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Prevalence of Extensively Drug Resistant *Salmonella typhi* and its Susceptibility against Meropenem, Tigecycline, Fosfomycin and Azithromycin among Clinical Isolates from a Tertiary Care Hospital Laboratory

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ABSTRACT

The emergence of Extensively Drug-Resistant (XDR) *Salmonella typhi* in Pakistan has posed a significant public health challenge. Traditional antibiotics, including ampicillin, chloramphenicol, and fluoroquinolones, have become ineffective due to widespread resistance, necessitating the exploration of alternative treatment options. **Objective:** To assess the in vitro efficacy of four antibiotics fosfomycin, tigecycline, meropenem and azithromycin against XDR *Salmonella typhi* strains isolated from patients in Karachi, Pakistan. **Methods:** A cross-sectional study was conducted at the Department of Microbiology, Ziauddin University Hospital, Karachi, over six months. Blood samples from both inpatients and outpatients aged 1 to 60 years were collected for culture and sensitivity testing. Antibiotic susceptibility was determined using the standard disc diffusion method. Data were analyzed to evaluate the effectiveness of the selected antibiotics against XDR *Salmonella typhi*. **Results:** The susceptibility patterns of *Salmonella typhi* and XDR *Salmonella typhi* revealed that meropenem, azithromycin, tigecycline, and fosfomycin were effective in all tested samples. Conversely, antibiotics such as ampicillin, aztreonam, cefixime, ceftriaxone, chloramphenicol, co-trimoxazole, and ciprofloxacin demonstrated resistance, with varying patterns observed between *Salmonella typhi* and XDR *Salmonella typhi*. The distribution of XDR and Non-XDR *Salmonella typhi* cases by gender and age, with no significant association found between these variables and XDR status. **Conclusions:** Meropenem and azithromycin remain effective against XDR *Salmonella typhi*; however, fosfomycin and tigecycline present promising alternatives. These findings underscore the need for continuous surveillance and the development of new treatment strategies to combat the rising threat of XDR *Salmonella typhi* in Pakistan.

INTRODUCTION

Salmonella typhi is a gram negative enteric rod of the family *Enterobacterales*. When individuals become infected with Salmonellae they might present with a range of clinical symptoms, including Enteric fever, gastroenteritis and septicemia. In some cases, suppurative lesions may also develop [1]. According to the WHO, approximately 9 million people contract typhoid annually, and 110,000 die from it each year as of 2019 [2]. Based on international data, there are about 22 million new manifestations of typhoid fever annually, resulting in around 200,000 deaths? The regions of South Central and Southeast Asia have the highest rates of sickness and mortality [3]. Among countries in this region, Pakistan has the highest occurrence of typhoid fever, with a rough annual incidence of 412.9 cases per 100,000 individuals. According to a study, the rate of typhoid fever in Pakistan is 15.5/1,000 cases, seemed notably higher compared to other regions [4]. The primary antibiotics used for treating infections caused by

