Incidence of *Salmonella typhi* in Blood Cultures and Antibiogram Patterns in Peshawar

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**ABSTRACT**

**Background:** Typhoid fever, caused by *Salmonella typhi*, has become a major public health problem worldwide, particularly in developing nations like Pakistan, as a result of the emergence of resistant strains. The aim of this study was to identify unique differences in antibiotic susceptibility across *Salmonella typhi* isolates isolated from human blood samples, providing insight into critical patterns impacting successful treatment approaches.

**Methodology:** 2000 patients’ blood samples collect whose sign and symptom like typhoid positive individuals were cultured on blood agar and MacConkey agar to detect *Salmonella typhi*. Biochemical assays were used to identify the bacterium, and the disc diffusion method on MHA agar was used to evaluate its susceptibility to eleven antibiotics.

**Results:** *Salmonella typhi* (5%) was identified from patients' blood samples. Ciprofloxacin resistance was found in 32% of those tested, followed by resistance to azithromycin (41%), ceftazidime (87%), cefotaxime (88%), sulactum/cefoperazone and piperacillin/tazobactam (89%), and chloramphenicol (97%) and Tigecycline, Imipenem, Gentamicin and Amikacin along with Meropenem shows 0% resistance toward bacterium otherwise there was no Funding.

**Conclusion:** According to our findings, *Salmonella typhi* very rapidly developing resistance to antimicrobial drugs as a result of virulence transfer and antibiotic resistance, this might lead to treatment failure in typhoid patients in Pakistan.

**Keywords:** *Salmonella typhi*, Resistance, Susceptibility, Blood Cultures, Multidrug-resistant.

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**INTRODUCTION**

*Salmonella* spp cause the Enteric fever and some other infection. There are many issues in treatment due the resistance of broad rang antibiotics which is called (MDR) multi drugs resistance but in some are completely resistance to drugs that is known is Extensively Drug Resistance (XDR) that is the worldwide public health issue but mostly in developing countries also in Pakistan. The prevalence of multidrug-resistant *Salmonella* strains increased from 1992 (approximately 20%) to 2015 (50%), while the resistance of *Salmonella typhi* to fluoroquinolones increased in 2002 and was recorded as 96.5% in 2015. A study conducted at Rajshahi Medical College in Bangladesh described that an isolated strain of *Salmonella typhi* is resistant to ciprofloxacin (62.5%) and ceftriaxone (20.7%), although 17% of MDR cases and XDR cases 3.7%. *Salmonella* specie is a group of bacteria, which is an aerobic and non- pore-forming, flagellated bacilli, belong to the gram negative bacteria which are the family member of Enterobacteriaceae. The *Salmonella* call are species which shape are rod, length are 2-3 micrometer and the diameter are 0.4-0.6 micrometer. They are non-lactose fermenting bacterium; *Salmonella* are growing on different media, for example. (MacConkey Agar, Blood Agar, Salmonella and Shegalla Agar (SS Agar), XLD Agar). The *Salmonella* species contains more than 2500 serotypes taxonomic groups, which define on the basis of the three types of virulence factor i.e., flagellar antigen H (they invade inside the cell), somatic antigens H (they replicate intracellular) and invasive antigen Vi (more toxin production). *Salmonella typhi* and Paratyphoid A are host-restricted pathogens, whose reservoir is humans. It is most common in many developing countries, with poor drainage system. *Salmonella* spp are multi-organs pathogen, characterize to inhibit the small intestine lymphatic tissue, spleen, liver and infected person blood stream. *Salmonella* species causes multiple complicated diseases, such as urinary tract infections and Enteric fever etc. *Salmonella* spp is leading causes of enteric fever, which is a food borne infectious disease which
used Fecal-Oral-Route for transmission, usually from eating and drinking of food and water which are contaminated. It is primarily cause of local intestinal inflammation, diarrhea and intestinal perforation etc. Upon ingestion into the body, its active replication takes place 7 to 10 days. After that they invade the epithelial cell of the small intestine, which spread in mesenteric lymph nodes and hepatic cells through the portal vein. Reticendothelial cell uptake and it spread in the body which causes the hepatomegaly, splenomegaly and also cause meningitis. The Typhoid fever mostly found in urban communities because this area has poor or weak Salmonelleanitation system. In past 20 years, It is estimated that typhoid fever causes 21.7 million illnesses and 216,000 deaths worldwide. According to estimates by the International Vaccine Institute, there are 11.9 million cases of typhoid fever and 129,000 deaths in developing countries in 2010. Furthermore, according to WHO report, 16 to 17 million peoples are affected from Salmonella typhi and the death rate elevates up to 600,000 deaths, all over the world. The death rate due to typhoid infection varies from location to location, but the mortality rate as high as 5 to 7% in regardless of the use of regular antibiotic treatment. In each year 500 deaths occur out of 2 to 4 million individuals infected with Salmonella typhi. Only 1-10% of cases are reported in Asia, Africa, Central and South America. According to the World Health Organization, In Pakistan, annually 412 individuals get infected with typhoid fever in every 100,000 cases. The aim of this study was to identify unique differences in antibiotic susceptibility across Salmonella typhi isolates isolated from human blood samples, providing insight into critical patterns impacting successful treatment approaches.

