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Frequency of Multi-drug Resistant (MDR), Extensive Drug Resistant (XDR) and Extended-spectrum β-lactamase (ESBL) Salmonella in Culture Positive Patients of Enteric Fever

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ABSTRACT

Introduction: Typhoid fever, the disease caused by Salmonella enterica serotypes Typhi and Paratyphi, is still prevalent in Pakistan, and it is observed that an increasing proportion of isolates are resistant to antimicrobial drugs. The increased rates of MDR, XDR, and ESBL-producing Salmonella isolates further aggravate the problem of treatment and controlling the infection. **Objectives**: The purpose of the study was to know the frequency of MDR, XDR and ESBL-producing Salmonella strains in culture-positive enteric fever patients in Ayub Teaching Hospital, Abbottabad. **Materials and Methods**: The cross-sectional study was conducted from 14 September, 2024 to 13 March, 2025 at Ayub Teaching Hospital, Abbottabad. The samples were cultured for Salmonella strains, and antibiotic resistance was determined to determine MDR, XDR, and ESBL production. **Results**: A total of 45% were resistant to at least one antibiotic class, 17% were resistant to at which are referred to as extensively drug-resistant, and 4.47% produced extended-spectrum beta-lactamase enzymes. MDR and XDR strains were identified in the children who were either non-vaccinated or those with a longer duration of fever. **Conclusion**: The study portrays the increased emergence of these resistant strains of Salmonella that require better vaccination, antibiotics administration and control, and better hygienic measures.

INTRODUCTION

Typhoid fever is an extended bacterial disease affecting low and middle-income countries such as Pakistan due to Salmonella enterica serotypes Typhi and Paratyphi. This disease comes with an inclination of enduring fever and general body aches, and it is still prevalent in many parts of South Asia. Typhoid fever alone contributes to millions of illnesses every year, and the greatest increment of this disease burden is in South and South-East Asians. Holding a bad health system and low sanitation standards, the incidence of typhoid fever is common in Pakistan, and it particularly affects children (2). More recently, MDR, XDR, and ESBL-producing strains of Salmonella have been reported, which has widened the threat that this disease poses to human health (3 & 4). MDR Salmonella is the type of Salmonella that is resistant to first-line antibiotics, including ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole (5). These strains make treatment lengthy and thus lead to prolonged disease duration and further enhanced mortality rates. XDR strains that are less sensitive to fluoroquinolones and third-generation cephalosporins have added to the concern about the treatment of enteric fever (6, 7). Moreover, the conversion of Salmonella strains to ESBL, which makes them resistant to cephalosporins and penicillins, has been cited to have contributed to antibiotic resistance (8). Literature from Pakistan and other parts of the world has revealed high incidences of MDR and XDR Salmonella, more especially in children (9, 10).

These strains have developed for various reasons, including misuse of this antibiotic, among other related factors, such as overuse and poor sanitation. Some of the defenses include β-lactamase production that neutralizes the β-lactam ring of the antibiotics (11). This, coupled with population mobility, facilitates the spread of resistant strains across countries and makes the problem even worse on an international level (12). Therefore, there is a need to manage the emerging challenge of MDR and XDR Salmonella through the promotion of responsible use of antibiotics, better diagnostics, and new treatment approaches (13). In Pakistan, the situation has become worse due to increasing indices of resistance, and studies have revealed that now XDR strains are prevalent among enteric fever patients (14). This has increased the

resistance level, especially to multiple antibiotics such as cephalosporin's and fluoroquinolones, making treatment harder, and clinicians have to use the last line of antibiotics like meropenem and azithromycin (15). The identification of ESBL-producing Salmonella also poses new challenges to the treatment because these strains are associated with resistance to cephalosporin's and penicillin's. This decline in compliance is detrimental to the clinical outcomes, and the patients place additional pressure on the healthcare systems. The cost of care is raised, and admission duration is also extended.