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Salmonella typhi, including chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, have gradually become less effective due to the development of resistance. As a result, fluoroquinolones including ciprofloxacin have been adopted as a possible treatment option [5]. The omnipresent spread of *Salmonella typhi's* H58 Multi-Drug Resistant (MDR) haplotype has been an explicitly agitating development in current years. This MDR variation has developed wide spread in Asia and a few parts of Africa, and it is unsusceptible to a number of antibiotics, such as ampicillin, co-trimoxazole, chloramphenicol, and some fluoroquinolones. Cephalosporins have therefore been employed as an empirical therapy option for typhoid infections arising due to this multidrug resistant strain [6]. There was a significant outbreak of typhoid fever that was reported in Pakistan in February of 2018. This strain of *Salmonella enterica* serotype *typhi* causing the outbreak was unsusceptible to many different antibiotics, such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol both third-generation cephalosporins and *fluoroquinolones* [7]. It was therefore termed as an extensively drug-resistant strain of *Salmonella typhi.* The XDR *typhi* strain is resistant to a wide range of antibiotics, including carbapenems [8]. It has become a major concern in Pakistan [9]. The Sindh region of Pakistan had 8,188 cases of typhoid fever between 2016 and December 2018, 5274 of which were classified as XDR *typhi,* according to data from the World Health Organization (WHO). Provincial Disease Surveillance and Response Unit reported these cases in 14 districts (PDSRU) [10]. In Sindh province, the highest number of XDR *typhi* cases were reported in Karachi, the provincial capital, accounting for 69% of all cases, Hyderabad district followed with 27% of the reported cases, while the remaining 4% were distributed across other districts in the province [8]. *Salmonella typhi* has the capability to rapidly transform from MDR to XDR by obtaining a plasmid, which imparts resistance to all standard treatment options. The outbreak of XDR *Salmonella typhi* in Pakistan has been attributed to a specific H58 clade which harbors an IncY plasmid that carries the CTX-M-15 gene bla, resulting in resistance against fluoroquinolones and ceftriaxone. This plasmid plays a critical role in the progression of resistance, enabling the organism to survive and persist in the face of various antibiotics treatment [8]. At present, meropenem and azithromycin are the two primary treatment options available for combating drug-resistant *Salmonella typhi.* However, a case of azithromycin resistance has been reported from India, indicating the need for continuing monitoring of treatment efficacy and the advent of antibiotic resistance in this bacterial pathogen [11, 12]. Given the limited options for treating Extensively Drug-Resistant (XDR) *Salmonella typhi,* this study seeks to evaluate the efficacy of four antibiotics: fosfomycin, tigecycline, meropenem, and azithromycin. The findings could inform their potential use in combating these highly resistant bacteria. The study particularly emphasizes the need to conserve azithromycin, due to its broad-spectrum effectiveness, and meropenem, given its high cost and critical role in treating XDR Salmonella typhi. Since both drugs have shown significant efficacy against XDR *Salmonella typhi,* we are investigating the in vitro effectiveness of fosfomycin and tigecycline as alternative treatment options for this infection.

The primary objective of this study was to assessed the in vitro efficacy of four antibiotics fosfomycin, tigecycline, meropenem, and azithromycin against XDR Salmonella typhi strains isolated from patients in Karachi, Pakistan..

METHODS

It was a cross-sectional study conducted at the Department of Microbiology at Ziauddin University Hospital, Karachi. The duration of the study was six months, from 1st January to 31st July 2021. After obtaining informed consent, blood samples for culture and sensitivity tests were collected from both inpatients and outpatients using a convenience sampling method. This method involved selecting samples based on their availability and accessibility within the healthcare settings where the study was conducted in Karachi, Pakistan. The study included both males and females with an age range 1 to 60 years. Blood samples for culture and sensitivity showing growth other than bacteria like fungus or yeast and repeat and duplicative sample from the same patient were excluded. Written approval was taken from the institutional ethical committee (Reference Code: 0611118ZIMIC) and permission from the management of Ziauddin Hospital was obtained. The sample size was calculated through WHO Sample size calculator, taking statistics for meropenem sensitivity as 87% margin of error as 9% and 95% Confidence interval, the sample size came out as 54 [1]. Additionally, the whole study included 204 blood samples among which *Salmonella typhi* included 57 blood samples while XDR included 147 blood sample. Before beginning any antibiotic therapy, all blood cultures were taken from a peripheral vein while following the correct aseptic procedures. Blood cultures were taken in regular BACTEC bottles for the adult population and in pediatric BACTEC bottles for the pediatric population. The blood to broth ratio was 1:10, and the sample was incubated for five days at 35.5°C ± 1.5°C in a BACTEC 9240 blood culture equipment. The BACTEC device detects the development of germs using a fluorescence sensing technology. A gram-stained smear of the broth was used to assess the microbial growth that the flag and audible sound of the device could detect. The bacteria were then subculture on 5% sheep blood agar, chocolate and MacConkey agar plates and incubated at 37°C for 18 to 24 hours in order to isolate the bacteria. The MacConkey agar plates were kept

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at 37°C aerobically in incubator while chocolate agar and sheep blood agar were incubated in capnophilic atmosphere (5-10 % CO_2), Identification of *Salmonella typhi* clinical isolates was performed by standard microbiological methods and their characteristic appearance on gram staining, the oxidase test, the catalase test, motility, Triple- Sugar Iron (TSI) fermentation, colony morphology and for the ultimate confirmation, biochemical tests of the analytical profile index (API 20 E) were used [13]. Antibiotic susceptibility pattern of the isolates was done by Modified Kirby Bauer disc diffusion method on Muller-Hinton-agar in accordance with CLSI recommendations [14, 15]. The Muller-Hinton agar plates were placed aerobically at 35°C ± 2 for 18-24 hours. The control strains used were of Staphylococcus aureus ATCC-25923® and Escherichia coli ATCC-25922®. Data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0. The results were presented as frequencies and percentages, with the post-stratification chi-square test applied to assess significance, setting the threshold at $p \leq 0.05$. Gender was stratified to control for its potential confounding effects on the relationship between antibiotic resistance and the presence of XDR Salmonella typhi. Quantitative variables, such as patient age, were summarized using summary statstics, including mean and SD. Qualitative variables, including gender, presence of XDR Salmonella typhi, and resistance profiles, were displayed using frequency distributions and percentages.