MATERIALS & METHODS
To investigate that how percent of patient have Salmonella typhi and Salmonella Paratyphi in last 3 months and also identify although how much of Salmonella samples are MDR and XDR patient in Hayatabad Medical Complex Peshawar the all patient which have positive growth of Salmonella. All blood samples collected via convienent sampling technique.

A total of 2000 patent blood samples were collected from Hayatabad Medical Complex Hospital Peshawar medical ward patient how symptoms like Salmonella typhi in which I was fund 100 patient Salmonella typhi positive patient which is 5% of 2000 patients. There are two types of blood culture bottles Biotic and Manual bottle we used in which 30 ml of Brain Heart Infusion Broth (BHI) which are liquid media then insert 5ml of venous blood from patient. After collection of sample give sample numbers which is for identification for the patient Salmonella sample easily then take these blood sample placed in incubators for 5-7 days at 37°C the Biotic battle take place in specific incubator which alert that in this bottle have growth and that is no growth but the manual bottle take place in sample incubator which want to check these all bottle time by time and set by own method. After checking out these bottles and the agglutination is present in blood then its means that bacterial growth is present and when the blood transparent that means there is no growth of micro-organism. The Salmonella sample have a growth present were again cultured for subculture on blood agar and MacConkey agar plates for 24 hours at 37°C incubate. After growth seen on agar plates make a gram staining for seen these bacterial colonies in microscope that is these are gram positive or gram negative and also the bacteria morphology cocci, rods or bacillus and many more. After subculture of colony the biochemical test perform for identification of microorganism spcies take indol, TSI and also Antibiotics take on Nutrient Agar (NHA) take place in incubator for 24 hours. It next day check the indol and TSI and also Antibiotics Sensitivity on that day any error in identification of bacteria take API (Analytical Profile Index) after checking and balance the result will be publish for patient.

On MacConkey agar and DCA plate colony color always yellow and on blood agar have 1-3 mm size colony with large grayish, their convex will be low and round, entire margin (the colony is separate with one another). Salmonella Spp on Blood agar 1-3 mm size colony with large grayish, their convex will be low and round entire margin.

Kits for identifying Gram-positive bacteria, Gram-negative bacteria, and yeasts are known as API identification products, We used API (Analytical Profile Index) 20E in Hayatabad Medical Complex Peshawar which is a biochemical panel used to identify and distinguish members of the Enterobacteriaceae family. After confirmed that the culture belongs to Enterobacteriaceae family. A fast oxidase test for cytochrome oxidase can be used to determine this. By marking each test as positive or negative on the tray lid, you may get the API reading table (colored chart). Holes are marked as triples by black triangles, and scores are assigned to them.

RESULTS
One hundred Salmonella typhi and Para Typhi Salmonella samples were obtained from typhoid patients at Hayatabad Medical Complex in Peshawar for this investigation. There were 68 (68%) men and 32 (32%) females among the total number of patients. The 1.06:1 male-to-female ratio looked negligible. (Fig. 1).

Patients are classified into three age groups: 1 to 10 years old, 11 to 20 years old, 21 to 30 years old, and 31 to 50 years old (Above the 31 years old). The age category of children has the most patients, followed by the age group of adults. The number of Senior Adult
patients was kept to a bare minimum. Male patients account for a larger proportion of all age groups than female patients.

