

Extensively Drug-Resistant Salmonella typhi XDR Infection at Rawalpindi Medical University and Allied Hospitals

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Abstract

Objective: To evaluate the prevalence of extensively drug-resistant Salmonella typhi (XDR) in RMU Allied Hospitals.

Study Design: A descriptive cross-sectional study.

Material and Methods: It is a cross-sectional, prospective study conducted at RMU Allied Hospitals from January 2019–December 2019. Blood culture samples were received in the pathology lab, they were collected by simple random sampling and processed by conventional incubation. Antibiotic susceptibility of the isolates was done on Muller Hinton agar using modified Kirby Bauer disk diffusion method and antibiotic zone diameters were measured according to CLSI guidelines.

Results: Out of the total 8045 cultures, 911 (11%) showed growth, among which 179 (20%) were Salmonella typhi and 135 (15%) XDR Salmonella. Meropenem revealed the highest sensitivity, Chloramphenicol, and Augmentin revealed the highest resistance.

Conclusion: Blood culture results revealed Salmonella typhi 20% with a significant number of XDR Salmonella 15%. Antibiotics susceptibility pattern exhibits Meropenem and Azithromycin as the only antibiotics for XDR Salmonella. Salmonella typhi infection has a significantly high prevalence among children as compared to adults. ($p=0.0017$) Injudicious use of antibiotics is one of the important aspects of the occurrence of antibiotic-resistant Salmonella.

Keywords: XDR Salmonella typhi, Antibiotics susceptibility, Kirby Bauer disk diffusion.

Introduction

Antibiotic-resistant *Salmonella typhi* is an increasing cause of morbidity and mortality in Pakistan.

In the 20th century, typhoid fever incidence decreased due to the vaccine introduction, improved treatment, better hygiene, and sanitation.¹ The first-line treatment of Enteric fever was very successful in the late 20th century with co-trimoxazole, chloramphenicol, and ampicillin. Then, resistance to chloramphenicol emerged in the early period of 1970, and around 1990 multidrug-resistant *Salmonella typhi* evolved. As a result, fluoroquinolones came as first-line antibiotic therapy for enteric fever.²

The emergence of Fluoroquinolone resistance led to the use of oral cefixime or parenteral ceftriaxone as the treatment of choice. This was soon followed by resistance to these two drugs labeling the organism as XDR (Extensively resistant *Salmonella typhi*). XDR denotes non-susceptibility to ampicillin, cotrimoxazole, chloramphenicol, fluoroquinolones, and 3rd generation cephalosporins. The evolution of XDR strains led to carbapenem (meropenem) and azithromycin as the only drug options.³

The Provincial Disease Surveillance and Response Unit (PDSRU) reported 5274 XDR *S. typhi* cases from 14 districts of Sindh, from 2016 to December 2018, according to the World Health Organization (WHO). These included 76% of cases from Karachi, 27% of cases from Hyderabad, and 4% cases from other districts of Sindh.^{4,5} Despite the control measures initiated by the local government, there was a noticeable increase in reported cases from 2017 to 2018.⁴ The international surveillance for XDR *S. typhi* identified a case from the UK and five cases from the USA.⁶ Recently, another case has been reported from Canada.⁷ All these patients had a history of travel to Pakistan.^{4,6,7}

This study was designed to evaluate the antimicrobial-resistant pattern of *Salmonella typhi* at RMU Allied Hospitals. The rationale of this study was to find out XDR cases of Enteric fever, to treat patient earliest, decrease hospital stay to prevent nosocomial infections and to prevent any life-threatening complications. The gold standard options for diagnosing enteric fever are blood cultures and stool cultures before the start of antibiotics.

Materials and Methods

Blood culture samples, selected by simple random sampling, received in the pathology lab of the respective hospitals from the paediatric and medical department, were incubated aerobically at 37°C. After 48 hours the first subculture was done on blood and MacConkey agar. Standard blood culture protocols were followed. Non-lactose fermenting (NLF) colonies were identified on agar plates, gram-negative bacilli were identified by Standard biochemical tests including API 20E.

Antibiotics susceptibility of the isolates was done on Mueller Hinton agar using the modified Kirby Bauer disk diffusion method. The antibiotic discs applied were ampicillin (10 µg) (short-acting penicillin), chloramphenicol (30 µg) (protein synthesis inhibitor), co-trimoxazole (1.25/23.75 µg) (sulfonamides), ciprofloxacin (5 µg) (quinolone group), ceftriaxone (30 µg) (cephalosporin), azithromycin (15 µg) (macrolide) and meropenem (carbapenem). Zones of inhibition after overnight incubation were measured according to CLSI (Clinical Laboratory Standards Institute) guidelines, 2019.