RESULTS

Table 1 presented the CLSI criteria for antibiotic resistance, detailing disc content and corresponding resistance thresholds for various antibiotics. For example, chloramphenicol is resistant if the zone is ≤12mm, while ciprofloxacin is resistant at ≤20mm. Similarly, resistance thresholds for other antibiotics like ampicillin, ceftriaxone, and meropenem are specified, aiding in the accurate assessment of antibiotic efficacy and guiding effective treatment choices [14, 15].

Table 1: Interpretative Criteria According to CLSI

Antibiotics	Disc Content (µg)	Resistance (mm)
Chloramphenicol	30	≤12
Ampicillin	10	≤13
Trimethoprim-Sulfamethoxazole	1.25/23.75	≤10
Ciprofloxacin	5	≤20
Ceftriaxone	30	≤19
Cefotaxime	30	≤22
Cefixime	5	≤15
Aztreonam [12, 13]	30	≤21
Meropenem	10	≤19
Azithromycin	15	≤12
Fosfomycin	15	≤14

Table 2 displayed the interpretative criteria for antibiotic resistance based on guidelines from the FDA or EUCAST. It specifies that tigecycline, with a disc content of 15 µg, is considered resistant if the zone diameter is 14 mm or less. This criterion helps in evaluating the effectiveness of tigecycline against bacterial strains and informs appropriate treatment decisions.

Table 2: Interpretative Criteria According to FDA or EUCAST

Variables	Disc Content (µg)	Resistance (mm)
Tigecycline	15	≤14

Table 3 presented a total of 204 blood samples were included. The patient population was made up of 59.3% male and 40.7% female. XDR *Salmonella typhi* was detected in 72.1% of patients, while *Salmonella typhi* was found in 27.9% of patients.

Table 3: Age and Gender Statistic of Patients

Variables	N (%) / (Mean)
Gender	
Male	121 (59.3)
Female	83 (40)
Age	
Age (all Patient)	11.42
Age (<10 Years)	4.72
Age (>10 Years)	22.93

Table 4 represented that meropenem, azithromycin, tigecycline, and fosfomycin were sensitive to all the isolates. 68.4% sensitive to ampicillin; 94.0% to aztreonam and ceftriaxone; 94.7% to cefixime; 54.4% to chloramphenicol; 47.4% to co-trimoxazole; and only 7.0% to ciprofloxacin.

Table 4: Frequency Distribution Susceptibility Pattern of *Salmonella typhi* (n=57)

Antibiotics	Sensitive (S) N (%)	Resistant (R) N (%)	Total
Meropenem	57 (100)	0 (0)	57
Azithromycin	57 (100)	0 (0)	57
Tigecycline	57 (100)	0 (0)	57
Fosfomycin	57 (100)	0 (0)	57
Ampicillin	39 (68.4)	18 (31.6)	57
Aztreonam	53 (94.0)	4 (7.0)	57
Cefixime	54 (94.7)	3 (5.3)	57
Ceftriaxone	53 (94.0)	4 (7.0)	57
Chloramphenicol	31(54.4)	26(45.6)	57
Co-Trimoxazole	27 (47.4)	30 (52.6)	57
Ciprofloxacin	4 (7.0)	53 (93.0)	57

Sensitive = S Resistant = R

Table 5 represented that meropenem, azithromycin, tigecycline, and fosfomycin were sensitive to all the isolates while ampicillin, aztreonam, cefixime, ceftriaxone, chloramphenicol, co-trimoxazole and ciprofloxacin were resistant to all the isolates against extensively Resistant *Salmonella typhi*.

