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Antibiogram Study and Resistance Mechanism of Salmonella typhi Isolated from Clinical Samples at District D.I. Khan

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ABSTRACT

The global issue of antibiotic-resistant bacteria (ARB) is a pervasive concern. The objective of this study was to isolate and identify the bacteria salmonella typhi (s. typhi) in blood samples from individuals who were diagnosed with typhoid fever. This study analyses 150 blood samples from patients who have presented with typhoid fever. Located in D.I Khan, the DHQ hospital is a medical facility. Questionnaires were employed to gather demographic data and ascertain antibiotic usage. The process of isolating bacteria and conducting antibiotic susceptibility tests was carried out using established microbiological methods. Multidrug resistant (MDR) microorganisms were chosen based on their resistance to three or

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more types of antibiotics. A total of 25 samples, accounting for 16.5% (n = 25), were isolated and identified as Salmonella Typhi. The prevalence between 20% and 60% was seen in the paediatric age range (1-5 years). Females had a higher prevalence (60%) of Salmonella typhi compared to males. Amoxicillin was mostly used to treat fever in patients. A significant resistance to amoxicillin (16.6%) and tetracycline (14.6%) was discovered in E. coli. Among Salmonella Typhi, both amoxicillin and ampicillin showed a resistance rate of 14.6% and 8% respectively, while cefotaxime had a resistance rate of 22% and imipenem had a resistance rate of 13%. The prevalence of the examined bacteria is high among the indiviauls aged 1-5 years and some of them exhibit multidrug-resistant (MDR) traits. This situation requires immediate attention from public health authorities. Therefore, it is necessary to conduct extensive investigations to determine the molecular epidemiology of these bacteria for the purpose of public health surveillance.

Keywords: Antibiogram, Blood culture, Multidrug-Resistant (MDR), Salmonella Typhi

INTRODUCTION

Salmonella belongs to the Enterobacteriaceae family of Gram-negative, microscopic, rod-shaped, facultatively anaerobic, aerobic, non-spore-forming, and non-capsulated microorganisms.

Humans can contract moderate to fatal salmonellosis from members of the Salmonella species; therefore, Salmonella must be isolated from a water sample at all times (*Abbas et al., 2019)*. Salmonella enterica Salmonella Typhi serotypes A, B, and C, as well as paratyphi serotypes A, C, are causative agents of typhoid fever, sometimes Known as well as enteric fever *(Adesoji et al.,* 2015). Typhoid fever, chills, nausea, diffuse abdominal pain, rash, anorexia, diarrhea, or constipation are symptoms of the fecal–oral mode of transmissionand hepatomegaly, splenomegaly, and relative bradycardia are frequently observed on physical examination.

Typhoid fever is still one of the leading causes of disease and mortality

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globally, particularly in low- and middle-income countries according to *(Adesoji et al., 2016)*. In full, poorly sanitary environments, typhoid fever is widespread. The (WHO) or World Health Organization, reported in 2014 that there were over 21 million typhoid cases annually worldwide, and that was about 222,000 deaths from the disease (*Ali et al., 2010*). The problem of antibiotic resistance has come to light more and more since the introduction of antibiotics in the middle of the 20th century (Anita et al; 2002).

The risk of resistance has increased due to a sharp decline in the rate at which new antibiotic classes are being introduced and a corresponding decline in funding for the field (*https://amr-review.org)*. The proliferation of highly pathogenic multidrug-resistant (MDR) bacteria in today's environment poses a challenge to the efficacy of healthcare Enterobacter salmonella an example of a subtype of the Gram-negative enteric pathogen is serovar Typhi, or S. Typhi. Enteritis salmonella. In addition to many other S. enterica serovars, S. Typhi is a human -limited disease that, to the best of our knowledge, is disseminated from person to person without the use of a zoonotic reservoir and has a limited capacity for long-term environmental survival *(Ayrikim et al., 2015)*.