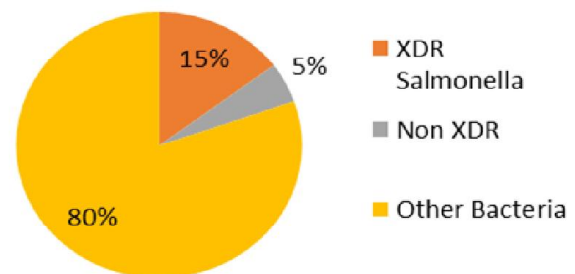


Figure 1: Blood cultures positive for Salmonella

Table 1: CLSI guidelines of antibiotics sensitivity zone diameters for *Salmonella*

Antibiotics	Sensitive	Intermediate sensitive	Resistant
Ampicillin	≥17	14-16	≤13
Chloramphenicol	≥18	13-17	≤12
Co-trimoxazole	≥16	11-15	≤10
Ciprofloxacin	≥31	21-30	≤20
Ceftriaxone	≥23	20-22	≤19
Azithromycin	≥13	-----	≤12
Meropenem	≥23	20-22	≤19

The antibiotic resistance pattern was noted. Those samples which were resistant to Ampicillin,

Chloramphenicol, Co-trimoxazole, Ciprofloxacin, and Ceftriaxone were labelled as XDR Salmonella typhi.

Inclusion Criteria: All blood culture samples received from the paediatric and medical departments.

Exclusion Criteria: Patients who were already taking antibiotics or those suffering from UTI or upper respiratory infection were excluded.

Results

From January 2019-December 2019 at RMU allied Hospitals, out of the total 8245 cultures, 911 (11%) showed bacterial growth, among which 179 (20%) were Salmonella typhi and 135 (15%) were XDR Salmonella (Figure 1). Out of 179 isolates, 140 (78%) were from Paeds and adult positive cases were 39 (22%) (Table 2). The antibiotic sensitivity pattern of Salmonella typhi isolated from blood culture shows the least resistance pattern to Meropenem (MEM) followed by Azithromycin (AZM), Co-trimoxazole (SXT), Ciprofloxacin (CIP), Ceftriaxone (CRO) and Chloramphenicol (C), and the highest resistance to Augmentin (AUG) (Table 3, Figure 2).

Table 2: Prevalence of Salmonella isolates in blood cultures

Age Group	Positive	Percentage	P-Value
Children	140/610	22%	0.0017*
Adult	39/301	13%	

*Highly significant. The probability of Salmonella infection is significantly high in children.
Total cases=179

Table 3: Antibiotics sensitivity pattern of Salmonella typhi isolated from blood cultures.

Antibiotics	Sensitive	Percentage
Meropenam	179	100
Azithromycin	162	90.4
Co-trimoxazole	74	41.2
Ciprofloxacin	71	39.6
Ceftriaxone	62	34.9
Augmentin	6	3.1
Chloramphenicol	18	10

Table 4: Antibiotics resistance pattern of Salmonella typhi isolated from blood cultures.
n=179

Antibiotics	Resistance	Percentage
Meropenam	0	0
Azithromycin	17	9.6
Co-trimoxazole	105	58.6
Ciprofloxacin	108	60.33
Ceftriaxone	117	65.3
Augmentin	173	96.6
Chloramphenicol	161	89.9

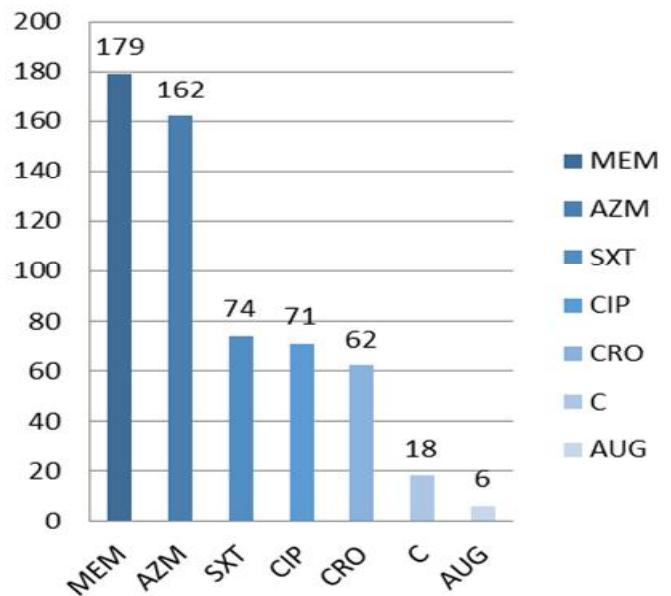


Figure 2: Antibiotic Sensitivity Pattern of Salmonella typhi

Discussion

The present study revealed 15% XDR Salmonella. There was no resistance to Meropenem, 9.6% of cases were resistant to Azithromycin which is quite an alarming situation and 64.1% were resistant to ceftriaxone. A study conducted at a hospital in China from 2005-2011 showed 546 cases of Salmonella typhi. 2% (12 cases) isolates were XDR, 20% showed resistance to Ciprofloxacin and 4% were ceftriaxone resistant. Among these 12 cases, 2 cases were resistant to Azithromycin.⁸

