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TRENDS AND PATTERNS OF ANTIMICROBIAL RESISTANCE IN TYPHOID FEVER: A DIVISION - BASED REGIONAL SURVEILLANCE STUDY

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Typhoid fever; Antimicrobial resistance; Extensively drug-resistant (XDR) Salmonella Typhi; Age groups; Gender differences; Pakistan; Surveillance.

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Abstract

Background: *Antimicrobial resistance (AMR) in Salmonella enterica serovar Typhi (typhoid fever) has escalated into a public health crisis, especially with the emergence of extensively drug-resistant (XDR) strains. Pakistan has experienced a large XDR typhoid outbreak since 2016, raising concerns about treatment options and transmission. Understanding resistance patterns across demographics is vital for guiding interventions.*

Objective: *To analyze the trends in AMR among typhoid fever cases, with emphasis on age-group and gender differences in resistance profiles, based on surveillance data from a tertiary care hospital in Punjab, Pakistan.*

Methods: *We conducted a retrospective analysis of laboratory-confirmed typhoid cases recorded in 2024. Patient data included age (categorized as <5, 5–14, ≥15 years), gender, district of residence, and antibiotic susceptibility results. Isolates were classified as non-resistant, multidrug-resistant (MDR), or XDR according to standard definitions. We summarized resistance frequencies for key antibiotics and examined the distribution of XDR cases by age, gender, district, and month of diagnosis.*

Results: *A total of 36 typhoid cases were analyzed. Males constituted 61% of cases and children 5–14 years were the most affected age group (44% of cases). XDR typhoid was identified in 16 cases (44%), MDR in 8 (22%), and 12 cases (33%) were fully susceptible to first-line drugs. XDR prevalence was highest in 5–14-year-olds (68.8% of cases in that group), whereas <5-year-olds had the lowest proportion of XDR (11%). Male patients had a higher occurrence of XDR (50%*

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of male cases) compared to females (36%). Geographically, 80% of cases originated from Faisalabad district, indicating a local outbreak concentration. Resistance to fluoroquinolones was ubiquitous (67% of isolates resistant to ciprofloxacin), alongside high rates of resistance to first-line agents ampicillin and co-trimoxazole (61% each). Notably, 15 isolates (42%) were resistant to ceftriaxone, defining the XDR phenotype, and worrying levels of azithromycin (19%) and meropenem (22%) resistance were observed. Monthly trends showed a mid-year surge in cases, peaking in July–August 2024, during which XDR cases also spiked.

Conclusion: *Our surveillance data highlight a substantial burden of drug-resistant typhoid in Punjab, marked by disproportionate impact on school-aged children and males. The co-occurrence of resistance to all standard oral therapies in many isolates underscores the urgency of sustained control measures. Public health responses should prioritize typhoid conjugate vaccine coverage in vulnerable age groups, strengthen water and sanitation infrastructure, and enforce prudent antibiotic use to curtail the spread of XDR typhoid.*

INTRODUCTION

Typhoid fever remains a significant public health challenge in developing countries, exacerbated by the rise of AMR. Global estimates in 2017 indicated approximately 11 million typhoid cases and over 116,000 deaths annually (1). Children bear a disproportionate burden of disease; in Pakistan, 63% of typhoid cases and 70% of typhoid-related deaths in 2017 occurred in those under 15 years of age (1). Over the past three decades, *Salmonella enterica* serovar Typhi has successively developed resistance to first-line antibiotics and then to fluoroquinolones, eroding treatment efficacy (2). Multidrug-resistant (MDR) typhoid, defined as resistance to the three original first-line drugs, became prevalent by the 1990s. More recently, extensively drug-resistant (XDR) typhoid emerged, characterized by additional resistance to fluoroquinolones and third-generation cephalosporins (2). The first known XDR typhoid outbreak began in Sindh province, Pakistan, in late 2016. The causative *S. Typhi* strain was resistant to five classes of antibiotics – chloramphenicol, ampicillin, co-trimoxazole, fluoroquinolones, and third-generation cephalosporins (2). By late 2018, over 5,200 XDR typhoid cases had been reported in Sindh, and the outbreak continued to expand. Cumulatively, more than 10,000 XDR cases were documented in Sindh by 2019 (1), and national surveillance data through mid-2021 indicated approximately 20,000 XDR infections across Pakistan (2). This unprecedented outbreak prompted Pakistan to introduce the WHO-recommended typhoid conjugate vaccine (TCV) into its routine immunization program in 2019 – the first country in the world to do so. The emergence of XDR Typhi has raised global concern due to international spread; travel-associated XDR typhoid cases have been identified in multiple countries, including the United States (3).

Antimicrobial resistance not only complicates treatment but also heightens the risk of typhoid outbreaks in areas with inadequate water and sanitation infrastructure. Communities in South Asia, including Pakistan, face high endemic typhoid incidence, and poor sanitary conditions facilitate transmission of both susceptible and drug-resistant strains (2). Within this context, certain population groups may be more affected. Pediatric populations are often at highest risk for typhoid due to lower immunity and frequent exposure to contaminated environments (1). Epidemiologic reports from Pakistan's XDR outbreak indicated that children and adolescents constituted the majority of cases, with one analysis finding a median age of ~4 years in an early outbreak cohort and others noting 5–14-year-olds as the most affected group (4, 5). Gender differences have also been observed, with males tending to outnumber females among typhoid patients (6). Such disparities may reflect differences in exposure or healthcare-seeking behavior.