Although S. Typhi can survive in food and water tainted with human feces, there isn't a stage of the bacterial life cycle that is adaptable to the environment like spore production. Clinically silent transmission in certain people (carriers), who might be sick for months or even years and periodically release contaminated feces into the environment, affects the persistence of S. Typhi in human populations *(Asmatullah et al.,2015)*. If an infection is not appropriately treated, it may become worse and cause a carrier state, where the infected individual can spread the resistant strain to others and becomes infectious. In the previous few years, Because of the increase of salmonellae that are resistant to ampicillin, chloramphenicol, and co-trimoxazole, or multidrug-resistant (MDR) strains of bacteria, fluoroquinolones and third-generation cephalosporins are now frequently used as first-line medications *(Bhutta et al., 2019)*.

The word "typhoid fever" was originally used in 1829 by Pierre Louis, who saw lesions in the lymph nodes of the abdomen in people who had died of

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"gastric fever. In 2000, typhoid fever was determined to have caused 216,000 deaths and 21.7 million illnesses worldwide *(Brooks et al., 2005)*. But these numbers reach Given that the majorityof patients receive therapy The prevalence of typhoid fever is higher in children and young adults. who either receive no therapy at all or are treated as outpatients and is probably not accurately expressing the actual burden of disease? There are between 200 and 300 cases of Salmonella enterica serotype typhi in the US per year; bout 80 percent of these cases are from visitors who have just returned from an endemic area (De *2005)*.

Typhi is an important risk factor to disease and mortality in a large number of nations with low or middle incomes. (LMICs) (*Bhutta et al., 2019)*. According to findings from the Institute for Health Metrics and Evaluation Global Burden of Disease Study, typhoid disease caused 10.9 million illnesses and 116.8 thousand deaths globally in 2017. (Dekker *et al., 2018)*. When typhoid cases and deaths worldwide were classified according to geographical sub-regions of the United Nations (UN), Southern Asia accounted for 68.0% of cases, while just five sub-regions of the UN— Southeast Asia, Western Africa, Southeast Africa, and Southeast Asia collectively accounted for 96.0% of cases (*Di et al., 2010)*.

When individuals consume water or food tainted through the excrement of someone who is losing During Salmonella Typhi an acute contamination, recovering, or long-term transportation, bacteria might spread due to inadequate sanitation (*Crump et al., 2015)*. The people who are most likely to contract typhoid disease are those with low access to microbiologically pure water, poor sewage systems, and dense population densities (*Ellen et al., 2012)*.

According to earlier research, children are especially vulnerable to typhoid disease (*Myanmar et al., 2017 and Leekha et al., 2011)*. Despite causing up just 39.0% of the population under research, children aged 15 and younger accounted for 75.0% of confirmed blood cultures cases of Typhoid fever disease, according to a study that published data from population-based surveillance in five Asian LMICs (*Elumalai et al., 2014)*

The clinical diagnosis of typhoid is challenging because the symptoms

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that follow Salmonella Typhi infection are frequently gradually and lack a distinct therapeutic manifestation. A fever is the most common symptom of infection, but other symptoms include headaches, muscle weakness, constipation, abdominal pain, nausea, and, more rarely, diarrhea, a painful throat and dry cough. Throat

The ability of a bacteria to withstand being exposed to an antibiotic such might have already killed it or halted its development is known as antimicrobial resistance (AMR) in microbiology (*Evidence et al., 2003)*. In addition to being associated with worse clinical outcomes for infected persons, the presence of AMR can result in significant delays before proper treatment is started Therefore, in order to enhance patient outcomes and make well-informed treatment decisions, testing the antibiotic susceptibility of isolated Salmonella Typhi is required.

The current study aimed to isolate and identify salmonella typhi (s. typhi) in blood sample among patients presenting with typhoid fever symptoms in D.I. Khan District hospital.

MATERIALS AND METHOD

The research being conducted was cross-sectional at the D.I. Khan Department of Microbiology and the FVAS Institute of Microbiology. Blood samples were taken from DHQ Hospital D.I. Khan patients. The main focus of this study will be the patients infected with salmonella typhi infection. A number of the many procedures done to ensure the safety of all 150 blood samples (n = 150) was to determine the proper amount of blood to be added in the blood culture vial (10 ml; never keep it below 15 °C for adults and 1-6 ml for children according to age). Blood cultures from several hospital wards were incubated in the laboratory using BACTEC 9050 blood culture equipment (*Ellen et al., 2012)*.