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Table 5: Frequency Distribution Susceptibility Pattern of Extensively Drug-Resistant *Salmonella typhi*(n=147)

Antibiotics	Sensitive (S) N (%)	Resistant (R) N (%)	Total
Meropenem	147 (100)	0 (0)	147
Azithromycin	147 (100)	0 (0)	147
Tigecycline	147 (100)	0 (0)	147
Fosfomycin	147 (100)	0 (0)	147
Ampicillin	0 (0)	147 (100)	147
Aztreonam	0 (0)	147 (100)	147
Cefixime	0 (0)	147 (100)	147
Ceftriaxone	0 (0)	147 (100)	147
Chloramphenicol	0 (0)	147 (100)	147
Co-Trimoxazole	0 (0)	147 (100)	147
Ciprofloxacin	0 (0)	147 (100)	147

Sensitive = S Resistant = R

Table 6 represented distribution of XDR and Non-XDR *Salmonella typhi* cases by gender. Among males, 89 cases (73.6%) were XDR *Salmonella typhi* and 32 cases (26.4%) were Non-XDR *Salmonella typhi*. Among females, 58 cases (69.9%) were XDR *Salmonella typhi* and 25 cases (30.1%) were Non-XDR *Salmonella typhi*, total 83 cases. The p-Value for the comparison between genders was 0.566, indicating no significant difference in the distribution of XDR and Non-XDR *Salmonella typhi* between males and females. The analysis also included stratification by age, categorizing patients into two groups: those aged ≤ 120 months and those aged > 120 months. The distribution of XDR and Non-XDR *Salmonella typhi* cases across these age groups showed no significant association, as indicated by a p-value of 0.103.

Table 6: Distribution of XDR and Non-XDR *Salmonella typhi* Cases by Gender

Gender	XDR *Salmonella typhi* N (%)	Non-XDR *Salmonella typhi* N (%)	Total	p-Value[(a)]
Male	89 (73.6)	32 (26.4)	121	0.566**
Female	58 (69.9)	25 (30.1)	83	
Total	147 (72.1)	57 (27.9)	204	
≤120 months	98 (76.0)	31 (24.0)	129	0.103**
>120 months	49 (65.3)	26 (34.7)	75	
Total	147	57	204	

Note: (a)**Chi Square test

Figure 1 mentioned that the four antibiotics meropenem, tigecycline, fosfomycin, and azithromycin exhibited 100% effectiveness against XDR *Salmonella typhi*, as all tested isolates were found to be sensitive to them. It also showed varying degrees of resistance, which demonstrates the challenge in treating XDR infections and reinforces the critical role of these effective antibiotics in managing resistant cases. The percentage of XDR *Salmonella typhi* isolated that were sensitive or resistant to various antibiotics, including meropenem, tigecycline, fosfomycin, and azithromycin. The data highlighted the effectiveness of these antibiotics against XDR strains.

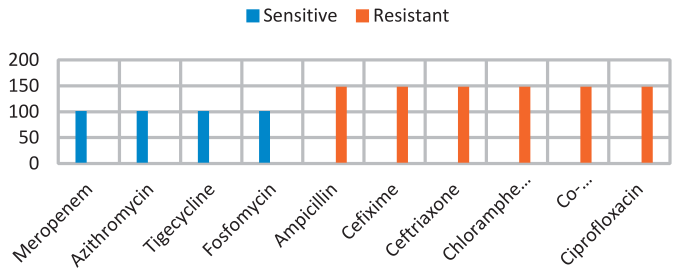

Figure 1: Susceptibility Pattern of Extensively Drug-Resistant *Salmonella typhi*

Figure 2 represented that antibiotic discs create zones of inhibition, which were clear were as around the discs where bacterial growth was prevented. The size of these zones indicates the effectiveness of the antibiotic against the bacteria. Antibiotic discs were placed on Muller-Hinton agar inoculated with XDR Salmonella typhi, and zones of inhibition were measured to determine bacterial resistance or sensitivity to the antibiotics tested.