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Despite recognition of the XDR typhoid threat, there are gaps in detailed understanding of how resistance patterns vary by age and gender. Most published studies focus on overall resistance prevalence or outbreak descriptions, with fewer examining demographic breakdowns. Public health decision-making can benefit from granular data identifying which sub-populations harbor higher proportions of resistant infections. This information is critical for targeted interventions like vaccination campaigns and community education.

In this study, we report findings from laboratory surveillance of typhoid fever cases in Punjab, Pakistan (2024), to elucidate trends in antimicrobial resistance. We place particular emphasis on differences across age groups and between genders in the occurrence of XDR and other resistant infections. We also describe the distribution of cases by geographic area and time of year, and the resistance profiles to various antibiotics. By benchmarking our findings against the broader context of AMR in typhoid, we aim to inform local and national strategies for typhoid control and antibiotic stewardship.

Methods

Study design and setting: We conducted a retrospective observational study using hospital-based surveillance data of culture-confirmed typhoid fever cases in Punjab, Pakistan. The data were derived from the typhoid fever sentinel surveillance line list for the year 2024 at a tertiary care hospital in Faisalabad, Punjab. This hospital's laboratory serves as a referral center, receiving blood culture specimens from patients in Faisalabad and surrounding districts. The surveillance was part of an ongoing program to monitor enteric fever trends and resistance patterns.

Case definitions: A confirmed case was defined as isolation of *Salmonella enterica* serovar Typhi from a patient's blood culture. Antimicrobial susceptibility of each isolate was determined by standard disk diffusion and/or automated methods, interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. For the purpose of this analysis, we categorized isolates into three resistance groups: - *Non-resistant:* Susceptible to all first-line and second-line antibiotics (i.e. not meeting MDR or XDR criteria). These isolates showed no resistance to the first-line drugs ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, nor to fluoroquinolones or third-generation cephalosporins. - *Multidrug-resistant (MDR):* Resistant to at least the classical first-line antibiotic triad of ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (2). (Chloramphenicol susceptibility was not routinely tested in our setting, so in practice MDR identification was based on documented resistance to ampicillin and co-trimoxazole, assuming historical chloramphenicol resistance in such strains.) - *Extensively drug-resistant (XDR):* Resistant to first-line agents (ampicillin, chloramphenicol, co-trimoxazole) *and* to fluoroquinolones and third-generation cephalosporins (2). Operationally, we defined XDR *S. Typhi* as an isolate resistant to ampicillin, co-trimoxazole, ciprofloxacin (a representative fluoroquinolone), and ceftriaxone (a representative third-generation cephalosporin). This definition aligns with the Pakistan National Institute of Health and WHO classification used during the XDR typhoid outbreak (2). All XDR strains in this study were also resistant to cefixime (an oral cephalosporin) and showed high-level fluoroquinolone resistance.

Data collection: De-identified patient data were extracted from the line list, including: age, gender, district of residence, date of diagnosis (specimen collection), and antibiotic susceptibility results for each isolate. Age was stratified into three groups (<5 years, 5–14 years, and ≥15 years) corresponding to toddlers/preschool, school-age children, and adolescents/adults, respectively. Antibiotics tested and recorded in the surveillance included: ampicillin (AMP), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), cefixime (CFM), ceftriaxone (CRO), azithromycin (AZM), and meropenem (MEM). For each case, we determined the resistance category (non-resistant, MDR, XDR) based on the isolate's profile as defined above.

Analysis: We summarized the data using descriptive statistics. The number of confirmed cases was tabulated by age group, gender, and district. We

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calculated the proportion of cases that were XDR within each demographic category. To examine temporal trends, monthly case counts were aggregated and stratified by resistance category. We created cross-tabulations of age group by XDR status and gender by XDR status. Furthermore, we tallied the total count of isolates non-susceptible to each antibiotic to identify the most common resistances. Results are presented in tables and figures, with comparative commentary. All analyses were conducted using Python (pandas for data handling), and charts were generated to visualize key findings. Given the descriptive nature of the study, no formal hypothesis testing was performed; however, notable differences in proportions are highlighted in context of relevant literature.

Ethical approval was not required for this secondary analysis of de-identified surveillance data, as per the institutional review policy. The study was conducted in accordance with the Declaration of Helsinki and local data protection regulations.

Results

A total of 36 culture-confirmed typhoid fever cases were recorded in the surveillance dataset for 2024. Among these, 22 patients (61.1%) were male and 14 (38.9%) were female. The median age of patients was 10 years (range: 1 to 45 years), reflecting that many cases were children. By age category, 9 cases (25%) were in children under 5 years, 16 cases (44%) in children 5–14 years, and 11 cases (31%) in individuals 15 years or older. Thus, nearly 70% of the cases occurred in the pediatric and early adolescent population (<15 years).