By using isolated culture methods, the bacteria were separated from the collected samples. Only MacConkey's agar and nutrient agar media were used for the isolation, measurement and maintenance of pure cultures. One (1) gramme of blood was collected from typhoid patients and enriched in five milliliters of Rappaport Vassiliadis R10 broth. The sample was then subculturing on Salmonella-Shigella (SS) agar and MacConkey agar, and the

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plates were incubated at 37°C for twenty-four hours (*Nan et al.,2015)*. After 24-48 hours of incubation, the plates were examined for the existence of colonies. Salmonella like colonies were used to prepare pure cultures. These colonies included those on MacConkey Agar (Abu-Resha et al., 2019), which appear pale or nearly colorless, and those on XLD selective media (*Nalbantsoy et al., 2010),* which appear smooth and red with a black colony Centre due to the ability of salmonella. Similarly, colorless colonies on Shigella agar ss (*Aburesha RA et al., 2019*) were collected under aseptic conditions and kept on nutrient agar for later biochemical analysis. Under an oil immersion lens (100x), the bacteria were visible as pink, short rod coccobacilli that were gram-negative, flagellated, and non-spore providing (*Aburesha RA et al., 2019)*. The microscopically characteristics after staining with gramme stain revealed conformity with those that another study has reported for S. Typhi. According to (*Aburesha RA et al., 2019 and Mortazavi et al., 2019)*. Traditional biochemical tests that are essential for identifying S. Typhi are Simmons citrate (positive), H2S production test (positive), indole test (negative), urease test (negative), and motility test (positive)

Traditional microbiological techniques, such as gramme stain and colony morphology, were used for identification. In short, this condition on blood agar and lactose fermentation on MacConkey's and Salmonella Shigella agar (*Oxoid Ltd., UK)* were the main characteristics examined to identify the isolates. After developing pure colonies, Gramme staining was used to identify between Bacteria that are Gram-positive and Gram-negative (*Chesbrough et al., 2005)*. This is an essential first step in the process of identifying bacteria.

Gramm staining was used to separate the bacterial species into Gram-positive and Gram-negative groups, according to Holt et al. (1994). After adding a drop of distilled water to the glass slide, the bacterial colony was spread out onto it using a sterile wire loop. Crystal violet and Gramme iodine were added to the slide after it had been heated for a duration of one minute each. Add a decolorizer for ten seconds after the iodine, and then safranin for one minute. The colored slides were examined under a microscope to check for the presence of any microorganisms. According to (Smith & Hussey et al.,2005), Gram-negative bacteria shown up as pink colonies, while gram-

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positive bacteria were identified by purple colonies.Many biochemical methods, such as the IMViC test, catalase test, and oxidase test, were used to biochemically detect bacterial colonies (Chesbrough et al., 2005). The isolates' identification and characterization were made simple by these tests. The following biochemical tests were used in this research (*Nahab et al.,* 2018).

A small amount of the bacterial colony's inoculum was removed using a put onto a spotless glass slide with a sterile wire loop. After that, a 3% hydrogen peroxide drop was introduced to the slide. Bubbles began to form in 5–10 seconds, indicating that the experiment was successful.

Paper was filtered using an oxidase reagent. The test bacterial colony was dispersed out over the filter paper using a sterile wire loop. A vivid blue or purple color that appeared within 10 to 30 seconds indicated that everything had worked well.

Tryptophan broth was placed in sterilized test tubes, which were filled with growth obtained from an 18–24-hour culture. After that, the tubes were incubated for 24 to 28 hours at 37°C. Following Kovac's reagent was added throughout incubation to the broth culture and the presence or absence.

RESULTS AND DISCUSSION

150 blood cultures in total were obtained from various hospital wards located in D.I. Khan. Out of the 150 blood cultures, 61 (40.6%) belong to the pediatrics age group (children under 5 years age). According to reports, gender was known a total of 70 men and 80 women. The positive sample of men 7(4.6%) and then female is 8(12%). However, 30 (20%) of the 150 blood cultures were from infants. Out of 150 samples, only 25 (16.6%) showed bacterial growth in rural areas, whereas 125(83.3%) in urban showed no.

