ORIGINAL ARTICLE

CURRENT ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG BLOOD CULTURE ISOLATES OF *SALMONELLA ENTERICA* SEROVAR TYPHI IN MUZAFFARABAD, PAKISTAN

Mumtaz Ahmad Khan, Jamila Farid*, Muhammad Idris*, Raja Ijaz, Sehrish Mumtaz***, Muhammad Shakil Sadiq†**

Department of Pathology, AJK Medical College, Muzaffarabad, *Ayub Medical College, Abbottabad, **Department of Surgery, AJK Medical College, ***Department of Medicine, Abbas Institute of Medical Sciences, †Department of Anatomy, AJK Medical College, Muzaffarabad, Azad Jammu and Kashmir

Background: Antibiotic resistance is a major problem in *Salmonella enterica* serovar Typhi (*S* Typhi), the causative agent of typhoid fever. The resistance has developed to three first line drugs (ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) in early 1980s, and labelled as multidrug resistant (MDR). Several cases of MDR *S* Typhi have also become resistant to fluoroquinolones and third generation cephalosporins, and are referred as extensively drug resistant (XDR). The aim of this study was to determine the current susceptibility pattern of *S* Typhi in our setup. **Methods:** This prospective study was carried out in the Department of Microbiology, Abbas Institute of Medical Sciences, Muzaffarabad, from January 2018 to December 2019. All blood culture samples from febrile patients were inoculated in Oxoid Signal blood culture system (Remel, Lenexa KS) and incubated at 37 °C. Positive culture bottles were sub cultured on Blood agar and MacConkey agar, and incubated for overnight at 37 °C. The isolates were identified as *S* Typhi by colony morphology, serotyping, and biochemical tests using API 20E. Susceptibility testing was performed using the Modified Kirby Bauer disk diffusion method. The results were interpreted according to Clinical Laboratory Standards 2018. All XDR isolates were confirmed by determining the MICs of ciprofloxacin and ceftriaxone by using the E-test strips. **Results:** A total of 360 blood culture specimens were submitted, 146 (40.5%) specimens yielded growth of *S* Typhi. The resistance rates of *S* Typhi for ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole were 82%, 88% and 89% respectively. Percentage of MDR isolates in *S* Typhi was found very high (108, 74% isolates). A high rate of resistance (78, 53% isolates) was found to ciprofloxacin. Frequency of XDR isolates in *S* Typhi was high (40, 27% isolates). All isolates were found sensitive to azithromycin and meropenem.

Keywords: *S* Typhi, Multidrug resistant (MDR), Extensively drug resistant (XDR), Drug resistance

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INTRODUCTION

Typhoid fever remains a significant public health problem in under resourced countries and is one of the leading causes of mortality in developing countries.[1] It is caused by *Salmonella enterica,* Serovar Typhi (*S* Typhi). In developing countries the prevalence of antibiotic-resistant *S* Typhi strains has increased significantly in recent years. Due to irrational use of antibiotics resistance has been developed in three first-line drugs (ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) in the early 1980s. Serovar Typhi strains with resistance to these three antibiotics are labelled as multidrug resistant (MDR).[2] The emergence of MDR strains of *Salmonella spp* has complicated the treatment of enteric fever and has become one of the greatest challenges for physicians. Multi-Drug Resistant (MDR) isolates were reported from South-East-Asia and Africa. Several cases of MDR *S* Typhi have also developed resistance to fluoroquinolones.[3] The main therapeutic option was the use of fluoroquinolones for MDR isolates. Among the fluoroquinolones, ciprofloxacin and ofloxacin have become the most widely used antimicrobial agents. However, over the past two decades, a dramatic rise in fluoroquinolone-resistant *S* Typhi isolates has been seen globally.[1] Third-generation cephalosporin and azithromycin emerged as main drugs to treat MDR and fluoroquinolone resistant *S* Typhi. Among third generation cephalosporins, ceftriaxone, cefotaxime and cefoperazone are effective therapeutic alternative in multidrug resistant and quinolone resistant *S* Typhi infected cases.[4] A large proportion of ceftriaxone-resistant cases has recently been reported from Pakistan and India leaving limited therapeutic options.[2,5] Since November 2016, outbreaks of ceftriaxone-resistant *S* Typhi have been reported in the province of Sindh, Pakistan.[2,6] These isolates were found resistant to three first line drugs, fluoroquinolones, and third-generation cephalosporins and labelled as XDR.[7,8] These resistant isolates are H58 haplotype, harbouring qnrS fluoroquinolones resistant gene and blaCTX-M-15 ESBL genes, rendering it non-susceptible to fluoroquinolones and ceftriaxone.[9] The Provincial Disease Surveillance and Response Unit (PDSRU) of Sind reported 5274 XDR *S* Typhi cases from 2016 to