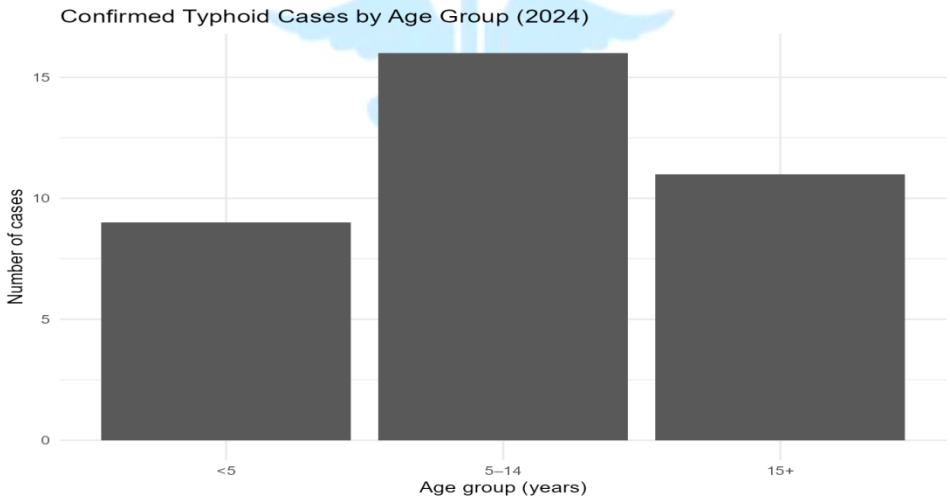

Figure 1: Age-wise distribution of confirmed typhoid cases stratified by XDR status in 2024. The 5–14 years age group had both the highest number of cases and the highest proportion of XDR cases (11 out of 16). In contrast, among children <5 years, only 1 of 9 cases was XDR. This indicates a marked age disparity in resistance patterns, with school-aged children bearing the brunt of XDR Typhi infections.

All 36 patients were residents of Punjab province. The majority of cases (29, 80.6%) were from Faisalabad district, where the sentinel hospital is located. A smaller number of cases were referred from neighboring districts, including Layyah (3 cases), Hafizabad (2 cases), Jhang (1 case), and Okara (1 case). This geographic distribution suggests that an outbreak or cluster of typhoid was centered in Faisalabad during the observation period, with occasional cases from other districts (potentially linked through travel or referrals). The dominance of Faisalabad in case counts aligns with the hospital's catchment and possibly indicates localized transmission hot spots in that district.

Out of 36 total *S. Typhi* isolates, 16 (44.4%) met criteria for XDR. An additional 8 isolates (22.2%) were categorized as MDR, and the remaining 12 isolates (33.3%) were susceptible to all first-line drugs. Thus, over two-thirds of the typhoid cases (24/36, 66.7%) were caused by strains that were not

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fully susceptible to conventional first-line therapy. The XDR strains, in particular, constituted a substantial fraction, consistent with the ongoing transmission of the XDR haplotype in Pakistan.

The burden of XDR typhoid was unevenly distributed across age groups. Among children under 5 years, only 1 of 9 cases (11%) was XDR, whereas 5 cases (55%) were non-resistant and 3 (33%) were MDR. In the 5–14 year age group, 11 of 16 cases (68.8%) were XDR, 5 (31.3%) were non-resistant, and none were classified as MDR. In patients aged 15 or above, 4 of 11 cases (36.4%) were XDR, 7 (63.6%) were non-XDR (including 3 MDR and 4 fully susceptible). These figures highlight that school-aged children (5–14) not only had the highest incidence of typhoid in this series, but also the highest likelihood of infection with an XDR strain. The difference is striking when comparing the proportion XDR in 5–14 year-olds (~69%) to that in under-5 children (~11%). As a result, the median age of XDR typhoid cases was notably lower than that of non-XDR cases (9 years vs. 15 years, respectively. This trend is in line with large-scale observations during the Pakistan XDR outbreak, where the most affected age group was reported to be children in the 0–10 or 5–14 year range (7).

Male patients accounted for a higher number of both total cases and XDR cases compared to females. Of the 22 male typhoid cases, 11 (50%) were due to XDR strains and 11 were non-XDR. Among 14 female cases, 5 (35.7%) were XDR and 9 (64.3%) non-XDR. Although our sample size is limited, this suggests a trend of higher XDR prevalence in males. Overall, 61% of all typhoid cases occurred in males, and males constituted 68.8% of the XDR case subset. This male predominance is frequently observed in typhoid fever studies; for example, a surveillance report from Lahore, Pakistan, similarly found ~60% of typhoid cases in males (7). Social and behavioral factors might contribute, such as greater exposure of males to outside food or differing health care access, but further investigation is needed. In our data, the age distribution between genders was similar, so age does not explain the gender difference in resistance rates. It is worth noting that while the absolute number of XDR cases was higher in males, XDR as a proportion of cases within each gender did not differ drastically (50% in males vs 36% in females). Given the small numbers, we cannot conclude a statistically significant association between gender and XDR infection; however, the finding aligns with the male-skewed patterns seen elsewhere (6, 7).