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FREQUENCY AND PERCENTAGE OF SALMONELLA ISOLATED

Table 1 Patient Demographics

Variable	Response	Frequency	Percentage %
Age(years)	<1	30	20.0
	1-3	59	39.3
	4-5	61	40.7
Total	150	150	100.0
Gender	Male	70	46.7
	Female	80	53.3
Total	150	150	100.0
Area of residence	Urban	125	83.3
	Rural	25	16.7
Total	150	150	100.0

Study table 1 revealed the demographic characteristics of the respondents. The bulk of the participants were females between the ages of 4 and 5 years, residing in metropolitan areas respondents. The bulk of the participants were females between the ages of 4 and 5 years, residing in metropolitan areas.

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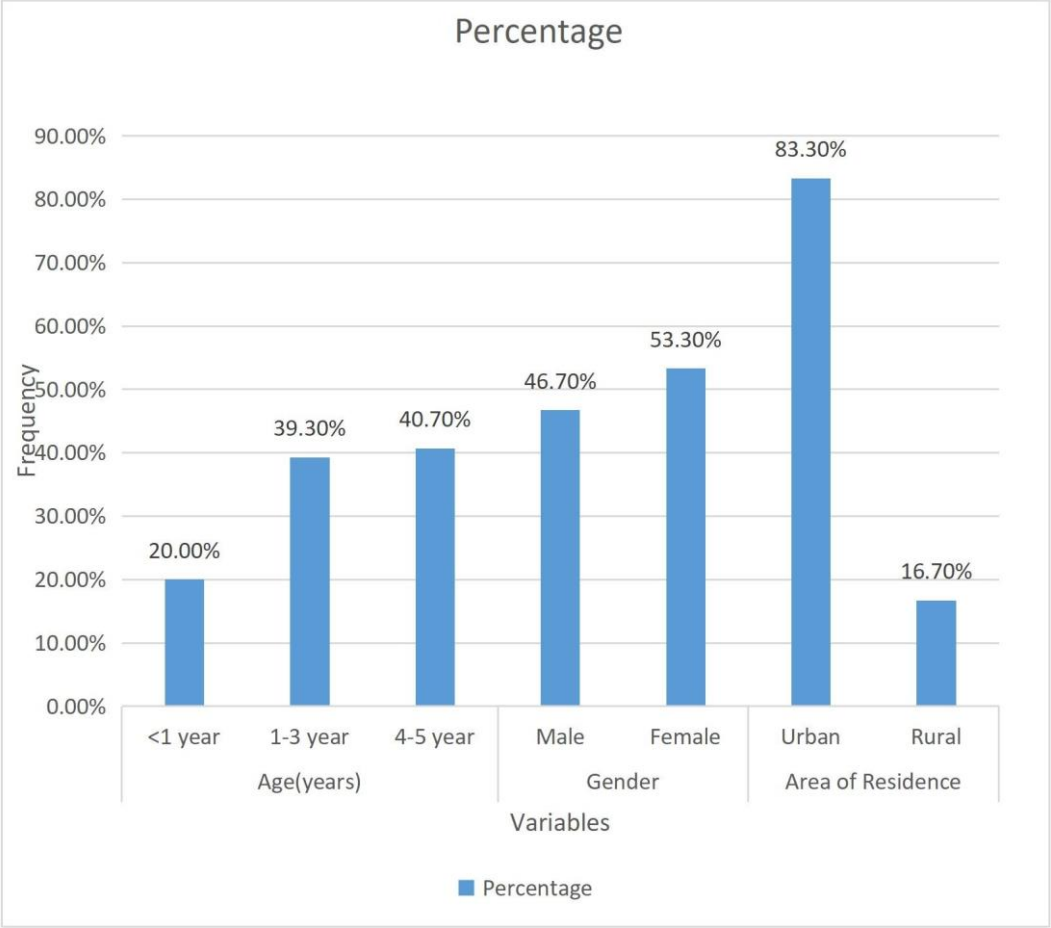

Figure 1: Graphical Representation of Demographic Variables

The Demographics of paediatric patients and the geographic distribution of the blood culture sample.