Figure 1: Percentage of *Salmonella typhi* and Para Typhi isolates males and females

Table 01: Distribution of the patients in different age groups

<table>
<thead>
<tr>
<th>Age Groups (Years)</th>
<th>Total Patients %</th>
<th>Male Patients %</th>
<th>Female Patients %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (1-10 years old)</td>
<td>68 %</td>
<td>54 %</td>
<td>14 %</td>
</tr>
<tr>
<td>Adolescence (11-20 years old)</td>
<td>25 %</td>
<td>12 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Adult (21-30 years old)</td>
<td>1 %</td>
<td>1 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Senior Adult (Above the 31 years old)</td>
<td>6 %</td>
<td>1 %</td>
<td>5 %</td>
</tr>
</tbody>
</table>

In observed towards Tigecycline, Imipenem, Gentamicin and Amikacin are highest rate of antibiotic susceptibility which are (100 %) then other antibiotics which will be also high sensitivity rate like Ciprofloxacin (68%), Azithromycin (59%), Ceftazidime (13%), Cefotaxime (12%), Sulactum/cefoperazone and Piperacillin/Tazobactam (11%) and Chloramphenicol (3%) in total 100 patient sample.

DISCUSSION
This research is to fill the gaps in domestic research, to try to estimate the prevalence and resistance of enteric fever in fourth months, 100 of *Salmonella typhi* positive state in 2000 patients which was 5%, the Bacteria was isolated from the suspected 100 patient who visited the tertiary hospital in Peshawar. In addition, we are also trying to predict whether there will be sensitivity of intestinal pathogens to conventional first-line drugs reappears drug. Despite the fact that there was no relation among gender overall illness incidence, our research observed that male had a greater incidence of cases (68%) than females (32%). Previous studies have indicated a somewhat different biological sex ratio which the incidence rate of male was (50.1%) and the incidence rate in female was (47.5%) (Peer et al 2021). In Southeast Asian countries, such as Pakistan, enteric fever is a significant public health issue. According to Kariuki et al 2021 in each year there are 596 *Salmonella typhi* isolated in 100,000 people which are 0.59%.

The incidence of Tigecycline sensitive rate in this study is 100% is compare the past study the 97% sensitive rate. Among the sensitive rate of Ciprofloxacin is (68%) nonetheless the sensitive rate was (84%) and the sensitive rate of Chloramphenicol is (3%) nonetheless the sensitive rate was (50%) The incidence of Amikacin sensitive rate is (100%) nonetheless the sensitive was (71.9%). The Azithromycin is (59%) in our study but the sensitive rate (46%). The incidence of Sulactum/cefoperazone sensitive rate in this study is 11% is comparing the past study the 36% sensitive rate. Among the sensitive rate of Piperacillin/Tazobactam is (11%) nonetheless the sensitive rate was (53.33%), Ceftazidime sensitive is (13%) in our study but (63.6%) according to the past study in this study the Cefotaxime is (12%) sensitive but the sensitive rate was (93.2%)

CONCLUSIONS
The incidence rate of *Salmonella typhi* was 5% in Hayatabad Peshawar well sense to be alarming for society as well as for Doctors, Teachers, Students and many other professionals from society. Furthermore frequent use of antibiotics leads to MDR and XDR strains of bacterium that is prediction about *Salmonella spp* going to be more prevalent on era.

Competing Interest declaration
There are no Competing Interests.

Funding Declaration
I certify that any study related to "Prevalence of *Salmonella typhi* in Blood Culture and Sensitivity Samples and its Antiibogram Pattern at Tertiary Care Hospital, Peshawar" is being sponsored solely by the private sector. For this project, no outside money has been obtained. I guarantee impartiality and honesty in the research by taking full responsibility for it.
Table 2: Observed resistance / susceptibility pattern of *Salmonella typhi* with age groups and P- value which was calculated by using SPSS

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitive % in ages</th>
<th>Resistance % in ages</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
<td>11-20</td>
<td>21-30</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>38</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Amikacin</td>
<td>68</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Ciproflaxacin</td>
<td>46</td>
<td>16</td>
<td>-</td>
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<tr>
<td>Tigecycline</td>
<td>68</td>
<td>25</td>
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</tr>
<tr>
<td>Gentamicin</td>
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<td>1</td>
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<tr>
<td>Cefepime</td>
<td>10</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>11</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>3</td>
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</tr>
<tr>
<td>Imipenem</td>
<td>68</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Sulactum/cefoperazone</td>
<td>11</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>11</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

REFERENCES