been sporadic reports of third-generation cephalosporin-resistant MDR S. typhi. 3rd Generation cephalosporin resistant strains of MDR S typhi showed ESBL production [11]. This new resistance mechanism significantly limits the treatment options and raises a concern about the increasing threat of resistant strains in resource-limited settings.

METHODOLOGY

Total of 327*Salmonella typhi* isolates were recovered from blood cultures from patients at CRC lab Lahore, over the period about five years from Jan 2020-dec 2024. In the study one positive culture for every patient was selected and included. Samples were cultured using MacConkey agar, chocolate and blood agar and these plates were aerobically incubated at 37°C for 24 hours. Upon no observation of growth of bacteria past 48 hours of incubation, subcultures were taken on 5th day and 7th day. identification Isolate was based on colony morphology, Gram staining, and biochemical identification by API-20 E system.

For antimicrobial susceptibility Kirby-Bauer disc diffusion method was performed according to CLSI guidelines. The antibiotics included ampicillin 10 mg/L, chloramphenicol 30 mg/L, trimethoprim 1.25 mg/L, sulfamethoxazole 25 mg/L, ceftriaxone 30 mg/L, cefotaxime 30 mg/L, ceftazidime 30 mg/L, ciprofloxacin 5 mg/L, and nalidixic acid 30 mg/L, among others.

Zones of inhibition (ZOI) were measured by Vernier calipers to determine the point at which complete bacterial growth inhibition occurred. Sensitivity and Resistance was determined according to the CLSI sensitivity chart.

For the production of ESBL a double disc synergy test (DDST) was conducted using co-amoxiclav (a combination of amoxicillin and clavulanic acid) Zone of the isolates show ZOIs less than 22 mm for ceftazidime or less than 27 mm for cefotaxime,

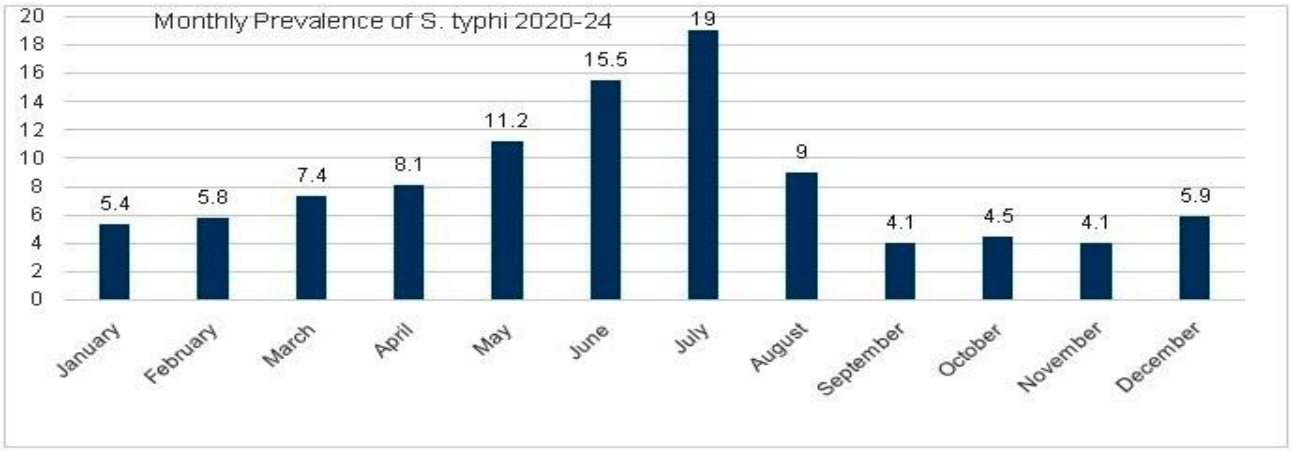
For quality control, the study used a number of reference strains under the same conditions. Among them were *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC 27853), *Enterococcus faecalis (E. faecalis)* (ATCC 29212) *Escherichia coli(E. coli)*(ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Acinetobacter baumannii(A. baumannii)*(ATCC 29212).