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Table 2 The Demographics of paediatric patients and the geographic distribution of the blood culture sample

Variable	Response	Positive (%)	Negative (%)	Total (%)	$\times^2$	Df	P value
Age(years)	<1	0 (0%)	30(20%)	30(20%)			
	1-3	7(4.6%)	52(34.6%)	59(39.3%)	14.222	2	.001
	4-5	18(12%)	43(28.6%)	61(40.6%)			
Gender	Male	7 (4.6%)	63(42%)	70(46.6%)			
	Female	18(12%)	62(41.3%)	80(53.3%)	4.200	1	.040
Area of residence	Urban	0 (0%)	125(83.3%)	125(83.3%)	150.000	1	.000
	Rural	25(16.6)	0(0%)	25(16.6%)			

The study table 2 presented the occurrence of S. typhi and its correlation among the demographic of participants. The study found a significant relationship ($p<0.05$) between the presence of Typhi bacteria and being a female living in urban regions, aged 1-3 years.

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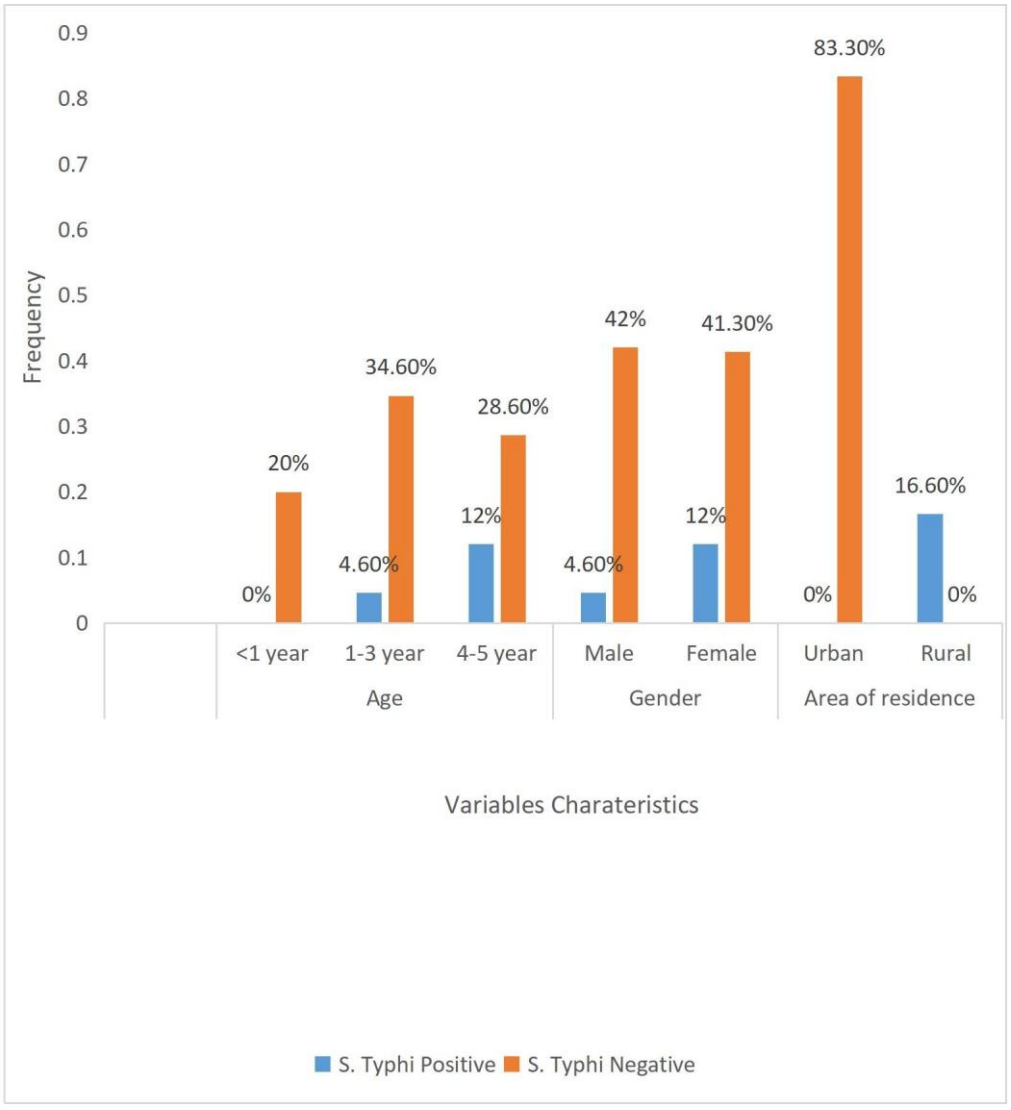

Figure 2: The geographical distribution of the blood culture sample and the demographics of the paediatric patients

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Table 3 Sensitivity pattern of isolated salmonella

By using a disk diffusion method, the percentage of Salmonellae resistant to various antibiotics Table (3).