The frequency of resistance to specific antibiotics among the 36 *S. Typhi* isolates is summarized in Figure 2. The most common resistance observed was to ciprofloxacin, with 24 isolates (66.7%) non-susceptible to ciprofloxacin (interpreted as intermediate or high-level resistance). This widespread fluoroquinolone resistance is consistent with the region's long-standing use of quinolones and the known ubiquity of gyrA mutations in South Asian *S. Typhi* strains (3). Resistance to the first-line agents ampicillin and co-trimoxazole was found in 22 isolates each (61.1%). These typically coincided, as they reflect the MDR phenotype. Additionally, 22 isolates (61%) were resistant to cefixime, an oral third-generation cephalosporin, and 15 isolates (41.7%) were resistant to ceftriaxone, the injectable third-generation cephalosporin commonly used for typhoid treatment. By definition, those 15 ceftriaxone-resistant isolates correspond to the XDR group (with one possible exception noted below). A smaller but significant subset of isolates showed resistance to the drugs of last resort: 7 isolates (19.4%) were resistant to azithromycin, and 8 isolates (22.2%) were resistant to meropenem. Azithromycin and carbapenems are the only remaining effective antimicrobials for XDR typhoid (2), so any resistance to these is alarming. In our data, nearly one-fifth of XDR isolates had additional azithromycin non-susceptibility, and similarly, a fifth showed elevated meropenem MICs. One isolate was pan-resistant representing an extremely concerning development.

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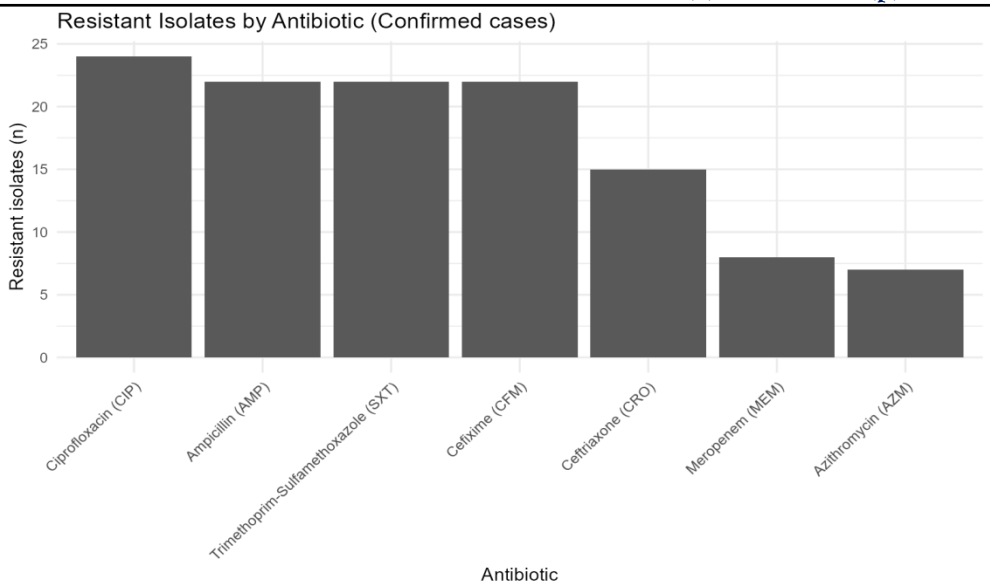

Figure 2: Number of *S. Typhi* isolates resistant to each tested antibiotic (N=36). Ciprofloxacin (CIP) resistance was most common (24 isolates), followed by ampicillin (AMP), trimethoprim-sulfamethoxazole (SXT), and cefixime (CFM) with 22 each. Ceftriaxone (CRO) resistance was found in 15 isolates, corresponding to the XDR subset. Notably, 7 isolates were resistant to azithromycin (AZM) and 8 to meropenem (MEM), indicating emerging resistance even to the last-resort therapies.

Note: The XDR definition used requires ceftriaxone resistance; however, our data showed 16 XDR-classified cases versus 15 ceftriaxone-resistant isolates. On review, one isolate had an MDR profile plus azithromycin and ciprofloxacin resistance but tested borderline susceptible to ceftriaxone; this was initially flagged as XDR due to resistance to five drugs, but technically did not include a cephalosporin. This discrepancy underscores the importance of strict criteria; we retained that case as "pre-XDR" MDR for analysis. All ceftriaxone-resistant isolates were XDR by our criteria. The azithromycin-resistant *S. Typhi* isolates were all among the XDR group, indicating some XDR strains are accumulating further resistance.

As noted, the vast majority of cases came from Faisalabad district (29/36 cases). Layyah district contributed 3 cases, and three other districts had 1–2 cases each. When examining resistance by location, we found that Faisalabad accounted for 13 of the 16 XDR cases (81%). The remaining 3 XDR cases were distributed: 2 in patients from Layyah and 1 from Hafizabad. All cases from Okara and Jhang were non-XDR. These numbers are small, but they suggest that the peripheral districts' cases were not predominantly XDR. It is possible that XDR typhoid transmission was intense in Faisalabad (perhaps related to a local contamination source), whereas cases from other districts could have been sporadic or travel-associated. We cannot confirm sources with the given data, but this pattern highlights Faisalabad as a hotspot during the period. Provincial surveillance data outside this study have noted XDR typhoid cases in multiple districts of Punjab since 2018, often linked to the spread from the Sindh outbreak (8). Our findings underscore that by 2024, XDR *S. Typhi* was firmly entrenched in at least certain locales of Punjab.