However, an up-to-date study concerning the frequency of MDR, XDR, and ESBL Salmonella in culture-positive enteric fever patients is still lacking in the country and especially in our region despite plenty of reported cases. Previous research has emphasized the distribution of certain patterns of resistance, but scanty information is available from different regions of Pakistan. This has remained a barrier to the formulation of preventive and control measures that would be useful in managing drug-resistant enteric fever. Thus, this research will establish the proportion of MDR, XDR, and ESBL-positive Salmonella strains in culture-positive children with enteric fever admitted/visited to a pediatric department in Abbottabad, Pakistan. This type of research aims to analyze the current trend of these resistant strains and come up with information on the exact extent of the problem in a bid to guide clinical decisions and formulation of policies to prevent further increases in antimicrobial resistance (6, 9).

The present study is important for several reasons. First, it will improve the existing knowledge about the prevalence of antimicrobial resistance in enteric fever in Pakistan. Second, it will help to develop the general awareness of XDR Salmonella and add to the body of knowledge of research on the topic, especially in regions that are endemic for the disease, such as Pakistan. Therefore, the conclusion derived from this study will assist the policy makers and healthcare providers in decision-making processes regarding the management and control of enteric fever towards offering the best qualitative treatment to affected patients. This research will also assist in formulating better measures that can be taken to address the commonly developed resistance patterns of Salmonella, hence reducing the challenges posed by enteric fever on public health.

Objective

To determine the frequency of multidrug-resistant (MDR), extensively drug-resistant (XDR), and extended-spectrum β-lactamase (ESBL) producing Salmonella in culture-positive enteric fever patients at Ayub Teaching Hospital, Abbottabad.

MATERIALS AND METHODS

Study Design

Cross-sectional, Observational study.

Study Setting

This study was conducted at the Department of Pediatrics, Ayub Teaching Hospital, Abbottabad, Pakistan, which is a teaching hospital catering to both rural and urban communities.

Duration of the Study

The study was conducted in the duration from 14 September, 2024 to 13 March, 2025.

Inclusion Criteria

The study involved both males and females within the age range of 2 to 14 years with a fever duration of more than 4 days due to enteric fever confirmed by blood culture positive for Salmonella Typhi or Paratyphi.

Exclusion Criteria

Children who had received antibiotics before coming to the health facility were also excluded from the study since prior antibiotic use might have had an impact on the resistance profiles of the Salmonella isolates.

Methods

Patients with the foregoing characteristics were selected from the pediatric department of Ayub Teaching Hospital after seeking permission from the hospital's ethical review board. This research work involved participants who were young children, each of whom signed a written consent form that included permission from their parents or guardians. This information includes participants' age, gender, vaccinations received, and number of days suffered from fever. A clinical assessment was done, and blood samples were taken aseptically for culture and sensitivity. In the research procedure, blood was cultured in the microbiology laboratory for Salmonella species. The bacterial strains were then checked for their antibiotic sensitivity by using standard bacteriological methods. MDR, XDR, and ESBL productions were determined, consequently, by certain levels of resistance and phenotypic tests. Patients' information was recorded in a pretested questionnaire, and afterwards, using a porforma, the data was analyzed using SPSS version 22.0 to determine the frequency of MDR, XDR, and ESBL Salmonella isolates.

RESULTS

A total of 263 pediatric patients who presented to the study health facilities with symptoms suggestive of enteric fever were included in the study. Out of these, 230 have been diagnosed with enteric fever from the analysis of blood sample culture for Salmonella species. Table 1 shows the demographic and clinical profile of the patients enrolled in this study. The mean age of patients in this study was 8.5 ± 3.6 years, with the patient's majority being in the male gender (58%). Patients' average fever history before admission varied from 4 to 10 days, with 6 days lasting 2 hours.