Antibiotics n=10	Response	Positive (%)	Negative (%)	x^2	Df	P value
Ampicillin	S	22 (14.6%)	0 (0%)	128.906	1	.000
	No	3 (2%)	125(83.3%)			
Total	128	22 (14.6%)	150 (100%)			
Polymixin	R	22 (14.6%)	0 (0%)	128.906	1	.000
	No	3 (2%)	125(83.3%)			
Total	128	22 (14.6%)	150 (100%)			
Meropenem	R	22 (14.6%)	0 (0%)	128.906	1	.000
	No	3 (2%)	125(83.3%)			
Total	128	22 (14.6%)	150 (100%)			
Amoxicillin	S	25 (16.6%)	125(83.3%)	128.906	1	.000
	No	25 (16.6%)	125(83.3%)			
Total	150		150 (100%)			
Piperacillin	R	25 (16.6%)	125(83.3%)	128.906	1	.000
	No	25 (16.6%)	125(83.3%)			
Total	150		150 (100%)			
Tetracycline	R	22 (14.6%)	0 (0%)	128.906	1	.000
	No	3 (2%)	125(83.3%)			
Total	128	22 (14.6%)	150(100%)			
cefotaxime	S	22 (14.6%)	0 (0%)	128.906	1	.000
	No	3 (2%)	125(83.3%)			
Total	128	22 (14.6%)	150 (100%)			
Imipenem	R	13(8%)	0 (0%)	128.906	1	.000
	S	9 (6%)	0 (0%)			
	No	3 (2%)	125(83.3%)			
Total	128	9+13(17.6%)	150 (100%)			
Ceftazidime	R	22 (14.6%)	0 (0%)	128.906	1	.000
	No	3 (2%)	125(83.3%)			
Total	128	22(14.6%)	150(100%)			
Ciprofloxacin	R	22(14.6%)	0	128.906	1	.000
	No	3(2%)	125(83.3%)			
Total	128	22(14.6%)	150(100%)			

R=Resistant, S=Sensitive

10 antibiotics were used and each one showed its unique resistant and sensitivity value according to CLSI guidelines. The inhibitory zone was measured and noted. In due to the above table, of the 150 samples that were

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tested for imipenem, 13 (8%) were resistant and 9 (6%), were sensitive. Ampicillin is the antibiotic with the highest number 22 (14.6%) of sensitivity out of the 10 commonly used antibiotics. On the other hand, ampicillin is the medication with the greatest number of resistances that is 100% resistance. A p-value in the final column shows whether or not the drugs used are significant. A statistical measure known as the P-value is used to verify a hypothesis by comparing it to observed data. We are able to determine which drugs are statistically significant or not by comparing the P-value with 0.000. From the above, we could say that all antibiotics have the same p-value, showing their significance.

In this work, we tried to solve the developing problem of Salmonella enterica resistance. The most serious public health risk in is typhoid illness which arises from the drug-resistant Typhi (Salmonella gram-negative) bacteria emerging Asian nations and a prevalent bloodstream infection source in the Middle East *(*Klemm *et al., 2018)*. One of the most common reasons for death among children is bacterial infections. Studying the bacterial profile and resistance to antibiotics pattern of the isolates can help pediatricians treat typhoid infections effectively.

In present study 12% of blood cultures occur in children aged 4 to 5, but 50% of newborn blood cultures are positive in another research (*Myat et al., 2020)*. Knowledge of common the susceptibility of bacteria obtained from blood cultures to antibiotics in specific environment improves in the selection of antibiotics. Salmonella typhi was the common bacteria that was isolated from blood cultures in the present study. The research was compared with (*Mzungu et al., 2016*) study on Bacteriologic Profile and Blood culture isolates antibiogrammed from a children's hospital in Kabul *(Nahab et al., 2018)*. By using standard biochemical tests isolates were identified and positive growths were examined. The frequency of positive blood cultures among the 150 blood cultures obtained from in-patients is due to this blood culture was 12% in our research.