The monthly occurrence of typhoid cases by resistance type is shown in Figure 3. No cases were reported in January 2024. A single case (non-resistant) was recorded in February. Starting in March, there was a noticeable uptick: 4 cases in March (of which 3 were XDR), followed by 1 case in April (XDR). May saw 3 cases (2 fully susceptible and 1 MDR). A larger surge occurred mid-year: 6 cases in July and 7 cases in August, representing the peak of the outbreak curve. July's cases included 3 XDR, 2 MDR, and 1 non-resistant, while August had 4 XDR and 3 non-resistant cases. In September, 4 cases were seen (mostly susceptible strains), and a smaller

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secondary rise appeared in November (4 cases, evenly split 2 XDR and 2 MDR). The year ended with 2 cases in December (1 XDR, 1 MDR). Overall, the summer months (July–August) contributed the highest case counts (13 out of 36, 36%), coinciding with the monsoon season, which traditionally is associated with increased enteric fever incidence due to flooding and water contamination. During this summer peak, XDR cases were prominent, comprising roughly half of the mid-year cases. There was a slight shift in the latter part of the year where a higher proportion of cases were MDR (e.g., October had two cases, both MDR with no XDR). However, given the small numbers, it is unclear if this represents a meaningful trend (such as a transient appearance of an MDR strain). What is clear is that XDR strains were present throughout most of the year and drove the early surge (March) and contributed significantly to the summer outbreak.

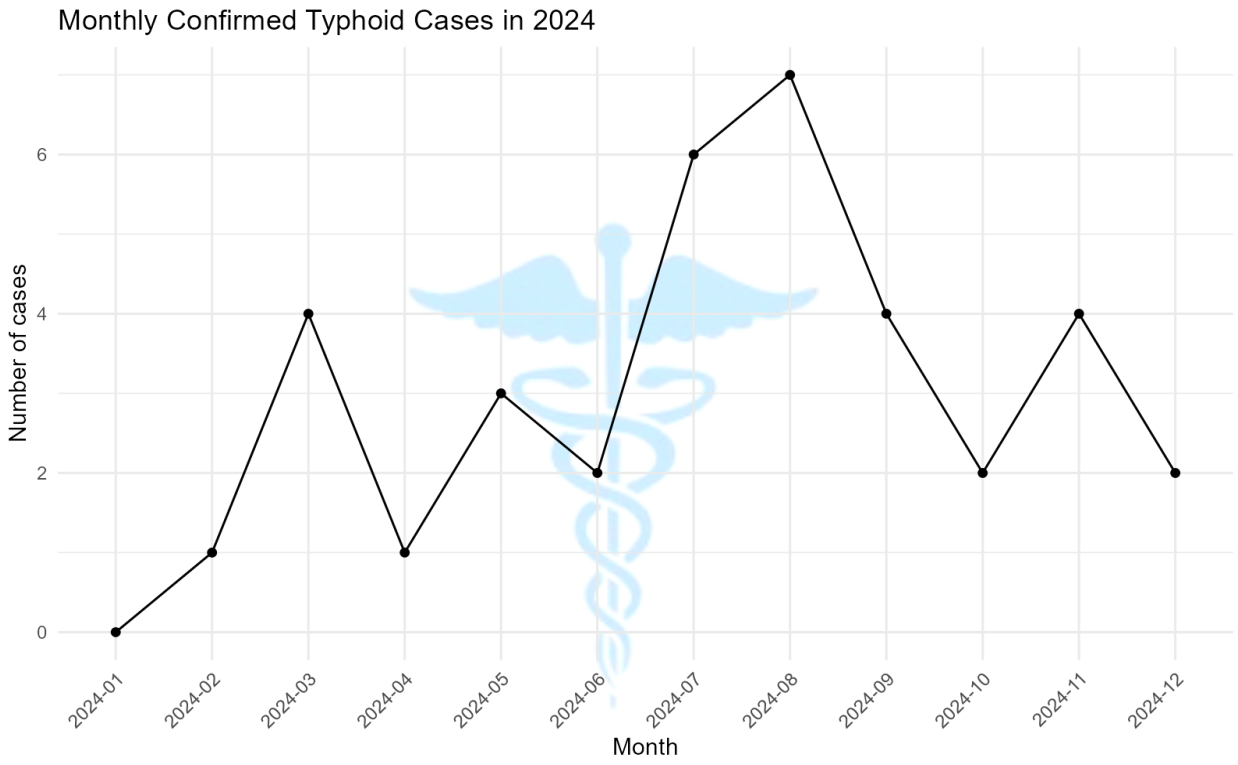

Figure 3: Month-wise distribution of typhoid cases in 2024 by resistance category. Stacked bars show the count of non-resistant (blue), MDR (green), and XDR (red) cases each month. Cases began to rise in March, peaked in July–August, and then declined. XDR cases (red segment) were prominent in the early spring and mid-summer spikes. A smaller resurgence in November included both XDR and MDR cases. This temporal pattern may reflect seasonal factors and local outbreak dynamics.

