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ORIGINAL ARTICLE

Frequency and Antimicrobial Resistance Patterns of *Salmonella Enterica* Isolates in a Tertiary Care Setting

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ABSTRACT

Background: Typhoid fever is endemic in South Asia and is the most common bacteremic illness in children in Pakistan, with rates as high as 1000 cases per 100000 child-years having been reported from Karachi. The occurrence of multidrug-resistant (MDR) *Salmonella enterica* strains has been highly reported from developing countries. Fluoroquinolone resistance has also been highly reported. Clinical cases of cephalosporin-resistant *Salmonella enteric* isolates have also been reported recently leading to spread of XDR strains.

Methodology; Between 1st January-31st December 2021, a total of 1385 blood sample were processed for identification of enteric fever isolates. The blood samples were inoculated in the tryptic soy broth bottles. The strain identification was initially performed by Gram staining, and by using API 20E. For serovar confirmation, agglutination assays were performed using antisera. The antimicrobial susceptibility testing for the *Salmonella enterica* isolates was performed using the disc diffusion method according to the (CLSI) standards for the following antibiotics; ampicillin, amoxicillin, piperacillin-tazobactam, ceftriaxone, cefotaxime, cefepime, imipenem, meropenem, ciprofloxacin, chloramphenicol and trimethoprim-sulfamethoxazole

Results: A total of 425(30.68%) samples gave positive growth. Sixty-two (14.58%) isolates were identified as *Salmonella enterica* species. Eight isolates (12.90%) were from < 5 years of age group, 35(56.45%) from 5 to 15 years age group. Species differentiation revealed, 52(83.87%) were *Salmonella typhi* and 10(16.12%) were *Salmonella paratyphi A.* Sensitivity pattern of the isolates (n=62), indicated 29(46.77%) were multi-drug resistant (MDR), while 32(51.61%) were extensively-drug resistant (XDR).

Conclusions: Both governmental and nongovernmental organizations in Pakistan need to actively promote vaccination campaigns and healthy hygiene habits and discourage irrational use of antibiotics to prevent the mortality and morbidity associated with increasing antibiotic resistance in pathogens. Safe water supply, personal hygiene and effective antibiotic policies can help us to control the disease therefore reducing antimicrobial resistance.

Keywords: Antimicrobial resistance, salmonella enteric, bacteremic illness

INTRODUCTION

There is worldwide distribution of Enteric fever with an estimated 17 million cases in 2015, with highest ratio of cases in South Asia[1]. There is major burden of Enteric fever cases in developing countries with about 200,000 deaths annually[3]. Enteric fever is endemic in South Asia and in Pakistan it is the most common bacterial infection in children. High rate of infection as 1000 cases per 100000 children had been reported in a study from Karachi[4]. Enteric fever is transmitted via oro-fecal route, mostly through fecal contaminated food or water consumption[5].

During last many years, the emergence of multidrug-resistant (MDR) *Salmonella enterica* strains have been highly reported from developing countries. Ampicillin, chloramphenicol and co-trimoxazole (trimethoprim-sulfamethoxazole) were the treatment of choice till the 1970s, known as first line drugs against Salmonella enterica[2]. Then fluoroquinolones remained second-line drug of choice in enteric fever for many years, but now fluoroquinolone resistance has also been frequently reported in developing countries.[3]The third-generation cephalosporins are now in use for enteric fever treatment, however, outbreaks of cephalosporin-resistant *Salmonella enterica*have also been documented recently[4,6].

In two years(from 2016 to 2018) World Health Organization (WHO) documented, 5274 XDR Salmonella Typhi cases from 14 districts of Sindh. The distribution was, 76% cases from Karachi, 27% cases from Hyderabad and 4% cases from other districts of Sindh.[6]The first outbreak of ceftriaxone resistant *Salmonella enterica* was reported in two sub-districts of Hyderabad, Sindh, in 2016[2,4].

Inspite of steps taken by the local government, there was progressive rise in documented cases in two years period (2017 to 2018)[7]. The international surveillance for XDR *Salmonella typhi* registered one case from England and five cases from United states and one case from Canada recently. All the documented cases had travel record of Pakistan[8,9,10].

The objective of the study is to determine the frequency and antibiotic resistance patterns of *Salmonella enterica serovar* Typhi (S. Typhi) and Paratyphi A, B, and C isolatesin blood culture specimens from Punjab Rangers Teaching Hospital Lahore.