Similarly, from our research as compared to Syed *(S.M et al., 2023)* study on antibiogram of blood culture in pediatric patients and positive blood culture was in-line 13.5%.so they are significant. From this study indicates

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that Positive blood culture was among highest age group from 4 to 5 years 18(12%) while according to research done in Gujarat, India 18.62% amongst the near result of our study. Typhoid fever is a prevalent sickness in underdeveloped nations, affecting both urban and rural regions. The number of deaths annually from the disease is over 500000, with over 120 million cases reported globally (*Nair et al., 2013*).

Resistant to multiple drugs Iraq and several Asian nations, including Pakistan and India, have high rates of S. typhi widespread disease. Reports of MDR S. typhi have also been made in several regions of the world, including United States, United Kingdom. Multi-drug resistant S. typhi cases have recently emerged in several nations throughout the world. The present results following testing with antibiotics, the zone of inhibition for each of the 10 samples was examined to determine if the tested antibiotics were sensitive or resistant. The disc diffusion method, a Kirby-Bauer method modification, was used to test the isolated strains of Salmonella for Mueller-Hinton agar antibiotic susceptibility (India's Hi-Media) according with conventional protocols. The Clinical and Laboratory Standards Institute (CLSI) has recommended. We examined the resistance of major clinical antibiotic included ampicillin 10µg, polymixin300µg, meropenem 10µg, trimethoprim 5µg, piperacillin 100µg, cefotaxime 30µg, ciprofloxacin 5µg, ceftriaxone30µg. Tetracycline and piperacillin show a high resistance to Salmonella isolates. Amoxicillin and cefotaxime, on the other hand, are less sensitive.

However, resistance to other drugs, cefotaxime, and imipenem was identified in this study, with resistance rates of 22(14.65%) and 9(6%), respectively, higher than in previous studies, with the exception of tetracycline outcome. Ciprofloxacin shown strong antibacterial activity against the isolates of Salmonella. Ciprofloxacin was reported to be effective against all 150(100%) isolates. Similar findings were reported in previous research resistance to tetracycline, respectively, from South India (Suresh et al., 2006), Nigeria (*Sofia et al., 2006*).

The current study's results also showed that Salmonella isolates had 100%, 100%, and 100% resistance rates to ciprofloxacin, polymixin, meropenem, and tetracycline, among other frequently prescribed


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antimicrobials. The difference can result from different laboratory procedures being used. Even so, compared to ampicillin, there was a lower percentage of resistance found for amoxicillin, cefotaxime, and tetracycline 22 (14.6%).

In a comparable way, we can now determine the antibiotic's importance by comparing the P value. A statistical measure used to verify a hypothesis against observed information is known as a P-value. We can determine whether or not a medicine is statistically significant by comparing its P-value to 0.000. As we can see from the above, the drugs tetracycline, ampicillin, amoxicillin, cefotaxime, and ciprofloxacin have p-values less than 0.000, showing that they are significant. A limitation of this research was that it was impossible to determine strain dynamics (different bacterial isolates) or perform additional test for susceptibility procedures that would have revealed different distribution of strains and antibiotics on the spot.

CONCLUSION

In conclusion, this study found that Salmonella typhi was commonly detected in children between the ages of 1 and 5. The findings of the antibiotic susceptibility tests conducted in this study revealed a significant level of resistance among the isolates, particularly towards amoxicillin (16.6%) and tetracycline (14.6%) in E. coli. In the study area, Salmonella Typhi had a resistance rate of 14.6% to amoxicillin and 8% to ampicillin. Cefotaxime had a resistance rate of 22% and imipenem had a resistance rate of 13% in relation to fever. Additionally, it was noted that 16.6% of bacteria exhibited traits of being resistant to many drugs. Therefore, it is necessary to conduct thorough investigations in order to determine the molecular epidemiology of these bacteria that are resistant to antibiotics, for the purpose of public health surveillance.

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