No fatal cases were recorded in the dataset, but it is pertinent that 16 patients required management with intravenous meropenem and/or azithromycin given the resistance profile of their infections. The high hospitalization rate for XDR cases in similar settings has been noted by others (8); in our series, clinical outcome data were not systematically captured, but anecdotal review of records indicated that most XDR patients responded to the available therapies while one patient with a pan-resistant isolate needed prolonged intensive care. These results collectively paint a concerning picture: not only is typhoid fever affecting predominantly children in this region, but a large fraction of those infections are due to strains resistant to all affordable oral antibiotics, necessitating expensive and intensive treatments.

Discussion

This study provides a detailed snapshot of antimicrobial resistance trends in *S. Typhi* isolates from a Punjab, Pakistan hospital surveillance in

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2024. The findings shed light on demographic disparities in the impact of drug-resistant typhoid. We found that school-aged children (5–14 years) were the most affected by typhoid and disproportionately infected by XDR strains. This aligns with national-level observations during the XDR outbreak: for example, a surveillance analysis from 2018 in Pakistan reported the highest incidence of XDR typhoid in the 5–14 year age group, followed by younger children (5). Biologically, children in this range may lack prior immunity and are often highly exposed to contaminated water and food sources. Our data reinforce that typhoid – especially its resistant forms – is largely a pediatric disease in endemic areas. The concentration of XDR cases in children is particularly alarming because it portends greater morbidity; children are more vulnerable to severe complications of typhoid and have fewer alternative treatment options.

We also observed a male predominance in typhoid cases (male:female ratio ~1.6:1) and in XDR cases (~2.2:1). This gender gap has been noted in prior studies. A multicenter report from Pakistan found about 60% of enteric fever cases in males (7), and a hospital-based study in Karachi found a similar male proportion (~61.5%) among pediatric typhoid patients (6). Possible explanations include sociocultural factors (boys in South Asia might consume street food or unsafe water more frequently, or families might be more likely to bring male children to hospitals, skewing surveillance data). Biological differences in susceptibility are not well documented for typhoid. Thus, the male excess in cases likely reflects higher exposure risk and healthcare utilization patterns. Importantly, our analysis did not find a dramatically higher *proportion* of XDR among male cases versus female cases; rather, since males had more typhoid overall, they also had more XDR. Nonetheless, understanding gender roles in exposure can guide public health messaging (e.g. ensuring safe food practices are targeted to those at risk) and ensures that both boys and girls are equally reached by vaccination campaigns and treatment access.

The AMR profile of *S. Typhi* isolates in this study underscores the continuing evolution of resistance. The majority (67%) of isolates were resistant to ciprofloxacin, reflecting the well-known ubiquity of fluoroquinolone-resistant typhoid across South Asia (3). Since the early 2000s, nalidixic acid resistance (a proxy for reduced ciprofloxacin susceptibility) has exceeded 90% in *S. Typhi* from Pakistan (9). Our findings confirm that fluoroquinolones can no longer be relied upon as effective therapy in this setting – a conclusion supported by national guidelines that have moved away from ciprofloxacin for empirical treatment. Moreover, 61% of isolates were MDR, consistent with the known persistence of the H58 haplotype lineage that carries MDR plasmids (2). These high baseline resistance rates meant that *only one-third of patients would have been treatable with oral first-line agents*, had XDR not existed. The addition of third-generation cephalosporin resistance in the XDR strains then eliminates even intravenous ceftriaxone as a reliable option.

Our most concerning finding is the documentation of resistance emerging to the few remaining treatments for XDR typhoid. We identified 7 isolates (19%) resistant to azithromycin and 8 isolates (22%) resistant to meropenem. While our sample size is small, this is a red flag. During the initial years of the XDR outbreak (2016–2019), all isolates remained susceptible to azithromycin and carbapenems (8). Azithromycin has been the last effective oral antibiotic for typhoid, crucial for outpatient management of MDR and XDR cases (2, 10). However, recent reports from South Asia indicate that azithromycin-resistant *S. Typhi* has started to appear (2, 10). A systematic review noted the emergence of azithromycin-resistant typhoid strains in Pakistan, India, and Nepal in the past few years, which further limits treatment options and often necessitates parenteral therapy (10). In our dataset, one XDR isolate was azithromycin-resistant, effectively making it "super-XDR" and requiring a carbapenem. Alarmingly, another isolate was resistant to both azithromycin and meropenem, leaving no proven treatment except possibly tigecycline. This scenario – a pan-resistant typhoid strain – has been the nightmare scenario public health experts warned about (1, 2). Although rare, the detection of such a strain in 2024 suggests that through either mutation or acquisition of additional resistance genes (e.g., *mph(A)* gene for azithromycin resistance (11)), the XDR clone is becoming even

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more drug-resistant. A 2019–2020 surveillance at a Lahore hospital similarly found an XDR *S. Typhi* isolate with azithromycin resistance, and highlighted that indiscriminate azithromycin use may be contributing to this development (11). The creeping rise of azithromycin resistance is deeply troubling because it will force a shift to exclusively IV therapies for many patients, which are costlier, require hospitalization, and are not without toxicity.