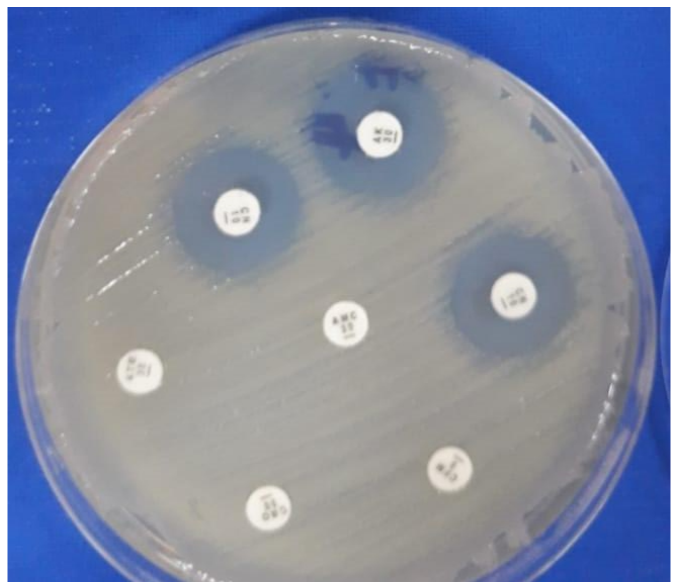

Figure 2: Modified Kirby Bauer Disc Diffusion Method on Muller-Hinton-Agar (Disc Showing Antibiotics).

DISCUSSION

The intent of this study was to address the issue of the emerging Extensively Drug Resistant (XDR) strain of *Salmonella typhi*. The previous treatment options for this strain, namely ampicillin, ciprofloxacin, ceftriaxone, chloramphenicol, and trimethoprim sulfamethaxazole, were once effective but their increasing resistance has made them less reliable. As a result, two new treatment options, meropenem and azithromycin, have been introduced. Azithromycin was an orally-administered antibiotic that was commonly used to treat typhoid fever, including in children. In our country, this antibiotic has been the preferred choice due to its effectiveness and low resistance rates. However, a recent case of extensively drug-resistant *Salmonella typhi* has been presented in a

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patient with suspected endocarditis and a prosthetic valve replacement [16, 17]. This strain was resistant to azithromycin, which was the first such case in our country. Fortunately, the patient responded well to intravenous meropenem treatment and had a smooth recovery. The resistance to azithromycin in *Salmonella typhi* was often linked to mutations in the AcrB efflux pump and the msrA gene [16-19]. Tigecycline was a broad-spectrum glycylcycline antibiotic that has been approved for the treatment of complicated skin and intra-abdominal infections, as well as community-acquired bacterial pneumonia. It has demonstrated in-vitro activity against both gram positive and gram-negative organisms. Due to its effectiveness against Multidrug-Resistant (MDR) Gram-negative bacteria, distinctly carbapenem-resistant Enterobacterales tigecycline was regarded as a last-resort antibiotic for the therapy of serious illnesse [20]. Fosfomycin was an antibiotic that kills bacteria by blocking the early stages of cell wall synthesis. It has a wide spectrum of activity for various types of Gram-positive pathogens, such as methicillin-resistant Staphylococcus aureus (MRSA), as well as drug-resistant Enterobacterales and Pseudomonas aeruginosa strains that produce Extended-Spectrum β-Lactamases (ESBLs) or were resistant to carbapenems. Fosfomycin was a likely choice due to its broad spectrum cover against the resistant organisms for the treatment of severe infections [20]. It was very beneficial in treating urinary tract infections as it can reach large amounts in urine. Despite the fact that fosfomycin was effective in treating resistant organisms, it's important to remember that there were few were restrictions on its usage. If it was continuously prescribed on routine infective cases it can lead to the development of resistance, also there was inadequate research regarding its safety and efficacy in specific populations, such as children and pregnant women Regardless of these disadvantages fosfomycin has proven to be a vital weapon against these resistant bugs. Based on its distinctive mode of action it provides to be an intriguing avenue for additional study and development [11]. Although often used to treat urinary tract infections, fosfomycin has shown benefit in the treatment of a variety of other infections, such as endocarditis, bacteremia and pulmonary infections [20]. In the dealing of Extensively Drug Resistant *Salmonella typhi* usage of meropenem and azithromycin along with tigecycline and fosfomycin can be used as an effective strategy.

CONCLUSIONS

As anticipated therapeutic drugs, azithromycin and meropenem have demonstrated good efficacy for the treatment of Extensively Drug Resistant *Salmonella typhi*. Additionally, fosfomycin and tigecycline have shown favorable in vitro results and can be adapted as an option in such cases.

Authors Contribution

Conceptualization: ZI
Methodology: FIA, HZ, YMP
Formal analysis: HZ
Writing, review and editing: AF, LF

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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