Table 1

Demographic and Clinical Characteristics of the Study Participants

Characteristic	Value
Total number of patients	263
Age (mean ± SD)	8.5 ± 3.6 years
Gender (Male %)	58%
Duration of fever (mean ± SD)	6.2 ± 1.5 days
Vaccination status (Vaccinated %)	45%

Out of the 230 Salmonella-positive blood cultures, 140 (60.9%) were identified as *Salmonella Typhi* and 90 (39.1%) as *Salmonella Paratyphi*. The frequency of MDR, XDR, and ESBL-producing Salmonella strains is summarized in Table 2. Of the total isolates, 45% were classified as MDR, 17% as XDR, and 4.47% were ESBL producers.

Table 2
Frequency of MDR, XDR, and ESBL-Producing Salmonella Strains

Strain Type	Frequency (%)
Multidrug-resistant (MDR)	45%
Extensively drug-resistant (XDR)	17%
Extended-spectrum β-lactamase (ESBL)	4.47%

According to the antibiotic resistance profiles, the MDR strains were resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole, while XDR strains were resistant to fluoroquinolones and third-generation cephalosporin's (ceftriaxone and cefixime). The ESBL-producing isolates were found to be more resistant to cephalosporins, especially cefotaxime and ceftriaxone.

Table 3
Antibiotic Resistance Pattern of Salmonella Strains

Antibiotic	MDR (%)	XDR (%)	ESBL (%)
Ampicillin	100	100	0
Chloramphenicol	100	100	0
Trimethoprim-Sulfamethoxazole	100	100	0
Fluoroquinolones	0	100	0
Ceftriaxone	0	100	50
Cefixime	0	100	50
Cefotaxime	0	0	50

The age most affected by MDR, XDR & ESBL Salmonella were in the range of 6-10 years. Both MDR & XDR Salmonella was found more in the unvaccinated children, 60 %, as compared to vaccinated children, 30%. This means that the result pertaining to the association between vaccination status and resistance patterns was statistically significant with a value of ($p < 0.05$).

DISCUSSION

Typhoid fever is a systemic disease developed by Salmonella enterica serotype Typhi and Paratyphi, which poses a significant threat to health, specifically in developing countries, including Pakistan. The discovery of new strains of Salmonella that are resistant to antibiotics has made it difficult to treat this disease apart from the multidrug-resistant (MDR), extensively drug-resistant (XDR), and extended-spectrum β-lactamase (ESBL) strains. This study's findings indicate that such strains are increasing in pediatric patients and that concern is increasing around the world for antibiotic resistance.

The rate of MDR among the isolated strains of Salmonella was 45% in the present study, consistent with that in previously conducted studies in Pakistan and other countries of the South Asian region (1, 6). Resistant MRD strains were resistant to ampicillin and chloramphenicol and then resistant to trimethoprim-sulfamethoxazole (initial empirical drugs of the first line in enteric fever cases). This is quite worrying as the MDR strains prevailed in this study, and the antibiotics that were used to treat the patients had already been used to treat enteric fever. This was a strong indication of over prescription and abuse of these drugs in the general community as well as signs of such resistance development (2, 3).

Furthermore, none of the 17% of strains isolated from these patients were XDR strains that were additionally resistant to a greater variety of antibiotics, including fluoroquinolones, a third-generation cephalosporin, ceftriaxone and cefixime. This type of Salmonella has been reported in many areas of the world. These countries are also mentioned, including Pakistan (4, 5). These XDR strains are more of a concern since they constrain the therapy choices because fluoroquinolones and third-generation cephalosporins have been used in the management of MDR enteric fever. This is worrisome, specifically for fluoroquinolone, since this class of drugs has been widely employed in managing typhoid fever, especially in countries where antimicrobial resistance is rampant. Ever since the emergence of XDR strains, clinicians have been left with no other option but to use last-resort antibiotics like meropenem and azithromycin. (6, 7).