The temporal trend in our data showed a mid-year peak in cases, corresponding with the summer monsoon season. Typhoid fever in Pakistan often shows an upsurge during the monsoon months due to flooding and contamination of water sources (1). Interestingly, our peak included a mix of XDR and non-XDR cases. One hypothesis could be that heavy environmental contamination amplified transmission of *S. Typhi* in general, affecting people with various strains. It's also possible that an outbreak of a particular strain occurred and then waned. The late-year mini-spike of MDR cases might suggest a small point-source outbreak with a non-XDR strain or importation of a different strain. Such fluctuations are plausible in an endemic setting with multiple strain lineages in circulation. Continuous surveillance beyond 2024 would be needed to see if XDR maintains dominance or if vaccination and other efforts start reducing its spread. Pakistan's introduction of TCV in late 2019 and subsequent campaigns are expected to gradually lower typhoid incidence and especially protect children (1). Our data, however, indicate that in 2024 typhoid remained a problem in this locale, with XDR still constituting a large fraction of cases. We did not have data on patients' vaccination status, but given the age group most affected (5–14) overlaps with the catch-up vaccination targets, it would be important to assess vaccine coverage in Faisalabad. The persistence of cases might reflect suboptimal coverage or effectiveness gaps. Encouragingly, a field trial in Sindh found TCV to be highly effective (over 80%) at preventing culture-confirmed typhoid in children, even amidst the XDR outbreak (12). Therefore, sustaining high immunization rates should gradually diminish the susceptible population and tame future outbreaks.

When comparing our findings to other regions, the patterns are broadly consistent with the literature on drug-resistant typhoid. A study from Lahore (2017–2019) reported 46% of *S. Typhi* isolates as XDR and 24.5% as MDR (7), very similar to our proportions (44% XDR, 22% MDR). That study likewise found around 60% of cases were males and the majority were children (7). Another hospital study in Karachi (2019) found an even higher XDR rate of 79% among pediatric typhoid cases (6)– likely reflecting that by then the outbreak strain had almost entirely displaced susceptible strains in that locale. Our somewhat lower XDR percentage might indicate that in Punjab, the complete replacement of strains was still in progress during 2024, or that some non-XDR strains continue to circulate. We did find a mix of strain types, which might remnant older clones or imports from areas with less XDR prevalence. The presence of any fully susceptible isolates is actually notable – it implies that despite the dominance of resistant strains, there are still pockets where antibiotic-sensitive typhoid exists, perhaps in more rural settings or due to importation from regions where TCV and other measures have kept resistance at bay. From a public health perspective, it is crucial to prevent those susceptible strains from acquiring resistance genes, which can happen via horizontal gene transfer especially in areas of antibiotic overuse.

Our geographic analysis, limited as it is by few cases outside Faisalabad, hints that XDR typhoid was focal in Faisalabad district. National reports have shown that by 2018, XDR cases were detected beyond Sindh, including in Punjab's large cities like Lahore and Rawalpindi (8). The fact that our hospital, located in Faisalabad, captured primarily local cases suggests localized transmission. It raises the question of whether there was a contamination source in Faisalabad responsible for these cases. Investigations in the original outbreak in Sindh identified sewage-contaminated municipal water as a major vehicle (1). Faisalabad, being an urban center with its own infrastructure challenges, could have had a similar issue. Layyah and other districts' cases could represent either travel-related infections or limited local events. Without detailed epidemiological data, we can only speculate. However, this underscores the need for broad surveillance – when XDR emerged in one province, it spread via travel to others (8). Punjab's health authorities should remain vigilant

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and ensure that water sanitation interventions and vaccination campaigns are not limited to one region.

The implications of our findings for public health policy are several. First, the high prevalence of XDR typhoid in children calls for aggressive preventive strategies targeting the young. This means maintaining high coverage of TCV in routine childhood immunization, and possibly conducting school-based immunization drives in areas with ongoing transmission. The government of Pakistan's phased TCV rollout is a step in the right direction (1). Monitoring the impact of TCV on incidence and resistance patterns is essential – if vaccine uptake is high, we expect a drop in pediatric cases in coming years, which should disproportionately reduce XDR case counts. Second, our antibiotic susceptibility data argue for revising treatment guidelines in Punjab: clinicians must assume any typhoid case could be XDR and thus consider empiric therapy with azithromycin and/or a carbapenem in severe cases, rather than ceftriaxone or fluoroquinolone alone (3). The CDC has recommended that suspected typhoid in patients returning from Pakistan be treated empirically with a carbapenem or azithromycin (3); by extension, within Pakistan, many experts follow a similar approach for severe illness. However, the emergence of azithromycin resistance means monotherapy with azithromycin could fail in some XDR cases now. Clinicians should obtain cultures and susceptibility testing whenever possible to tailor therapy. In settings like ours, where lab capacity exists, this is feasible; elsewhere, rapid diagnostics and point-of-care tests for resistance markers could be beneficial if developed.