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METHODOLOGY

Bacterial Isolates: Between 1stJanuary-31stDecember 2021, a total of 1385 blood samples were processed for microscopic and biochemical identification of enteric fever isolates (Ethical approval / IRB Ref No. 06/2021). The suspected cases of typhoid fever were either admitted directly or referred to Punjab Rangers Teaching Hospital, Lahore, Pakistan. The blood samples were inoculated in recommended liquid culture media as soon after receiving the specimen. Bottles showing positive culture were sub-cultured onto Blood agar (Oxoid, UK), Chocolate agar (Oxoid, Hampshire, UK) and MacConkeys agar (Oxoid, Hampshire, UK), and incubated at 35-37 °C. Microscopy was done for preliminary identification of bacterial etiology after gram staining. Further biochemical analysis was done by Analytical Profile Index (API) 20E (Biomérieux, Lyon, France) for species confirmation. Agglutination analysis was done for serovarverification, by antisera (Denka Seiken Co Ltd., Tokyo, Japan) as per user manufacturer instructions.

Antimicrobial Susceptibility: The antimicrobial susceptibility testing for *Salmonella enterica* isolates was performed using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2021) standards for 1st, 2nd and 3rd line of antimicrobials. The isolates were labelled "multidrug-resistant" (MDR) and "extensively drug-resistant" (XDR); which were resistant to 1st line drugs and those showing resistance to more than 2 groups of antibiotics[12].

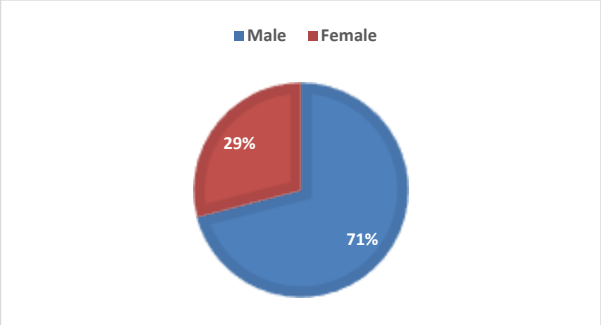
Statistical analysis: Statistically data was entered and evaluated using SPSS Version 22. Study data of gender, frequency of positive cases was represented as frequency percentage.

Graphically, Pi chart and bar graphs used to show distribution of cases. Chi square test applied to determine significance of qualitative variables. P-value of < 0.05 was taken as significant.

RESULTS

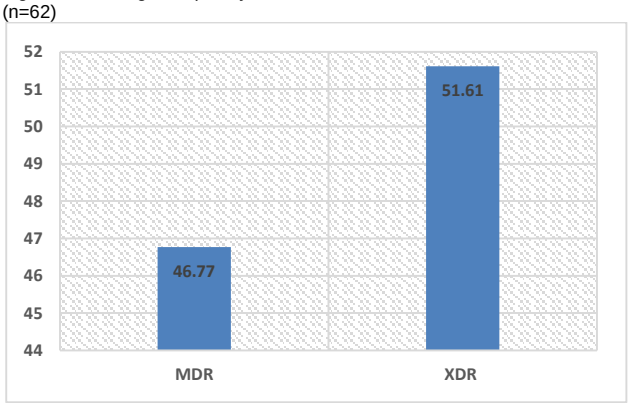
A total of 1385 blood samples were cultured, and 425 (30.68%) samples gave positive growth. Sixty-two (14.58%) isolates were identified as *Salmonella enterica* species. Figure 1 shows the frequency distribution of isolates according to gender. Eight isolates (12.90%) were from < 5 years of age, 35(56.45%) from 5 to 15 years, 12(19.35%) from >15 to 30 years and 7(11.29%) from above 30 years.

Fig 1: Gender wise frequency distribution of typhoid fever.



Species differentiation revealed, 52 (83.87%) were *Salmonella typhi* and 10 (16.12%) were *Salmonella paratyphi A*. Sensitivity pattern of all the isolates (N=62), indicated 29 (46.77%) were multi-drug resistant (MDR), while 32 (51.61%) were extensively-drug resistant (XDR) *Salmonella enterica* isolates (Fig. 2).

Fig 2: Percentage frequency of MDR &XDR Salmonella enterica isolates (n=62)



All the isolates were 100% sensitive to Azithromycin. Only one isolate of female child showed 100% sensitivity to all the recommended antibiotics. Sensitivity pattern of all the isolates (N=62) according to CLSI, 2020 is given in table 1.