RESULTS

A total of 327*S. typhi*-infected patients were identified during the study, with 55% of cases being male. Categorization of patients was into three major age groups: children of 5 to 15 years, adults from 16 to 60 years, and elders of 61 to 85 years. Most cases were observed among children aged 5–15 years (Table 1).

Age Group (Years)	Total Cases (%)	Male (%)	Female (%)
Children (5–15)	173 (52.5%)	92 (53.5%)	81 (46.5%)
Adult (16–60)	145 (44.8%)	82 (56.5%)	63 (43.5%)
Elder (61–85)	9 (2.7%)	6 (66.5%)	3 (33.5%)

Isolation of *S. typhi* was recorded in every month during the study period (January 2020 to December 2024). There was an increasing trend of the total *S. typhi* isolations from 2020up to 2024. The highest occurrence rates (11.2 to 19%) were seen during May, June and July which decreases during the cold and dry seasons. The lowest rates of incidence (4.1% to 5.8%) were recorded during November, December, January, and February. The monthly breakdown of cases is shown in bar chart 1.



Bar chart 1: Monthly isolation of MDR S. typhi from 2020 to 2024.

Table 2: Isolation Rate of MDR S. typhi.

Year	Total Cases	MDR Isolates	% MDR Isolates
2020	53	19	35.8%
2021	64	26	40.6%
2022	65	31	47.6%
2023	77	43	55%
2024	68	34	50%

Table 3: Susceptibility Pattern of S. typhi.

Antibiotic	Susceptible	Resistance
Chloramphenicol	30.3%	69.7%
Ampicillin	24.6%	75.4%
Trimethoprim/Sulfamethoxazole	21.5%	78.5%
Ciprofloxacin	35.1%	64.9%
Nalidixic Acid	8.6%	91.4%
Ceftriaxone	47.6%	52.4%
Cefotaxime	49.9%	50.1%
Ceftazidime	51.3%	48.7%
Gentamycin	96.6%	97.3%
Imipenem	95.8%	4.2%
Meropenem	97.4%	2.6%

DISCUSSION

Typhoid fever which is caused by *Salmonella Typhi* is declared as fourth common cause of death in Pakistan and according to WHO, 9 million people fall ill and 110,000 die annually due to typhoid fever.[12]In this study (55%) male patient have been reported which is accordance with the studies reported earlier. This is mainly due to more frequent outdoor interactions and consumption of street food. [13, 14] Moreover highest number of cases were found in patients ranging from age of 1 to 15 years old given their high interaction with contaminated surfaces and food and this study is in line with those published recently. [13] Maximum prevalence was observed in humid and hot months of June and July that dropped in the cold season which is also reported in China and different regions of Pakistan mainly because of ideal conditions for pathogen growth, increased water consumption and improper sanitation. [14-16] In the study isolation of MDR S. typhi gradually peaked from 2020 to 2024 during COVID-19 pandemic phase and as it gradually ended. The most important factors contributing to this were self-Medication, misuse of antibiotics and the development of diagnostic facilities in Pakistan. [17, 18] Improper infection control measures are also a major contributor to the upward trend of prevalence. Chloramphenicol, ampicillin and sulfamethoxazole (1st line antibiotics) nalidixic acid and ciprofloxacin (2nd line antibiotics) were resisted by a high number of isolations showing very low susceptibility. Reports of high resistance for 1st and 2nd line antibiotics have been recently published. [6, 19, 20] On the other hand imipenem and meropenem showed highest drug sensitivity for *S. typhi* and are considered as the only remaining treatment for *S. typhi* infection. [14, 20] ESBL production rate was found very low with only 3% of the total isolation during the timeline yet it has increased from the recent reports available.[21].

Conclusion: This study shows increase trend of MDR S. *Typhi* gradually from 2020 to 2024. ESBL production have been observed in the study which alarms us to control the prevalence of disease. Two decades earlier 1st line anti typhoid drug were effective but currently they are showing ineffectiveness against typhoid.

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