2018.[10] Few Cases of XDR *S* Typhi have been identified from the USA and UK who had travel history from Pakistan[7] which shows global threat of spread of resistance.[11] Some cases of XDR *S* Typhi were also recognized from Islamabad.[10] We still have inadequate information regarding the prevalence of XDR *S* Typhi in our country. The aim of this study was to determine the current susceptibility pattern of *Salmonella enterica* Serovar Typhi isolated from blood samples in our setup.

METHODOLOGY

This prospective study was carried out in the Department of Microbiology, Abbas Institute of Medical Sciences (AIMS), Muzaffarabad, from January 2018 to December 2019, after taking permission from the Hospital Ethical Committee. The AIMS Hospital receives patients from northern region of Azad Jammu and Kashmir. Informed consent was taken from all the patients. All the blood culture samples from suspected cases of enteric fever were inoculated in blood culture bottles (Oxoid Signal Blood Culture System, UK) and incubated according to manufacturer's instructions. Positive culture bottles were sub cultured on Blood agar and MacConkey agar. The media plates were incubated aerobically for overnight at 37 ºC. The isolates were identified as *S.* Typhi by Gram staining, colonial morphology, serotyping, and biochemical tests using API 20E galleries (Bio Merieux, France). Susceptibility testing was performed using the Modified Kirby- Bauer disk diffusion method. The results were interpreted according to Clinical Laboratory Standards 2018.[12] *Escherichia coli* ATCC 25922 was used for quality control of discs. All the extensively drug-resistant (XDR) isolates were confirmed by determining the MICs of ciprofloxacin and ceftriaxone by using the E-test strips (Ab Biodisk, Solna, Sweden) according to the manufacturers' instructions. *Escherichia coli* ATCC 25922 was used as the control strain. WHONET Version 5.6 was used for compilation and calculation of data.

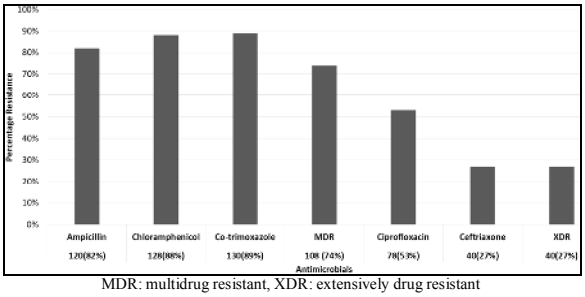
RESULTS

During study period a total of 360 blood culture specimens from suspected cases of enteric fever were submitted for culture and sensitivity testing. Out of these, 146 (40.5%) specimens yielded growth of *Salmonella* Typhi. Most (38%) of the isolates were from adults 16–40 years of age followed by (26%) children 5–10 years of age (Table-1).

The resistance rates of *S* Typhi for ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole were 82%, 88% and 89% respectively. Percentage of multidrug-resistant (MDR) isolates in *S* Typhi was found very high 74% (108 isolates). Resistance to ciprofloxacin against *S* Typhi was also found to be high (78, 53% isolates). XDR isolates in *S* Typhi were significant (40, 27% isolates). All isolates were found sensitive to azithromycin and meropenem. (Figure-1).