In another study at PNS Shifa Hospital Karachi, 292 isolates revealed Salmonella typhi. The majority of cases (91%) were resistant to Ciprofloxacin and 115 cases (48%) were XDR Salmonella. All were sensitive to Azithromycin and Meropenam.⁹

A similar study was conducted at Sheikh Zayed Hospital Lahore, which showed 43.4% XDR cases.¹⁰ Another study at Shifa International Hospital from Jan 2015-Dec 2018 revealed 30 cases (4%) of XDR Salmonella out of 832 Salmonella typhi cases.¹¹

The study at Sheikh Zayed Hospital Lahore had a preponderance of 57.1% (Males) and 42.9% (Females) and 80% males and 20% females at Shifa International Hospital. Our Study showed 58% XDR cases in males and 42% cases in females. Regarding age group, RMU Allied Hospitals have 78% cases from the Paediatric department, age < 12 years, and 22% in adults. This correlates well with the study at Shifa International Hospital and Shiekh Zayed Hospital where 70% and 81.7% were from the paediatric department respectively.^{10,11} So enteric fever is prevalent in children as compared to adults.

Pakistan is facing outbreaks of extensively drug-resistant (XDR Typhoid), defined as a case that is resistant to all first-line antibiotics, including ceftriaxone. Unrestricted, suboptimal, and insufficient duration of administration of antibiotics leads to, chromosome-borne and plasmid-borne, resistant genes against first-line antibiotics. Pakistan is alarmingly witnessing this problem.¹¹⁻²¹ Salmonella outbreak strain in Pakistan is XDR H58 haplotype. This is known for its ability to spread worldwide and to displace endemic Salmonella S. typhi. The therapeutic option, for this strain, is expensive parenterally administered antibiotics.¹⁴

Public health measures and widespread typhoid vaccinations are likely to modify the disease burden. WHO is likely to implement large scale vaccination against Salmonella.^{22,28} Water contamination, inadequate hygiene, and poor sanitation are held responsible for the Salmonella outbreak more so in the Pakistani scenario. In addition to the water and sanitation issues, several studies have confirmed the presence of XDR-Salmonella, serovars, in poultry and farm animals.^{15-19,23-27}

The paediatric age group has a sizeable representation in Salmonella outbreaks. It can be ascribed to earlier consultation of treatment by the guardians of children, the low trend of self-medication, and increased microbial load i.e. colony forming units per ml of blood in children as compared to adults.²⁶

The low specificity of different serological tests makes it imperative to adopt more definitive diagnostic modalities, like blood culture. Blood cultures isolate organisms and identify the most appropriate antibiotic to treat the impending resistant pattern. An important impediment, in this regard, is before antibiotic

therapy, which the majority of the patients already had, before embarking on blood culture tests. Bactec isolation techniques utilizing fluorescent technology and with the incorporation of antibiotic inhibitors ensures isolation of even smaller strains.¹⁰

Public Health Authority must ensure quality poultry products, supply potable water, to avoid mixing of sewerage lines and water supplies due to poor quality pipes. The joint effort of the Health community, government, and mass media campaign for the awareness of proper hygiene are important elements to control Salmonella infections in particular and other infections in general.

If these steps are not taken then XDR Salmonella will become a massive problem, patients will come with complications and health workers will not be able to combat it. As orofecal infections constitute major health problems so early preventive steps and awareness programs are mandatory.

Conclusion

- 1) Blood culture isolation revealed Salmonella typhi 20% with the recognizable number of extensively drug-resistant Salmonella typhi (XDR) strains 15%.
- 2) Antibiotics susceptibility pattern exhibits Meropenem and Azithromycin as the only antibiotics for XDR Salmonella.
- 3) Salmonella typhi infection has a significantly high prevalence among children as compared to adults. (p=0.0017)
- 4) Injudicious use of antibiotics is one of the important aspects of the occurrence of antibiotic-resistant Salmonella.

Recommendations

- 1) As XDR Salmonella are sensitive to Meropenem and Azithromycin, so clinicians should use these antibiotics with caution in other infections.
- 2) Public awareness programs should be conducted frequently, emphasizing the use of safe drinking water, quality poultry products, and frequent hand washing.
- 3) A mass vaccination program should be launched to combat Salmonella infection.
- 4) Food regulatory authorities have a vital role to check and treat carriers working in restaurants and bakeries.

- 5) It is required to adopt proper measures at the community level to circumvent the emergence of resistant Salmonella strains.

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