Table 1: Antibiotic susceptibility outline of Salmonella enterica isolates (n=62)

Antibiotics	Sensitive%	Resistant%
First line Drugs		
Ampicillin	01 (1.61)	61 (98.38)
Co-trimoxazole	32 (51.61)	30 (48.38)
Piperacillin-Tazobactem	38 (61.29)	24 (38.70)
Chloramphenicol	32 (51.61)	30 (48.38)
Second line Drugs		
Ciprofloxacin	8 (12.90)	54 (87.09)
Ceftriaxone	30 (48.38)	32 (51.61)
Cefipime	35 (56.45)	27 (43.54)
Third line Drugs		
Imepenem	51 (82.25)	11 (17.74)
Meropenem	60 (96.77)	02 (3.22)

DISCUSSIONS

According to our data, age wise occurrence of cases indicates dominance by children, as is also documented by some other studies from different regions of Pakistan. This correlates with some other studies as well[3],[6,7,8]. High incidence in children should be cause of concern and effective vaccination should be initiated and monitored in school going age group. There is predominance of male gender which indicates that the parameters for existence and spread of XDR Salmonella enterica should be re-evaluated.Same indicated in another local study[4].

Out of 62 isolates, 52 were Salmonella Typhi and 10 were Salmonella Paratyphi A[12,13]. Various studies have documented the presence of MDR Salmonella enteric all over Pakistan. MDR strains are resistant to the first line antibiotics, which are chloramphenicol, ampicillin, and trimethoprimsulfamethoxazole[12,14]. Antibiotic stewardship should have been considered many years back when this issue started emerging.

Recently, Extensively drug-resistant Salmonella enterica isolates have emerged as an important notifiable health issue in Pakistan. These are labeled as XDR Salmonella enterica when they show resistance to chloramphenicol, ampicillin, trimethoprim sulfamethoxazole, f luoroquinolones and third-generation cephalosporins.[3,13,15]In our set up MDR Salmonella enterica isolates are 46.7% and XDR strains are 51.6% which is quite relatable to other local and international studies[17,18].

If we see the antibiotic sensitivity pattern of our Salmonella enterica isolates to first line drugs only one isolate was sensitive to ampicillin and 48.38% isolates were resistant to each chloramphenicol and cotrimoxazole. MDR strains have been an issue since many years. These findings were relatable to some other studies[16,17].

Among second line drugs, ciprofloxacin was highly resistant against Salmonella enterica isolates,(87.09%) which is in consistent with many studies locally and internationally[13,14,18,19].

This refers to irrational use of floroquinolones during past 10 to 15 yrs. Ciprofloxacin had been treatment of choice for enteric fever since 1990s due to its oral intake except for children but now 10% isolates are resistant in Asia and Africa[14,15,20].

Third generation cephalosporins, cefixime and ceftriaxone are currently treatment of choice forenteric fever due to their broader spectrum and MDR phenotypes& floroquinolone resistance among enteric fever isolates. In our study, almost fifty percent isolates were resistant to third generation cephalosporins (51.61%).This indicates alarming rise in antimicrobial resistance in Salmonella enterica isolates i.e., XDR strains. Many local and international studies have quoted cephalosporin resistant isolates of Salmonella enterica. Infact genome sequencing of resistant isolates is being done in various studies[4,18,19,20].

Regarding third line drugs for enteric fever treatment, meropenem was highly sensitive and only 2% isolates showed resistance. Azithromycin was 100% sensitive against Salmonella enterica isolates in our study. Azithromycin is preferred option nowadays for uncomplicated enteric fever. But its sensitivity patterns needs to be monitored closely due to threat of antibiotic resistance. These findings are in consistent with some recent data[20,21].

Keeping in view such increasing antimicrobial resistance patterns we need to take measures for general public awareness for health and hygiene, prompt diagnosis and documentation of cases by public health sector.

CONCLUSIONS

The spread of extensively drug resistant Enteric fever should be an warning sign that we are gradually going into pre-antibiotic era due to widely irrational use of antimicrobials.

This should be vital cause of concern for organizations in developing countries such as Pakistan, where antibiotic stewardship programmes are not up to date. Mass Vaccination campaigns need to be initiated and properly monitored and general

public awareness programmes about hand, food and water hygiene should be carried out on regular basis. Safe water resources, hand hygiene and effective antibiotic policies can help us to control the disease therefore reducing antimicrobial resistance.

Ethical considerations: Formal approval was taken from concerned authority to conduct the study. Confidentiality was maintained.

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