

## ORIGINAL ARTICLE

# FREQUENCY AND ANTIMICROBIAL RESISTANCE PATTERN OF SALMONELLA TYPHI ISOLATED FROM BLOOD CULTURES

<sup>1</sup>Murtaza Khan <sup>1</sup>Faisal Iqbal Afridi, <sup>2</sup>Jawaid Abu bakar Warind, <sup>3</sup>Qamar Jamal

<sup>1</sup>Department of Microbiology, <sup>2</sup>Department of Medicine,

<sup>3</sup>Department of Pathology, Ziauddin University, Karachi.

## ABSTRACT

**Background:** Enteric fever spread worldwide by Salmonella typhi (*S. typhi*). The causative agent, *S. typhi* is gram negative bacilli belongs to the family Enterobacteriaceae. It is a motile, facultative anaerobic bacilli. Multi-drug resistant (MDR) *S. typhi* is being accepted as most challenging enteric pathogen in developing countries like Pakistan. The accurate diagnosis of *S. typhi* is vital for treatment. This study evaluates frequency of *S. typhi* in blood cultures and antibiotic resistance against *S. typhi*. The study assist physician to decide better antimicrobial option to treat patients and also update resistant pattern of *S. typhi*. The aim of this study was to determine the frequency and antibiotic resistance pattern of Salmonella typhi isolated in blood cultures at tertiary care hospital laboratory.

**Methodology:** All Blood culture samples were received from febrile patients. An automated machine, BACTEC 9240 were used for blood culture processing. Identification of *S. typhi* from positive cultures were done by the help of gram stain, colony morphology, biochemical tests, and serological testing. Antimicrobial susceptibility testing was performed through Kirby Bauer method on Mueller Hinton agar according to Clinical Laboratory Standard Institute guidelines. Data analysis was completed by using Statistical Package for Social Sciences (SPSS) version-20.

**Results:** A total of 16010 samples of blood were cultured for bacterial isolation, Out of these, 411 (2.56%) turned out to be positive for *S. typhi* while 193 Isolates were MDR strains and 218 isolates were Non-MDR strains. None of the MDR strains were resistant to cefixime, ceftriaxone and aztreonam .

**Conclusion:** This study indicated that resistance in *S. typhi* is progressively high against first line drugs which draw attention towards monitoring of antimicrobial resistance. Rational use of antimicrobials should be implemented on the basis of culture and sensitivity results.

**KEYWORDS:** Enteric fever, Salmonella Typhi, Drug resistance

### Corresponding Author

Murtaza Khan,

Department of Microbiology,

Ziauddin University Hospital,

North Nazimabad Campus, Karachi.

E-mail: murtuza\_khans3005@yahoo.com

## INTRODUCTION

Salmonella typhi (*S. typhi*) has long been recognized to cause enteric fever in many parts of the world.<sup>1</sup> Enteric fever is the most common bacteremic sickness in highly endemic countries, such as Pakistan and other Asia's countries.<sup>2</sup> Accompanying

symptoms include headache, cough, and different gastrointestinal complaints, such as nausea, vomiting, and abdominal pain and some time bloody diarrhea. Often constipation is much more common than diarrhea except in children.<sup>3</sup> The incubation period of typhoid fever is usually 8-14 days. The duration of illness is about 4 to 5 weeks.

The causative organism can be isolated from blood in 80% of patient during first week of illness if blood is collected for culture before starting antibiotics.<sup>4</sup>

*S. typhi* is a common cause of morbidity and mortality in the developing countries, earlier study showed high rate of annual deaths specially in developing countries along with 700000 yearly deaths occurring worldwide with enteric fever.<sup>5</sup> Antimicrobials are essential for appropriate clinical treatment for *S. typhi*, but multi-drug resistant (MDR) *S. typhi* is a major issue and emergence for public health in developing countries.<sup>6</sup>

MDR *S. typhi* (resistance to chloramphenicol, trimethoprim/sulfamethoxazole and ampicillin) have been widespread and hinder effective treatment and limit alternatives.<sup>7-8</sup> Quinolones was alternative drug for treating enteric fever after the *S. typhi* resistance occur to ciprofloxacin has been reported in many previous studies along with treatment failures with ciprofloxacin.<sup>9-10</sup> Third generation cephalosporins such as cefixime and ceftriaxone are alternative treatment options for enteric fever in developing countries including Pakistan, but some recent studies reported third generation cephalosporin resistant strains of *S. typhi*.<sup>11-12</sup>

In this study we determined the frequency and antimicrobial susceptibility pattern of *S. typhi*, isolated from the blood samples for culture and sensitivity of the patients suspected for enteric fever. Antimicrobial resistance is currently the most intimidating difficulty as regards to Infection control point of view as well as for treating physicians. This study also guides for the proper selection of antimicrobial against *S. typhi*