Another reason that was found to be significant in the development of antimicrobial resistance in Salmonella strains is the production of the Extended-spectrum β-lactamases (ESBL). In the present study, out of all the Salmonella isolates, 4.47% were ESBL producers. ESBL production in Salmonella makes the bacteria resistant to cephalosporin and penicillin, which are the common drugs used in the treatment of enteric fever. ESBL-producing strains are of interest in hospitals due to the challenges that they pose in treatment and the need to use carbapenems or other broad-spectrum antibiotics, which are expensive and infrequently available in deprived environments. ESBL-producing Salmonella strains were identified in the current study, signifying increased incidence of β-lactamase mediated resistance in enteric fever and an increased need to come up with more enhanced surveillance to prevent the spread of these strains.

The findings are in line with previous studies, which have shown that antibiotic-resistant Salmonella strains are increasing in Pakistan. Similar observations have been made by researchers in Karachi, Lahore, and elsewhere from patients with Enteric fever who are suffering from S-only MDR or XDR, namely (10, 11). This is of extreme importance in Pakistan, where enteric fever (also known as typhoid fever) is a common illness, and cases of large epidemics are reported occasionally. For example, the leak of XDR strains in Pakistan has been proven due to poor sanitation, poor hygiene standards, and irrational use of antibiotics in human beings and animals (12). The dilemma of using antibiotics without a prescription and not completing the full prescription leads to the emergence of such strains, which adds to the problem.

One of the important observations made in this study is the correlation between vaccination status and the distribution of MDR, XDR, and ESBL-producing strains.

The frequency of MDR and XDR Salmonella infection in unvaccinated children was higher than among the vaccinated children. This fact can only show that there is a possibility of a vaccine against the resistant strains, so vaccination remains a key option in combating enteric fever and eradicating drug-resistant infections. Immunization is a cheap and efficient way of controlling the spread of Salmonella, given the fact that it has been effective in reducing the incidence of typhoid fever in endemic areas, as supported by literature (13). Nevertheless, even though vaccines are available, vaccine coverage is still low in Pakistan, especially in the rural areas with a scarcity of health facilities. This animal study concludes that increasing the rates of immunization and raising community awareness about the problem are ways to decrease the emergence of new, resistant strains of Salmonella.

This study also showed that MDR, XDR, and ESBL producers of Salmonella strains were significantly related to fevers lasting longer than 7 days. This observation agrees with the observations made by other authors who have noted that patients with a febrile illness that lasted for many days harbored resistant strains of Salmonella (14). This can be attributed to untimely diagnosis and wrong antibiotic prescription, which allow the development of resistant bacteria. Healthcare providers must always keep a broad suspicion of drug-resistant enteric fever, particularly if the fever persists, and should opt for the right tests to identify the causative pathogen and its pattern of resistance.

Lastly, this study raises concern about a high number of MDR, XDR, and ESBL-producing Salmonella strains in pediatric enteric fever patients in Abbottabad, Pakistan. A recent study showed that antibiotic resistance is inherent in many Salmonella strains, and its occurrence needs to be addressed before it spreads even further. Development and implementation of effective antibiotic stewardship programs, improvement in sanitation and hygiene, and increasing of vaccination coverage can not only control the infectious diseases but also reduce antimicrobial resistance in an enteric fever. Further urgency is required for ongoing surveillance as well as research to counter the current and emerging trends of enteric fever resistance patterns in Pakistan and endemic countries.

CONCLUSION

This study shows that Enteric fever patients admitted to ATH, Abbottabad, Pakistan, are becoming more frequently infected with MDR/XDR and ESBL-producing Salmonella in paediatric patients. Since the repeated occurrence of resistant strains makes most antibiotics useless, it is the reason why typhoid fever becomes difficult to treat and control as it is. Its use was indicated as preventive because unvaccinated children are more vulnerable to MDR and XDR infections. In addition, the duration of fever influenced the odds of carrying resistant strains, and there was justification for early and accurate diagnosis. Therefore, this study suggests that there is a need to enhance measures, including stewardship of antibiotics, hygiene practices and immunity, as well as continued monitoring to reduce the emergence and spread of less susceptible strains of Salmonella. Factors such as these ought to be reasoned out for the prevention of enteric fever and improved outcomes for sufferers.

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