Table-1: Age distribution of patients with *S* Typhi

Age (Years)	Number	Percentage
0–4	8	5
5–10	38	26
11–15	12	8
16–20	16	11
21–30	19	13
31–40	20	14
41–50	13	9
51–60	12	8
≥61	8	5



MDR: multidrug resistant, XDR: extensively drug resistant

Figure-1: Antimicrobial susceptibility pattern of *S* Typhi

DISCUSSION

Recent studies suggest that globally about 21 million people contract typhoid fever in a year.[13] In Pakistan, typhoid is more common in provinces of Sindh and Punjab, out of all those Asian countries where typhoid is prevalent.[14] Its spread is mainly attributed to unsafe potable water and unhygienic sanitary practices. In our country, an increasing trend of multi drug resistant and extensively drug-resistant *S* Typhi has been reported in many studies.[6,14] Irrational use of antimicrobials both in humans and poultry industry have significantly contributed in developing resistance to multiple antibiotics.[1] In our study, resistance rates of ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol to be 82%, 88% and 89% respectively. These findings are consistent with the data reported by Hussain *et al*.[6] A study from Nepal also showed a high resistance rate of chloramphenicol, co-trimoxazole, and ampicillin as 98.8%, 98.8%, and 97.6% respectively.[15] Another study in Kenya also reported a high proportion of the isolates were resistant to the conventional first line antibiotics ampicillin (72%), cotrimoxazole (70%) and chloramphenicol (72%).[16]

We found a high percentage of MDR isolates in *S* Typhi, (108, 74% isolates). This finding is comparable with findings of Hussain *et al*[6]. Mutai *et al*[16] also recorded 68% cases of MDR *S* Typhi in Kenia. However, the prevalence of MDR isolates was found significantly low in other studies.[17,18] Ciprofloxacin has been used as a drug of choice in the treatment of MDR cases. We found 53% cases resistant to ciprofloxacin.

Higher rate of ciprofloxacin resistance was seen in recent local study as compared to our study[6], whereas relatively low rates were observed in other regional and international studies.[16,19]

Frequent use of fluoroquinolones for trivial infections like diarrheal diseases and urinary tract infections is the main cause of resistance to these agents. Therefore, this drug is no longer recommended empirically in typhoid fever in Pakistan. The emergence of resistance to flouroquinolones in *Salmonella spp.* is mainly due to the mutation in the genes coding for DNA gyrase and topoisomerase IV. This resistance could also result from changes in drug entry and efflux.[20]

The emergence of MDR and quinolone resistant *Salmonella* has forced physicians to prescribe third generation cephalosporin typhoid fever. This study documented 27% XDR strains in *S* Typhi. These XDR isolates were resistant to three first line drugs, ciprofloxacin and to third-generation cephalosporin. Ceftriaxone resistance was not reported in Pakistan until 2016 when a large proportion of XDR *S* Typhi was reported from the cities of Hyderabad and Karachi.[2,21] The main therapeutic option is the use of either meropenem or azithromycin for XDR cases. In our study, all isolates were found sensitive to azithromycin and meropenem. However, sporadic cases of azithromycin resistance have also been reported in some local studies.[6] Azithromycin is effective for uncomplicated cases, while carbapenems may be used for patients with suspected severe or complicated typhoid fever.

The emerging MDR and XDR *S* Typhi strains are an eye opener for clinicians. Over the counter prescription and irrational use of antimicrobials may have driven the emergence of MDR and XDR strains. The use of antibiotics as growth promoters for livestock production may also be contributing for resistance in human pathogens. These resistance patterns warn us that such antibiotic resistance can spread globally that requires bold and decisive preventive public health measures. Because of emergence of such resistance associated with *S* Typhi, the role of preventing spread of this pathogen to the communities is paramount. Advocacy for rational use of antibiotics, mass immunization of prevalent communities with typhoid vaccine, public health education, practicing safe eating and drinking habits are vital in the prevention of MDR/XDR enteric fever.

CONCLUSION

The spread and emergence of Multidrug resistant (MDR) and extensively drug resistant (XDR) *Salmonella* Typhi is alarming and highlights the significance of strict antimicrobial resistance (AMR) surveillance programs with antimicrobial stewardship.

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Address for Correspondence:

Dr Mumtaz Ahmad Khan, Associate Professor, Department of Pathology, AJK Medical College/Abbas Institute of Medical Sciences, Muzaffarabad, Azad Kashmir, Pakistan. **Cell:** +92-323-5049579
Email: mumtazahmad8166@gmail.com

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Contribution of Authors:

MAK: Concept, Study design, Data collection and analysis, Manuscript writing and review
JF: Data analysis and interpretation, Manuscript writing and final review
MI: Data analysis and interpretation, Manuscript writing and final review
RI: Data analysis and interpretation, Manuscript writing and final review
SM: Manuscript writing and revision, Facilitation for reagents
MSS: Data analysis, Manuscript writing, Proof reading

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