## METHODS

This descriptive study was conducted over a period of one year from 1st January 2016 to 31st December 2016 at the Department of Clinical Microbiology of Dr. Ziauddin University Hospital. Blood samples for culture and sensitivities were collected from both in-patients and out-patients. All *Salmonella typhi* isolated from blood samples were included in this study. Positive Samples that showed other gram negative and gram positive organisms were excluded from the study. From institutional ethical committee, written agreement was taken. Knowledgeable consent was taken from the patient or any family member. The blood samples were collected in one aerobic and one anaerobic BACTEC blood culture bottles and inserted into automated machine, BACTEC 9240. Every BACTEC vial include a sensor which react with concentration of carbon dioxide released from the metabolism of microorganisms or the utilization of oxygen required for the growth of microorganisms. Fluorescence increasing monitored in every ten minutes by instruments sensor,

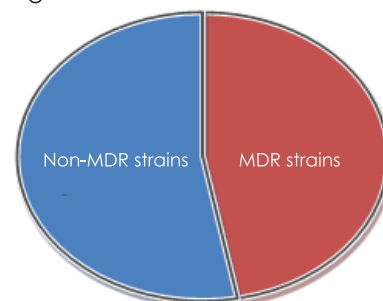
which is proportional to the increasing amount of carbon dioxide or the decreasing amount of oxygen present in the BACTECT vial. Positive interpretation shows the presence of microorganisms in the BACTEC vial. Every suspected vial taken out aseptically and performed gram staining from vial with control slide. The broth from the positive vial sub-cultured on Chocolate Agar, Sheep Blood Agar and Macconkey Agar. Macconkey was incubated at 37°C for 18-24 hours whereas chocolate agar and sheep blood agar incubated in 5% CO<sup>2</sup> incubator. After 18-24 hours incubation plates were examined, Non-lactose fermenting colonies on Macconkey's agar plate suspected for *S. typhi* were further identified by oxidase negative. Furthermore the serological tests (slide agglutination test) were done to confirm *S. typhi* according to standard microbiological technique.<sup>13</sup> The antisera used from Bact-Med Diagnostic co. Ltd containing specific antibodies against somatic antigen.

Results were interpreted followed by Clinical and Laboratory Standards Institute (CLSI) Guidelines and Antimicrobial susceptibility testing was performed on Mueller hinton media (Oxoid Ltd., England) using modified Kirby Bauer's disk diffusion method.<sup>14</sup> The antibiotics tested were ampicillin, aztreonam, ceftriaxone, cefixime, ciprofloxacin, trimethoprim-sulfamethoxazole, azithromycin and chloramphenicol.

Analysis of Data was performed by using Statistical Package for Social Sciences (SPSS) version-20.

## RESULT

A total of 16010 blood samples were received for culture and antimicrobial sensitivity during the study period. Out of 16010, 411 samples were found to be positive for *S. typhi*. The Frequency of *S. typhi* isolates were 411/16010 (2.56%). Out of 411, 193 (47%) isolates were identified as MDR strain as shown in Figure 1. Antibiotic Resistance pattern of other antimicrobials tested against *S. typhi* isolates is shown in Figure 2.



**Figure 1:** Percentages of MDR and Non-MDR *S. typhi*, 193 Isolates were MDR strains and 218 isolates were Non-MDR strains

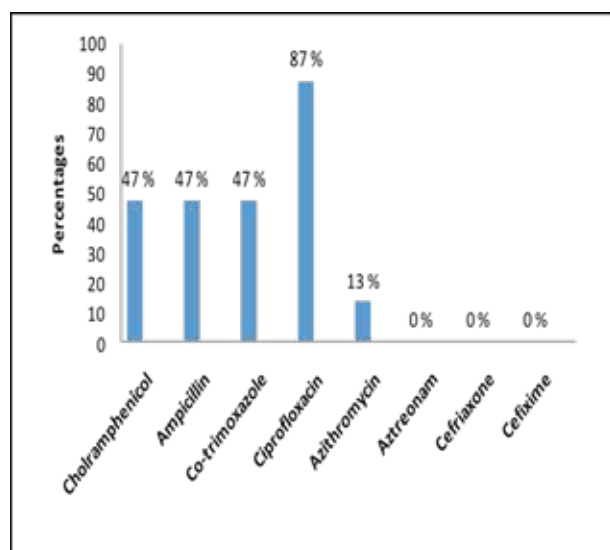


Figure 2: Antibiotic Resistant Pattern of *S. typhi*

## DISCUSSION

*S. typhi* causative agent of Enteric fever, it is a systemic and widespread infection with high rate of morbidity and mortality mostly in developing countries including Pakistan due to worse sanitation, overcrowding and underprivileged health care infrastructure.<sup>15</sup>