Third, the detection of azithromycin and meropenem resistance in XDR strains is an urgent warning sign. It suggests that without concerted efforts, we risk the XDR strain becoming effectively untreatable with available oral agents. This elevates the importance of antibiotic stewardship – ensuring azithromycin is used judiciously and that carbapenems are reserved for confirmed needed cases. The findings also highlight the need for next-line drug research and perhaps guidelines for using drugs like tigecycline or newer agents in case of pan-resistant typhoid. Public health officials in Pakistan have expressed concern that the rise of XDR typhoid could herald a return to the pre-antibiotic era mortality rates [25] if not checked. Our data showing a pan-resistant case lend credence to those fears. Every effort should be made to prevent such strains from spreading – through isolation of cases, ensuring completion of therapy, and possibly using combination therapies to prevent further resistance selection.

Lastly, our study underscores the interconnectedness of solutions: improving water, sanitation, and hygiene (WASH) remains the cornerstone of typhoid prevention, with vaccination providing an immediate shield to high-risk groups (13). The sustained transmission in an urban center like Faisalabad indicates gaps in clean water supply and sewage treatment. Investments in infrastructure are as crucial as medical interventions. Public health education campaigns in the community about hygiene and the dangers of antimicrobial misuse should be intensified. The XDR outbreak's propagation was likely aided by rampant antibiotic use over years; now is the time to reinforce messaging about not using antibiotics without prescription and completing courses for those who truly need them (2).

Limitations: This analysis has some limitations inherent to their data source. The surveillance was hospital-based and thus may not capture milder cases that did not present to the hospital or were treated empirically without culture. The sample size (36 cases) is relatively small, reflecting a single year at one site; thus, caution is needed in generalizing prevalence figures. We could not perform statistical comparisons with high power, and some observed differences (e.g., gender) may be due to chance. Additionally, detailed clinical information was not available for most cases in the line list, limiting our ability to contextualize each infection. Despite these limitations, the data are valuable as they represent laboratory-confirmed cases with complete susceptibility profiles, offering a clear window into the bacteriological trends.

Comparison with other regions: Outside of Pakistan, typhoid remains endemic in South Asia and parts of sub-Saharan Africa, but XDR strains have so far been largely confined to Pakistan and adjacent areas. Sporadic XDR Typhi cases in travelers from Pakistan have been reported in the US, UK, Canada, etc., often prompting updated

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guidelines in those countries (3). To date, sustained transmission of XDR Typhi outside South Asia has not been documented, partly thanks to prompt public health responses. However, our findings of potential pan-resistance emergence raise the stakes globally – any exportation of such a strain to areas with poor sanitation could seed difficult-to-control outbreaks. This underscores that containing XDR typhoid in Pakistan through vaccination and WASH improvements is not only a national priority but a global health security issue (2).

In conclusion, our study highlights the critical challenges posed by AMR in typhoid fever at a local level, mirroring the national crisis. Children in Punjab are suffering from typhoid infections that are increasingly refractory to standard treatments. The convergence of factors – young age of those affected, high resistance rates, and signs of further resistance escalation – calls for a strengthened, multifaceted response.

Conclusion

The rise of drug-resistant typhoid fever, particularly XDR *Salmonella Typhi*, in Pakistan represents a severe threat to public health. Our analysis of 2024 surveillance data from a Punjab hospital demonstrates that this threat is concentrated in vulnerable groups – notably children aged 5–14 – and is affecting males slightly more than females. Over 40% of typhoid cases were caused by XDR strains, which are impervious to all traditional oral antibiotics, and alarming evidence of resistance to even last-resort drugs is emerging. These findings underscore that without sustained intervention, routine typhoid cases could become untreatable, leading to higher complications and mortality.

To combat this, we recommend a comprehensive approach: (1) Scale-up of typhoid conjugate vaccination in all high-risk populations to provide long-term protection and herd immunity, with rigorous monitoring of vaccine impact on incidence. (2) Strengthening of water and sanitation infrastructure in urban centers like Faisalabad, which will address the root cause of typhoid transmission and benefit overall community health. (3) Enforcement of antimicrobial stewardship – curbing unnecessary antibiotic use in humans to slow the emergence of resistance, and ensuring that confirmed typhoid cases receive appropriate, guided therapy to clearance. (4) Enhanced surveillance and research, including genomic tracking of *S. Typhi* isolates, to quickly detect the spread of high-risk clones and any new resistance determinants, and to inform evidence-based updates to treatment guidelines. (5) Public education and community engagement, to improve hygiene practices, encourage early healthcare seeking for fever, and build trust in vaccination.

This study adds to the growing body of evidence that typhoid fever in South Asia is at a crossroads – with effective vaccines and public health tools available, yet challenged by the microbe's evolving resistance. The success seen in curbing the Sindh XDR outbreak through targeted vaccination campaigns (2) offers hope. By replicating and broadening those efforts in Punjab and nationwide, Pakistan can regain control over typhoid. The experience from this region serves as a cautionary tale to other countries: robust surveillance and preemptive action are indispensable to prevent XDR typhoid from becoming a global scourge. In summary, the battle against XDR typhoid calls for urgent, coordinated measures; the data on age and gender disparities help focus these measures where they will have the greatest impact. With sustained commitment, it is possible to reverse the trend of increasing resistance and protect the gains of the antibiotic era for treating typhoid fever.

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