Our study showed the frequency of *S. typhi* was 2.56%. As shown in this study conducted in Nepal showed frequency of *S. typhi* was 28.0%.<sup>16</sup> Data from a study done in Quetta, Balochistan showed, frequency in *S. typhi* was 18.8%.<sup>17</sup> A similar study published by district Quetta Pakistan showed frequency of *S. typhi* was 14.63%.<sup>18</sup> Another study in Taxila, a famous city of the Punjab, Pakistan observed frequency of *S. typhi* was 25.26%.<sup>19</sup> In India, a similar study showed significant burden of *S. typhi* and frequency was 9.7%.<sup>20</sup> Another similar study conducted in Civil Service Hospital, Minbhawan Kathmandu, Nepal, data of this study showed frequency of *S. typhi* was 9.2%.<sup>21</sup> Data collected from Indian and Chinese studies confirmed to cause of rising Frequency of *S. typhi* in last year's due to increased use of Vi polysaccharide vaccine.<sup>22</sup> Another similar study showed that *S. typhi* affected mostly young people about 92.9% outstanding interactions with drinking water and foodstuff which infected with *S. typhi*.<sup>23</sup> Comparable study in china proved that public living standards defiantly decreased incidence of *S. typhi* in populations.<sup>24</sup>

Epidemic issue of multidrug-resistant (MDR) strains (resistant to chloramphenicol, ampicillin and sulfamethoxazole-trimethoprim) became a major

concern from early 90s, resulting in extensive use of ciprofloxacin as first line therapy for enteric fever in MDR strain of *S. typhi*.<sup>25</sup>

Our study showed the MDR of *S. typhi* isolates was 47.0% while pattern of resistance of *S. typhi* with Ampicillin, Chloramphenicol, Co-trimoxazole, Azithromycin and Ciprofloxacin were 47.0 %, 47.0%, 47.0%, 13% and 87% respectively.<sup>26</sup> A similar study in India isolation of MDR strain of *S. typhi* was 3.4% and the pattern of antimicrobial resistance in this study was 5.0% to co-trimoxazole, 13 % to ampicillin and 10.0% to chloramphenicol and 95.8% to ciprofloxacin was observed.<sup>27</sup> Another similar study in India, MDR was found in 2% in *S. typhi* and antimicrobial pattern showed 12.5%, 5.0%, 2.5% and 97.5% resistance to ampicillin, chloramphenicol, co-trimoxazole and ciprofloxacin respectively.<sup>28</sup> In Nepal same studies showed the MDR in *S. typhi* was 1.08% and pattern of antimicrobial resistance 3.03% with ampicillin, 3.03% with chloramphenicol, 3.03% with co-trimoxazole, 81.82% with ciprofloxacin and 18.19% with azithromycin was observed.<sup>29</sup> Antimicrobial data from different region of Asia indicated that resistance rates of *S. typhi* in Bangladesh were 39.5% for Ciprofloxacin, 68.4% for Ampicillin, 57.9% for Chloramphenicol, 57.9% , 68.4% for Trimethoprim, and Sulfamethoxazole, in Vietnam resistant rate 00% for Ciprofloxacin, 80.4% for Ampicillin, 80.4% for Chloramphenicol, 80.4% for Trimethoprim, and Sulfamethoxazole, although in Indonesia they were 0.0% for Ciprofloxacin, 68.4% for Ampicillin, 68.4%, 57.9 Trimethoprim and Sulfamethoxazole, besides very low resistance were observed in Taiwan as compare to other region.<sup>30</sup> A study conducted in China, antibiotics resistance was recorded in *S. typhi* were, Trimethoprim resistance was 96%, Sulfamethoxazole 95%, Ampicillin 95%, Ciprofloxacin 66.2%, Nalidixic acid 80.58% and Chloramphenicol 62 %.<sup>31</sup>

This study shows that we have still better options like Cefixime, azithromycin, and ceftriaxone for treating *S. typhi* infections, if the isolates also reveal resistance to first-line antibiotics. The following two main factors are responsible for the emergence of multi drug resistant (MDR)

*S. typhi* in this region. The single most important factor in the increase of MDR *S. typhi* is certainly an indiscriminate use of antibiotics. Most physicians as well as Hakeems are responsible for encouraging the adventurous use of drugs without administering appropriate diagnostic procedures like blood culture test, etc. Sub therapeutic use of antibiotic is another factor contributing towards the emergence of MDR *S. typhi*. The bacteria develop rapid resistance when medicine in accurate potency is not administered or is discontinued before its total eradication.



## CONCLUSION

The present study showed that infection caused by *S. typhi* remains an important public health problem, particularly in developing countries including Pakistan due to many social, economic, and sanitary factors. Present study clearly indicated that high resistance against Ampicillin, Chloramphenicol, Trimethoprim, and Sulfamethoxazole and Ciprofloxacin were observed.

Options still available to treat infected people with appropriate antibiotics as indicated by sensitivity tests which should be employed to prevent the development of resistant strains of *S. typhi*. However, improved sanitation, vaccination, and cleanliness of drinking water will obviously help in a decline of the frequency of *S. typhi* infections in Pakistan.

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